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# MOLECULAR CHARACTERIZATION OF BEGOMOVIRUS INFECTING SOYBEAN (*GLYCINE MAX* L.) CROP IN CENTRAL INDIA

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## ABSTRACT

***Aim:*** *To detect bipartite begomovirus infection in soybean crop, characterize the pathogen and demonstrate Koch`s postulate on Nicotiana benthamiana.* ***Methods:*** *Symptomatic soybean leaves were collected from fields; total DNA was isolated followed by PCR detection of begomovirus by degenerate primers. RCA product of viral genome was digested by restriction enzyme, cloned in pBluescript (pKS+) cloning vector and sequenced. Evolutionary study of begomo virus was analyzed by constructing phylogenetic tree. Partial tandem repeats were constructed in pCAMBIA2300 plant binary vector for demonstration of koch`s postulate.* ***Results:*** *Soybean crop was infected by bipartite begomo virus along with associated betasatellite. MYMIV DNA-A (2736nt) showed maximum (99.8%) nucleotide sequence identity with Mungbean yellow mosaic India virus (MYMIV-[IN-Ak-14] KP779635). MYMIV DNA-B (2671nt) showed maximum (99.8%) nucleotide sequence identity with Mungbean yellow mosaic India virus (MYMIV-[IN-Sat-12] KP779634). MYMIV DNA-β (1366nt) showed maximum (97.3%) nucleotide sequence identity with Tomato leaf curl Gandhinagar betasatellite (ToLCGNB-[IN-GN-12] KC952006). Evolutionary studies of DNA-A, DNA-B and DNA-β showed close relationship with previously reported bipartite begomoviruses. Upon agro-inoculation of partial tandem repeats of DNA-A, DNA-B and DNA-β, typical symptoms of begomovirus infection were observed on N. benthamiana and verified by RT-PCR in terms of accumulation of viral copy number.* ***Conclusion:*** *Soybean crop was found to be infected by bipartite begomovirus along with associated betasatellite of Tomato leaf curl Gandhinagar betasatellite. Agro-inoculation of partial tandem repeats of DNA-A, DNA-B and DNA-β was capable to produce and increase the symptom severity on N. benthamiana. This study will help to develop a resistant variety of soybean cultivar to enhance soybean crop yield.*

## KEY WORDS

*Begomovirus, cloning, Mungbean yellow mosaic India virus, soybean.*

## INTRODUCTION

Geminivirus, belongs to *Geminiviridae* family, is the largest group of plant-infecting viruses [1]. Morphologically, Geminiviruses are twinned (geminate) incomplete icosahedral particle of 22 to 38 nm having single-stranded, circular DNA genome of ~2.7 kb [2]. On the basis of their genome organization, host range and insect vector, Geminiviruses are classified into nine genera-*Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus*, *Turncurtovirus, Capulavirus* and *Glabrovirus* [1]. Out of these genera, *Begomovirus* is the largest genus of *Geminivirideae* family and is exclusively transmitted by whitefly (*Bemisia tabaci* Gennadius) that cause economically significant loss of many dicotyledonous crops such as vegetables, cereals, pulses etc.

Based on the genomic component, begomoviruses are either bipartite having two components of the genome (DNA-A and DNA-B) or monopartite having single genome component (DNA-A) [3]. In bipartite begomoviruses, DNA-A has six ORFs, out of which two are present on viron sense strand and four on complementary strands that participate in different steps of virus life cycle such as replication, gene expression and vector transmission [4]. DNA-B has two ORFs which encode movement protein and nuclear shuttle protein for systemic and cell to cell movement of virus [5]. Opposite to bipartite begomoviruses, monopartite begmoviruses have a single component of genome DNA-A (~2.7 kb). Apart from DNA-A, monopartite begomoviruses are also associated with circular ssDNA betasatellite (DNA-β) of ~1.3 kb, essential for the induction of typical disease symptoms. Betasatellite has highly conserved single gene ORF (encodes βC1), an adenine-rich region and highly conserved satellite conserved region (SCR) [6]. βC1 protein determine pathogenicity (symptoms) and a RNA silencing suppressor (a host defense mechanism) [7].

Soybean (*Glycine max* L.), an important source of animal feed and vegetable oil worldwide, belongs to Fabaceae family which includes many economically and nutritionally important pulses. Soybean seed contains 40% protein (having essential amino acids), 20% oil, numerous vitamins (B complex, K), minerals (Calcium, Iron, Manganese, Copper and Phosphorus) carbohydrates, isoflavones and fibers [8]. India ranks fifth in world soybean production after USA, Brazil, Argentina and China with 7.00 million ton over 11.650 million hectares land [9]. In India, Madhya Pradesh (MP) is the largest producer of soybean with more than 50% of total soybean production of India [10].

However, since last few decades, soybean production has been reduced due to many biotic factors such as bacteria, fungi, insect, nematodes and virus which not only reduce the crop yield but also reduce the quality of soybean crop globally. Among all biotic factors, whitefly-transmitted begomoviruses (Family *Geminiviridae*) are the most fatal factor for soybean production in Indian sub-continent.

There are four species of bipartite begomoviruses *viz. Mungbean yellow mosaic virus*, *Mungbean mosaic India virus, Dolichos yellow mosaic virus*, *Horsegram yellow mosaic virus* are reported to cause yellow mosaic disease (YMD) in legumes [11]. The first report of YMD of soybean has been reported from the USA in 1935 [12]. Regarding Indian sub-continent, YMD of soybean has been reported for the first time in 1960 from central India [13]. YMD occurs all over the Indian sub-continent and is emerged as major threats to legume production including soybean. The precise figure of crop loss due to YMD is not available because the incidence of YMD varying area to area and crop to crop. However, annual crop loss in India due to YMD in mungbean, urdbean and soybean are estimated at USD 300 million [14, 15]. In addition, soybean crop loss in India due to YMD has been reported 105,000 metric tonnes in 1996 [16]. In the present communication, we have characterized bipartite begomovirus (MYMIV) along with betasatellite infecting to soybean crop in central India (MP).

## MATERIAL AND METHODS

### Sample collection and cloning of viral genome and betasatellite

On the basis of putative symptoms of begomovirus infection, infected soybean leaves were collected from the farmer`s fields of Madhya Pradesh (India) in 2016 (Figure1) and used for DNA isolation by Dellaporta method [17]. Begomovirus infection was detected by PCR with the help of degenerate primers AV494 and AC1048 [18] (Figure 2). Approximately 20 pg of total genomic DNA was used for Rolling circle amplification with the help of Templiphi DNA amplification kit (GE Health Care Life Sciences) followed by restriction digestion with *KpnI* and *BamHI*. Around 2.7 kb fragment which was eluted from the gel was cloned into pBluescript (pKS+) cloning vector that was linearised by *KpnI* and *BamHI* respectively. In order to confirm recombinant clones, positive clones were digested with a restriction enzyme at cloning site resulted into the release of ~2.7 kb along with 3.0 kb vector. For cloning of DNA betasatellite, total DNA was used for PCR amplification of ~1.3 kb fragments by universal beta primers [19] and ligated into pTZ57R/T vector (INSTA cloning kit, MBI Fermentas, USA).

**Fig.-1 Symptoms on naturally infected soybean crop by begomo virus.**

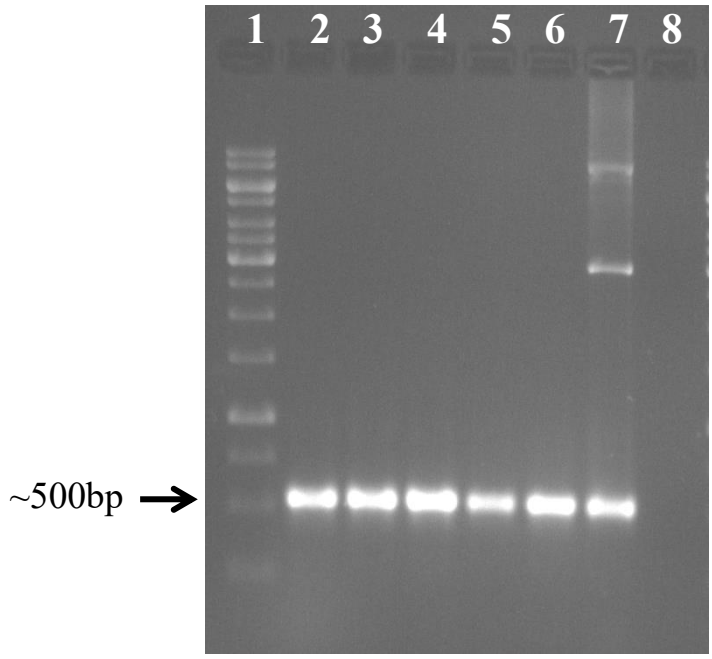


**Fig.-2 Detection of begomovirus infection in soybean leaf by degenerate primers. Lane 1: 1kbp DNA Marker, Lane 2 to 6: samples, Lane 7: positive control, Lane 8: Healthy plant**

**Sequence analysis of viral genomes and betasatellite**

Based on restriction analysis two of ~2.7 clones (pSOYA and pSOYB) and one of ~1.3 clone (pSOYb) were selected for full-length sequencing. The full-length sequences of above cloned were determined by dideoxynucleotide chain termination method with specific primer to the cloning vector and followed by primer walking (Chromous Biotech Pvt. Ltd., Bangalore, India). Sequencing results were examined for conserved nonanucleotide sequences and begomovirus-specific ORFs by using NCBI ORF finder. Analysis of sequences was performed by NCBI (http//www.ncbi.nlm.nih.gov) BLAST search. Full-length genome of viruses and satellites were aligned by ClustalW. Evolutionary studies

of viruses were analyzed by constructing a phylogenetic tree (dendrogram) by Mega7. Determination of sequence identity was performed by DNASTAR MegAlign software (version 10; DNASTAR, Lasergene Madison, WI, USA).

**Construction of partial tandem repeats of viral genome and evaluation of their infectivity**

Partial tandem repeats of clones pSOYA, pSOYB and pSOYb were constructed to demonstrate Kotch`s postulates in a glass-house. A 1690 bp fragment [(digested by *HindIII* (1113nt) - *KpnI* (60 nt)] of pSOYA, containing intergenic region was cloned in plant binary vector pCAMBIA2300 which was previously linearized by restriction enzyme *HindIII* and *KpnI*. Subsequently, full-length genome (2.7 kb) was ligated into pCAMBIA2300 in orientation of tandem repeat to prepare partial tandem repeat and designated as pMbA. Similarly, for pSOYB, An 1179 bp fragment [*PstI* (1620 nt) - *BamH*I (126 nt)] containing the intergenic region (IR) was cloned in pCambia2300 binary vector to generate a monomer. Subsequently, full length (2.7 kb) viral genome was cloned at *BamH*I restriction site and further mobilized into binary vector pCAMBIA2300 to yield partial tandem repeats of DNA-B and designated as pMbB. A 441 bp fragment [*HindIII* (1296 nt) - *XbaI* (370 nt)] of pSOYb, containing satellite conserve region was cloned in plant binary vector pCAMBIA2300 which was previously linearized by *HindIII* and *XbaI*. Subsequently, full-length betasatellite (1.3 kb) was ligated into pCAMBIA2300 in tandem repeat orientation to prepare partial tandem repeat and designated as pMbβ. All the plants were maintained in glass-house at 25 °C (±2) and 70% relative humidity in 16 hr of light and 8 hr of dark photoperiod cycle for 45 days. The symptom appearance and symptom severity were recorded.

**Detection of DNA-A, DNA-B and DNA-betasatellite from Agro-inoculated *Nicotiana benthamiana* by PCR and Real-Time PCR**

Detection of DNA-A, DNA-B and DNA-β in agro-inoculated *N. benthamiana* plant leaves were confirmed by PCR using degenerate primers AV494 and AC1048 [18] for DNA-A and universal beta primers [19] for DNA-betasatellite. The quantification of virus titer in agro-inoculated plants was performed by SYBR Green based Real-Time PCR with pMbA, pMbB and pMbβ specific primers. A standard curve was prepared by making a 100-fold serial dilution of a plasmid consisting of the full-length respective clone of pSOYA (DNA-A), pSOYB (DNA-B) and pSOYb (DNA-betasatellite). A total 20 µl volume of reaction mixture containing 10 µl SYBR Green (2X), 0.2 µM primers and 100 ng template was taken and PCR programmed was set up as initial denaturation at 95 $^0$C for 10 min followed by 40 cycles each consists denaturation at 95 $^0$C for 1 minute, annealing at 60 $^0$C for 30 sec., and extension at 72 $^0$C for 1 minute. PCR Amplification was performed in triplicates for each sample.

## RESULTS

**Genome organization of studied viral genome and betasatellite**

Sequence analysis revealed that viral genome pSOYA, pSOYB and pSOYb comprised of 2736 nucleotides, 2671 nucleotides, 1366 nucleotides respectively. All above nucleotide sequences were submitted to GenBank and their respective Accession no are MH324445, MH324446 and MH324447. The genome organization of studied viruses revealed typical bipartite nature of begomovirus. Like other bipartite begomoviruses DNA-A component, pSOYA possesses six ORFs, two on virion sense strand and four on the complementary sense strand. Viron sense strand ORFs, designated as AV1 and AV2 encode a coat-protein (30 kDa) and precoat protein (13.10 kDa), respectively. The ORFs on the complementary strand were designated as AC1 (encodes replicase; Rep 41.21 kDa protein), AC2 (encodes transcriptional activator; TrAP 17.07 kDa protein), AC3 (encodes replication enhancer; REn 15.65 kDa protein), and AC4 (encodes 11.35 kDa protein). An intergenic Region (IR) sequence was of 300nt and contained the characteristic inverted repeat capable of forming a stem-loop structure. The IR sequence had the upstream TATA box for the AC1 and the Rep protein. Similar to DNA-B component the second genomic component pSOYB had two ORFs and encodes one protein in each orientation (BV1 movement protein of 29.22 kDa and BC1 nuclear shuttle protein of 33.71 kDa). pSOYb genome organization was found like DNA-betasatellite and had single ORF βC1 (363 nt and encodes 13.51 kDa protein), SCR of 228 nt length and adenine-rich region, characteristic of its genome organization.

**Sequence analysis and phylogenetic relationship**

In order to analyze and compare the genome organization of studied virus, for each DNA-A, DNA-B and DNA-betasatellite, twenty-four full-length genome

sequences of DNA-A, DNA-B and DNA- betasatellite of begomoviruses infecting to soybean and other crops were selected which are available in NCBI GenBank database. DNA-A has showed maximum nucleotide sequence identity of 99.8% with *Mungbean yellow mosaic India virus*-[India-Akola-2014] (MYMIV-[IN-Ak-14], GenBank Accession no. KP779635) and minimum sequence identity 53.7% with Croton yellow vein mosaic virus-[India] (CroYVMV-[IN], GenBank Accession no. AJ507777) (Table 1). However, studied virus has showed the difference with soybean infecting begomoviruses, previously reported from India. The ORF AC1 showed the maximum amino acid identity of 99.6% with MYMIV-[IN-Ak-14] (Table 1) and its product replication associated protein (Rep) has showed a maximum sequence identity of 99.8% with MYMIV-[IN-Ak-14]. ORF AC2 encodes transcription activator protein (TrAP), showed 99.7% nucleotide sequence identity with MYMIV-[IN-Ak-14] (Table 1). ORF AC3 encodes replication enhancer protein (REn) was found to be 99.6% similar with MYMIV-[IN-Ak-14] (Table 1). The ORF AC4 showed maximum nucleotide sequence identity of 99.0% with MYMIV-[IN-Ak-14] (Table 1). The ORFs AV1 and AV2 encode coat-protein (CP) and pre-coat protein showed maximum nucleotide sequence identity 99.1% and 99.3% with MYMIV-[IN-Ak-14] respectively (Table 1) and their products showed 99.1% and 98.5% similarity with MYMIV-[IN-Ak-14]. In the evolutionary study, it shared the same clad of MYMIV in the phylogenetic tree (Figure 3). Intergenic region of studied virus shared maximum (98.2%) nucleotide identity with MYMIV-[IN-Ak-14] (Table 1).

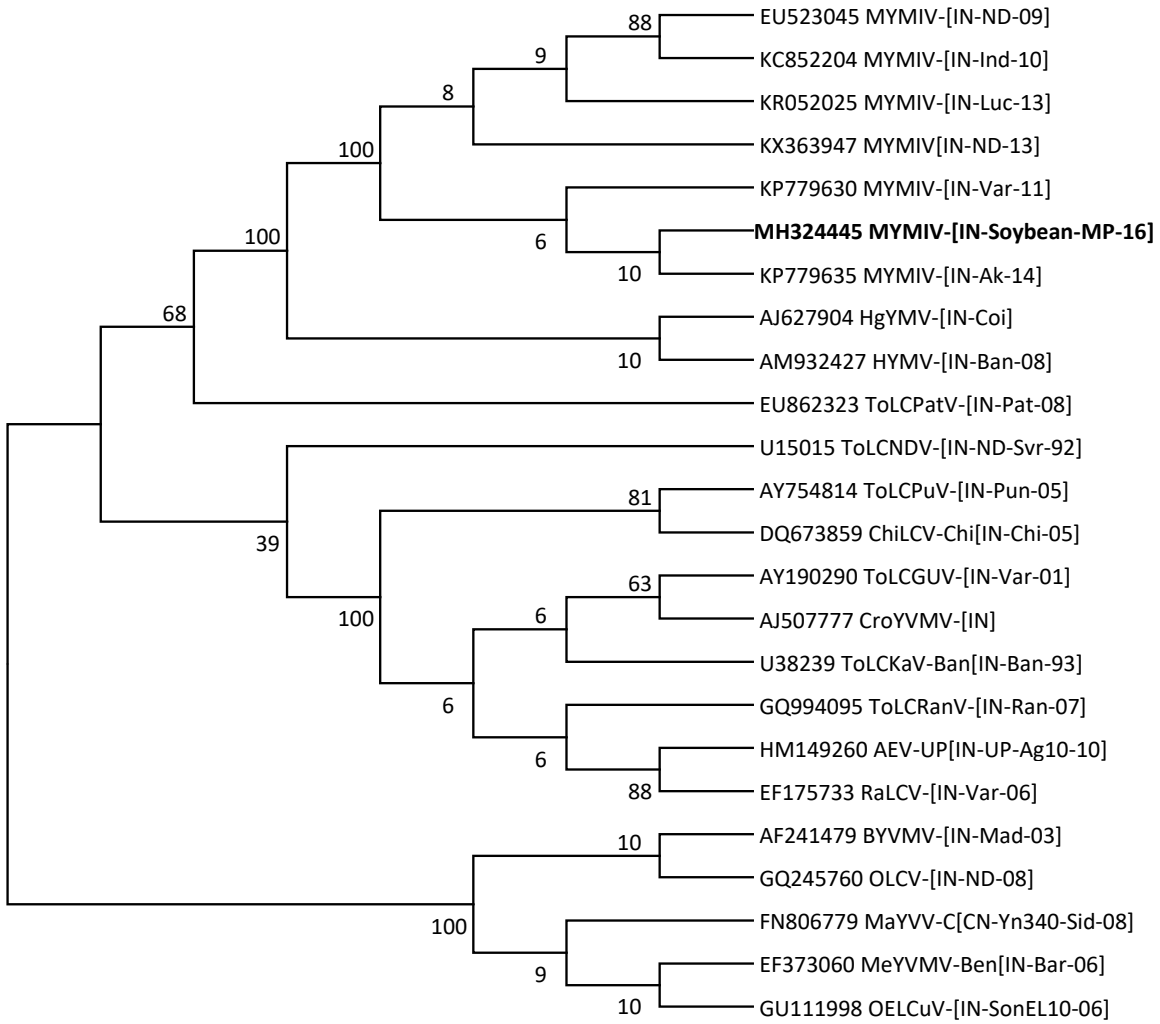


**Fig.-3: Phylogenetic tree of MYMIV-[IN-Soybean-MP-16] DNA-A genome constructed by the Mega 7, showing the relationship with other selected begomoviruses. GenBank accession numbers are indicated to the left of each virus name and abbreviations are as per Fauquet et al., 2008. Studied virus is in bold**

**Table 1: Percent nucleotide sequence identities of MYMIV-A [IN-Soybean-MP-16] with other selected begomoviruses.**

| S.N. | Name | Accession no. | Identity % | AC1 | AC2 | AC3 | AC4 | AV1 | AV2 | IR |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MYMIV-A [IN-Soy-MP-16] | MH324445 | | | | | | | | |
| 2 | MYMIV-[IN-Ak-14] | KP779635 | **99.8** | **99.6** | **99.7** | **99.6** | **99.0** | **99.1** | **99.3** | **98.2** |
| 3 | MYMIV-[IN-ND-09] | EU523045 | 97.7 | 98.5 | 96.2 | 95.6 | 97.7 | 97.4 | 98.0 | 95.9 |
| 4 | MYMIV[IN:ND:13] | KX363947 | 97.0 | 97.5 | 94.9 | 95.6 | 97.0 | 98.3 | 97.1 | 94.1 |
| 5 | MYMIV-[IN-Luc-13] | KR052025 | 96.9 | 98.2 | 94.9 | 95.8 | 97.7 | 97.3 | 98.2 | 95.9 |
| 6 | MYMIV-[IN-Var-11] | KP779630 | 96.7 | 97.3 | 98.5 | 98.3 | 97.3 | 97.7 | 98.0 | 88.2 |
| 7 | MYMIV-[IN-Ind-10] | KC852204 | 95.4 | 97.0 | 95.4 | 96.3 | 97.6 | 96.9 | 97.1 | 96.3 |
| 8 | HYMV-[IN-Ban-08] | AM932427 | 79.3 | 82.2 | 82.8 | 82.2 | 80.3 | 79.6 | 21.0 | 60.2 |
| 9 | HgYMV-[IN-Coi] | AJ627904 | 78.4 | 80.5 | 83.6 | 82.7 | 80.3 | 79.2 | 77.2 | 59.7 |
| 10 | ToLCRanV-[IN-Ran-07] | GQ994095 | 58.1 | 67.1 | 54.3 | 48.6 | 74.1 | 63.0 | 49.1 | 32.1 |
| 11 | ToLCGUV-[IN-Var-01] | AY190290 | 57.7 | 96.8 | 51.1 | 46.2 | 72.7 | 63.7 | 49.4 | 29.2 |
| 12 | ToLCPatV-[IN-Pat-08] | EU862323 | 57.7 | 64.0 | 54.7 | 50.6 | 64.0 | 66.1 | 50.6 | 26.9 |
| 13 | ToLCKaV-[IN-Ban-93] | U38239 | 56.8 | 67.1 | 51.1 | 46.2 | 72.7 | 63.0 | 49.4 | 29.2 |
| 14 | RaLCV-[IN-Var-06] | EF175733 | 56.4 | 63.3 | 54.1 | 48.1 | 64.7 | 62.6 | 50.6 | 27.7 |
| 15 | BYVMV-[IN-Mad-03] | AF241479 | 55.7 | 61.0 | 52.1 | 46.2 | 62.7 | 63.2 | 49.7 | 29.9 |
| 16 | ToLCPuV-[IN-Pun-05] | AY754814 | 55.6 | 64.5 | 50.9 | 47.7 | 70.7 | 62.1 | 45.3 | 29.5 |
| 17 | ChiLCV-Chi[IN-Chi-05] | DQ673859 | 55.5 | 64.7 | 51.4 | 46.2 | 71.0 | 60.4 | 47.4 | 31.0 |
| 18 | OELCuV-[IN-Son-06] | GU111998 | 55.4 | 62.3 | 50.3 | 48.9 | 62.7 | 61.6 | 50.6 | 26.2 |
| 19 | OLCV-[IN-ND-08] | GQ245760 | 55.4 | 62.0 | 49.7 | 47.9 | 65.7 | 63.8 | 48.8 | 28.4 |
| 20 | MaYVV-C[CN-Sid-08] | FN806779 | 55.2 | 62.2 | 48.8 | 46.7 | 71.4 | 62.9 | 44.7 | 25.5 |
| 21 | AEV-[IN-UP-Ag10-10] | HM149260 | 55.0 | 63.4 | 52.8 | 46.4 | 65.1 | 59.7 | 48.2 | 28.0 |
| 22 | ToLCNDV-[IN-Svr-92] | U15015 | 54.5 | 61.6 | 49.3 | 47.4 | 65.5 | 61.7 | 38.9 | 26.2 |
| 23 | MeYVMV-[IN-Bar-06] | EF373060 | 54.5 | 60.9 | 48.8 | 44.0 | 59.7 | 61.8 | 49.4 | 31.4 |
| 24 | CroYVMV-[IN] | AJ507777 | 53.7 | 57.9 | 52.8 | 47.4 | 52.7 | 63.9 | 48.5 | 25.8 |

**Table 2: Percent nucleotide sequence identities of MYMIV-B [IN-Soybean-MP-16] with other selected begomo viruses.**

| S. N. | Name | Accession no. | Identity % | BC1 | BV1 | CR |
|---|---|---|---|---|---|---|
| 1 | MYMIV-B [IN-Soy-MP-16] | MH324446 | | | | |
| 2 | MYMIV-[IN-Sat-12] | KP779634 | **99.8** | **99.0** | **99.9** | **99.5** |
| 3 | MYMIV-[IN-ND-13] | KX363948 | 94.9 | 96.5 | 97.1 | 91.5 |
| 4 | MYMIV-[IN-PM-09] | FR714861 | 93.9 | 93.8 | 96.8 | 91.6 |
| 5 | MYMIV-[IN-AK-14] | KP779636 | 93.3 | 95.1 | 95.6 | 89.8 |
| 6 | MYMIV-[IN-Jab-12] | LC271791 | 93.2 | 95.1 | 95.7 | 89.7 |
| 7 | MYMIV-[IN-Var-05] | DQ061273 | 92.3 | 94.9 | 93.3 | 87.1 |
| 8 | MYMIV-[NP-KB-10] | JN543396 | 92.2 | 95.4 | 95.8 | 86.2 |
| 9 | MYMIV-[IN-ND-Bg3-91] | AF142440 | 90.3 | 93.4 | 93.7 | 87.0 |
| 10 | MYMIV-[IN-MB-14] | KP319016 | 85.5 | 92.8 | 93.0 | 77.5 |
| 11 | HgYMV-[IN-Coi] | AJ627905 | 58.9 | 77.6 | 69.1 | 39.0 |
| 12 | HYMV-[IN-Ban-08] | AM932428 | 56.3 | 20.0 | 68.7 | 40.0 |
| 13 | MYMIV-[IN-Var-11] | KP779632 | 40.7 | 95.2 | 95.7 | 90.9 |
| 14 | MYMIV-[IN-10] | KP828155 | 40.6 | 95.8 | 96.9 | 88.0 |
| 15 | MYMIV-[IN-ND-08] | EU523046 | 40.5 | 96.0 | 96.8 | 88.8 |
| 16 | MYMIV-[IN-Har-01] | AY271894 | 34.3 | 94.6 | 96.4 | 90.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 17 | MYMIV-[IN-ND-05] | AY939925 | 33.8 | 95.3 | 96.0 | 87.5 |
| 18 | MYMIV-[IN-Meg-15] | KU950431 | 33.1 | 93.1 | 93.3 | 80.9 |
| 19 | MYMIV-[IN-Vam-06] | DQ400849 | 31.0 | 20.5 | 92.7 | 60.7 |
| 20 | ToLCPalV-[IN-Pal-047] | AM992534 | 30.0 | 37.5 | 29.2 | 24.1 |
| 21 | ToLCNDV-[IN-ND-Svr-92] | U15017 | 28.6 | 37.0 | 30.4 | 21.7 |
| 22 | ToLCGUV-[IN-Var-01] | AY190291 | 28.3 | 37.8 | 30.5 | 19.8 |
| 23 | HYMV-[IN-Ban-11] | KP752089 | 28.2 | 77.7 | 69.0 | 38.5 |
| 24 | SYMV-[IN-Mad-03] | AJ582267 | 26.0 | 77.3 | 70.2 | 39.1 |

**Table 3: Percent nucleotide sequence identities of ToLCGnB-[IN-Soybean-MP-16] with other selected betasatellites.**

| S. N. | Betasatellites | Accession no. | Identity % | Beta c1 | SCR |
|---|---|---|---|---|---|
| 1 | ToLCGnB-[IN-Soy-MP-16] | MH324447 | | | |
| 2 | ToLCGNB-[IN-GN-12] | KC952006 | **97.3** | **97.0** | **94.4** |
| 3 | CLCB-[IN-Lud-04] | AY765255 | 74.9 | 93.8 | 86.8 |
| 4 | PLCB-[IN-ND-05] | GU370715 | 73.1 | 93.0 | 87.3 |
| 5 | TYLCOB-[Om-Alk-13] | HG969298 | 72.6 | 93.3 | 86.0 |
| 6 | ToLCKarB-[IN-Guj-12] | KF515612 | 71.2 | 96.4 | 87.7 |
| 7 | ToLCB-[PK-RYK-97] | AJ316036 | 70.4 | 93.8 | 83.8 |
| 8 | ToLCB-[Om-14] | HG969210 | 69.0 | 93.6 | 79.2 |
| 9 | CPSLCB-[IN-ND-04] | AY728263 | 68.3 | 93.3 | 87.2 |
| 10 | TYLCB-[OM-Qur-08] | HE800543 | 68.2 | 95.0 | 72.8 |
| 11 | ToLCKarB-[IN-Jan-04] | AY754813 | 66.4 | 91.3 | 86.0 |
| 12 | ChLCuB-[PK-MC-97] | AJ316032 | 63.9 | 79.8 | 78.8 |
| 13 | ChiLCB-[IN-Raj-16] | KU376496 | 63.8 | 80.4 | 83.9 |
| 14 | ChiLCB-[PK-Mul-04] | AM279670 | 63.1 | 79.6 | 83.0 |
| 15 | PLCB-[IN-Guj-15] | KT253638 | 63.0 | 60.8 | 91.2 |
| 16 | ToLCRaB-[IN-Raj-03] | AY438558 | 62.1 | 78.4 | 86.4 |
| 17 | PaLCuB-[IN-ND-03] | AY244706 | 55.9 | 58.0 | 85.9 |
| 18 | ToLCPaB-[IN-Pat-07] | EU862324 | 52.0 | 64.7 | 81.3 |
| 19 | CroYVMB-[PK-Pun-06] | AM410551 | 47.4 | 56.0 | 80.3 |
| 20 | AYVB-[SG-95] | AJ252072 | 46.1 | 57.4 | 71.7 |
| 21 | RhYMB-[IN-Pha-14] | KP752092 | 44.3 | 53.5 | 78.1 |
| 22 | CLCuMuB-[PK-Mul-U89-97] | AJ298903 | 42.3 | 39.5 | 72.4 |
| 23 | FBLCuB-[IN-Kan-11] | JQ866298 | 41.7 | 53.0 | 73.2 |
| 24 | BYVB-[IN-Mut-00] | AJ308425 | 38.0 | 36.7 | 60.4 |
| 25 | HYVB-[UK-Nor1-99 ] | AJ316040 | 37.1 | 45.0 | 57.8 |

DNA-B comprised of 2671nt and showed maximum nucleotide sequence identity (99.8%) with *Mungbean yellow mosaic India virus*-[India-Satna-2012] (MYMIV-[IN-Sat-12] GenBank Accession no. KP779634) (Table 2). The ORF BC1 and BV1 shared 99.0 % and 99.9% nucleotide sequence identity with MYMIV-[IN-Sat-12] respectively (Table 2). CR of DNA-B had maximum nucleotide sequence identity of 99.5% with MYMIV-[IN-Sat-12] (Table 2). Phylogenetic analysis indicated a close relationship of DNA-B with MYMIV-[IN-Sat-12], sharing the same clade in the phylogenetic tree (Figure 4).

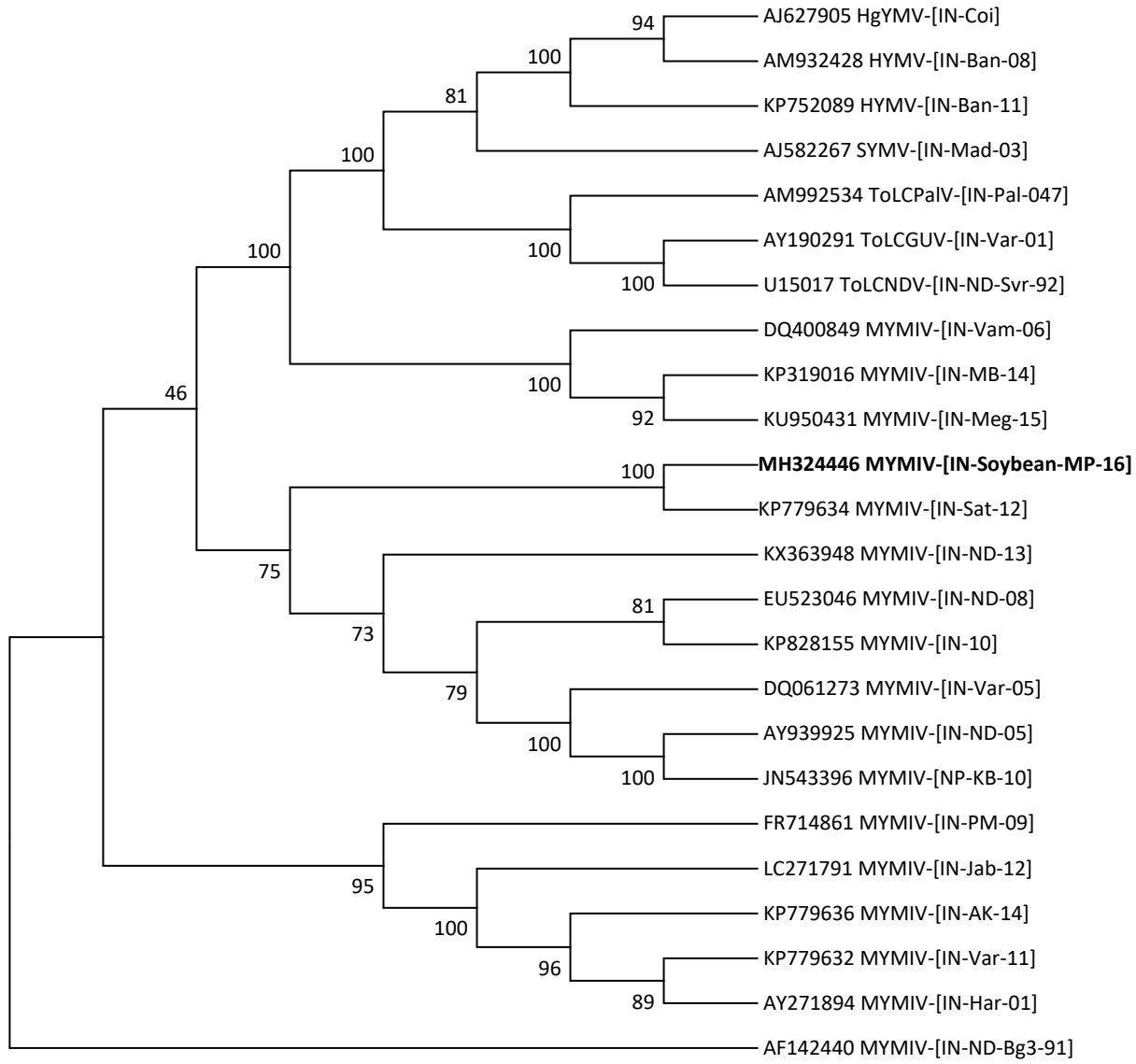


**Fig.-4: Phylogenetic tree of MYMIV-[IN-Soybean-MP-16] DNA-B genome constructed by Mega 7, showing the relationship with other selected begomoviruses. GenBank accession numbers are indicated to the left of each virus name and abbreviations are as per Fauquet et al., 2008. Studied virus is in bold.**

DNA-betasatellite consists of 1366nt, showed maximum nucleotide sequence identity (97.3%) with Tomato leaf curl Gandhinagar betasatellite-[India-Gandhinagar-2012] (ToLCGNB-[IN-GN-12] GenBank Accession no. KC952006) and minimum sequence identity of 37.1% with Honeysuckle yellow vein mosaic-[United Kingdom-Norwich1-1999] (HYVB-[UK-Nor1-99] GenBank Accession no. AJ316040) (Table 3). Similar to other begomovirus associated DNA-betasatellites, DNA-betasatellite has single ORF βC1 on the complementary strand and showed maximum amino acid similarity 98.3% with (ToLCGNB-[IN-GN-12], SCR with ToLCGNB-[IN-GN-12] (94.4%) (Table 3). Phylogenetic analysis also showed a close relationship of DNA-betasatellite with ToLCGNB-[IN-GN-12] sharing same clad in phylogenetic tree (Figure 5).

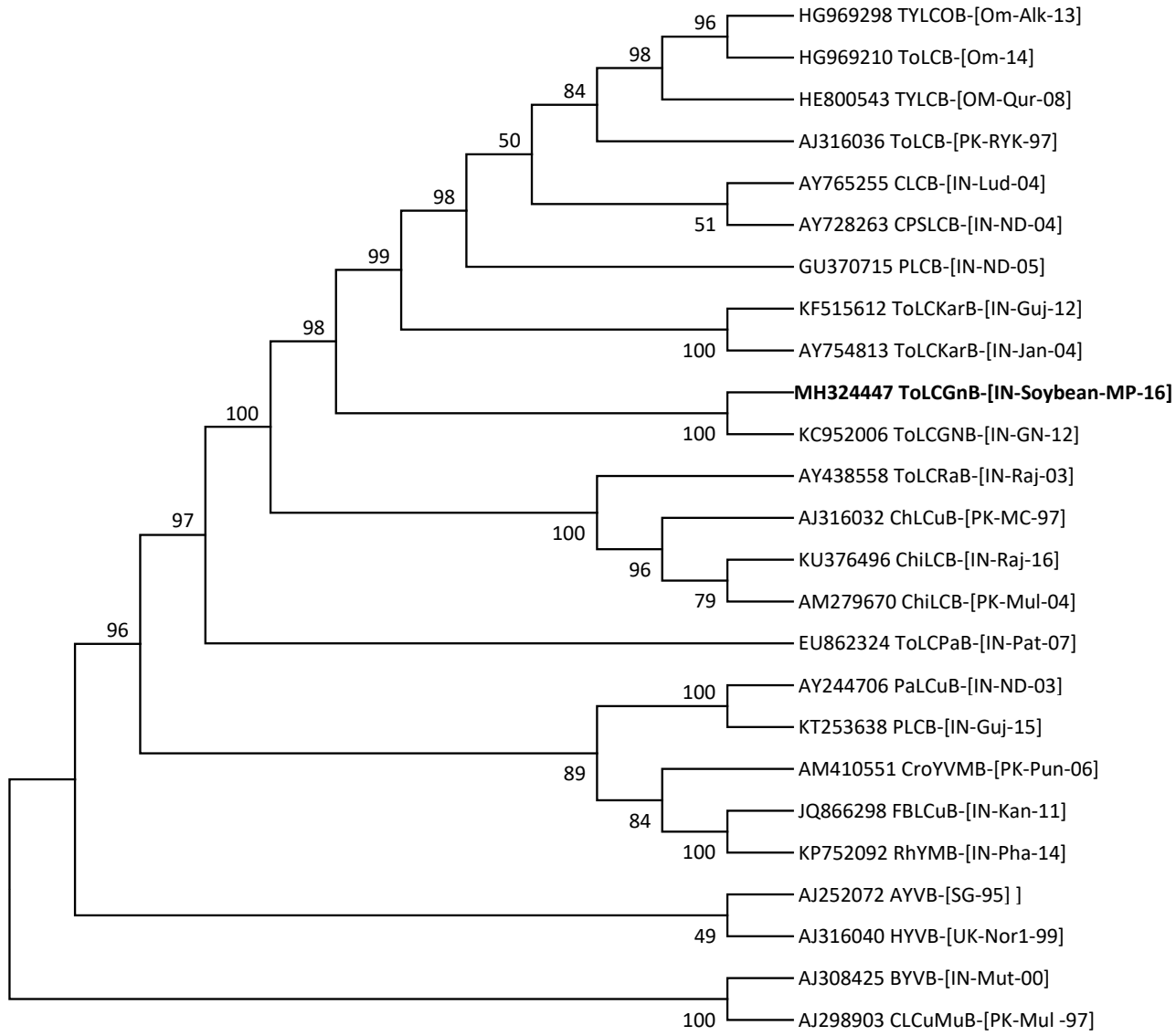


**Fig.-5: Phylogenetic tree of ToLCGnB-[IN-Soybean-MP-16] betasatellite genome constructed by Mega 7, showing the relationship with other selected begomoviruses. GenBank accession numbers are indicated to the left of each virus name and abbreviations are as per Fauquet et al., 2008. Studied virus is in bold.**

**Infectivity of pMbA, pMbB and pMbβ partial tandem repeats and detection of viral accumulation**

Partial tandem repeats pMbA was agro-inoculated on experimental plant (*N. benthmiana*) alone as well as in combination of pMbA+pMbB, pMbA+pMbβ and pMbA+pMbB+pMbβ. pMbA alone can produce mild symptoms in *N. benthamiana* including downward curling of newly young leaves at 21 dpi (Figure 6) whereas along with pMbB, similar symptoms were observed earlier at 16 dpi (Figure 6). Further, with the timespan severe symptoms were observed such as vein enation and vein clearing, twisting of petiole, severe downward curling in newly emerged leaves and stunted growth of the plant. Similarly, Agro-inoculation of pMbA+pMbβ also produce leaf curling symptoms but symptoms appearance was earlier (at 14 dpi) than pMbA+pMbB. Likewise, agro-inoculation of all three partial tandem repeats together (pMbA+pMbB+pMbβ) in *N. benthamiana*, showed symptom of twisting of petioles, downward leaf curling, yellowing of leaf lamina along with stunted growth at 10 dpi (Figure 6). The negative control *N. benthamiana* plants (inoculated with empty pCAMBIA vector) could not produce any symptoms at 30 dpi. Virus accumulation in *N. benthamiana*, agro-inoculated with different combinations were examined by RT-PCR (Figure 7). In

real time PCR data; it was observed that there was fold increased virus titer of DNA-A in pMbA+pMbB agro-inoculated plants in comparison to pMbA agro-inoculated plant (Figure 7). Interestingly, combination of pMbA+pMbβ had nearly three folds virus titer of DNA-A in comparison to pMbA+pMbB (Figure 7). Correspondingly, agro-inoculated of all three partial tandem repeats together (pMbA+pMbB+pMbβ) in *N. benthamiana*, had three folds higher virus titer of DNA-A in comparison to pMbA+pMbB (Figure 7).


**Fig.-6: Symptom induction upon Agro-inoculation of partial tandem repeats in different combinations on *N. benthamiana* at 30dpi.**

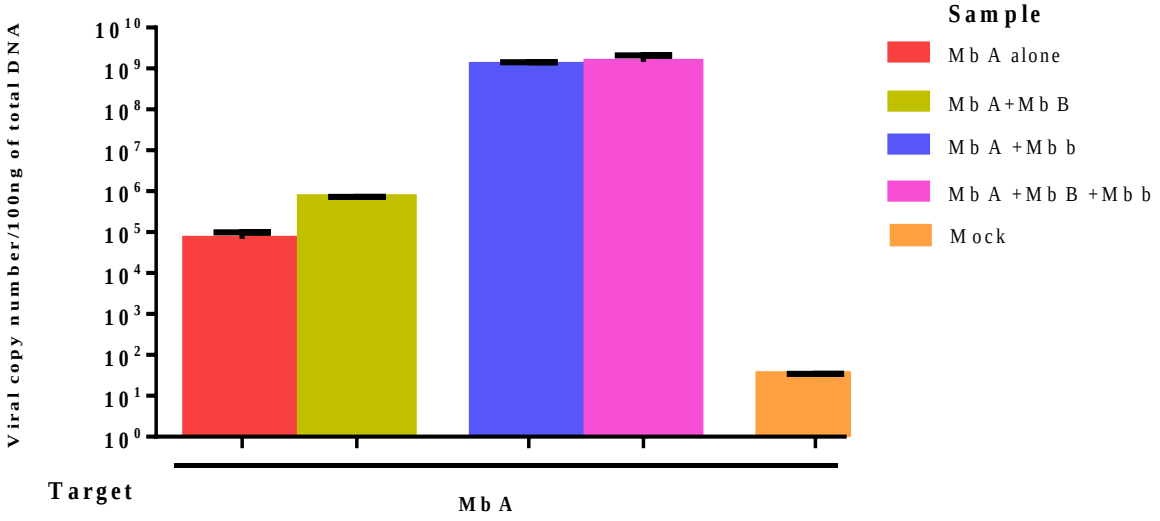


**Fig.-7 Graphical representation of relative viral DNA levels in the agro-inoculated plants using real-time PCR.**

## DISCUSSION

Pulses are consumed as a huge source of plant protein for human being worldwide. However, pulses are negatively affected by whitefly-transmitted begomoviruses that cause yellow mosaic disease (YMD). YMD has been emerged as an alarming threat to pulses productivity worldwide and predominantly found in pulse growing areas of southern Asia. The most affected hosts of YMD are soybean, mungbean, blackgram, cowpea, Dolichos, French bean, horsegram, lima bean, pigeon pea [20]. Although, earlier it was not clear about YMD of same pulse crop is caused by same strain/ isolate of begomovirus or different strain/ isolate of begomovirus but now literatures are available on that YMD is not host specific. *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV) are the main constrain of pulse production among four bipartite begomoviruses causing YMD in pulses [21].

In the present communication, we report the unique tripartite nature of begomovirus infecting to soybean

crop in Central India (Madhya Pradesh). Being the largest producer of soybean, the productivity of soybean in Madhya Pradesh has been hindered by begomoviruses and initial identification of begomovirus infection has been made but the molecular characterization was lacking. Sequence analysis of studied isolate MYMIV DNA-A and MYMIV DNA-B had the entire characteristic feature of old world bipartite begomoviruses having all ORFs, iterion (TAATATT/AC) for binding of replication initiation protein, TATA box and stem-loop farming structure [22, 23]. Unlike DNA-A, DNA-B is not self-dependent for replication and dependent on DNA-A. MYMIV DNA-A had iterion, upstream of nonanucleotide of CR that is important for trans-replication. Studied DNA-A and DNA-B had maximum nucleotide sequence identity of 99.8% that is much higher than ICTV criteria for new species (less than 92.0%). Therefore, studied MYMIV from soybean is identified as an isolate of MYMIV family and named as *Mungbean yellow mosaic India Virus*-[India-Soybean-Madhya Pradesh-2016] (MYMIV-[IN-Soybean-MP-16]). Unlike, previously reported bipartite begomoviruses infecting to soybean, in addition to DNA-A and DNA-B, we have also found betasatellite in the same leaf sample [24, 25]. DNA Betasatellite showed 97.3% sequence similarity with *Tomato leaf curl Gandhinagar betasatellite* (ToLCGnB) reported from Gujarat [26] which is much higher than ICTV criteria for new species (less than 92.0%). Therefore, studied betasatellite from soybean is identified as an isolate of ToLCGnB and named as *Tomato leaf curl Gandhinagar betasatellite*-[India-Soybean-Madhya Pradesh-2016] (ToLCGnB-[IN-Soybean-MP-16).

In old world, begomoviruses are also associated with betasatellite molecule, nearly half (~1.3 kb) of the genome that is dependent on viral DNA for replication and participate in symptom severity [7]. However, the presence of betasatellite with bipartite begomoviruses is rare the few bipartite begomoviruses like *Tomato Leaf Curl New Delhi Virus* in tomato *Mungbean Yellow Mosaic India Virus* (MYMIV*)* in cowpea has been reported the association of betasatellite [27, 28]. Similarly, we have also found betasatellite associated with bipartite MYMIV but in the soybean crop.

In order to demonstrate Kotch`s postulates, we have constructed partial tandem repeats of MYMIV DNA-A, MYMIV DNA-B and ToLCGNB DNA-β followed by infectivity experiment on *N. benthamiana* in a glass-house. MYMIV DNA-A showed mild symptoms upon agro inoculation on *N. benthamiana* whereas, co-inoculated with MYMIV DNA-B produced severe symptom as previous studies had showed in case of bipartite begomovirus [27]. DNA-B has nuclear shuttle protein and movement protein which help virus for intra cellular movement and long-distance movement in plant body (systemic infection in host body) that leads to symptom severity. Unlike, to bipartite begomoviruses the monopartite begomoviruses are associated with betasatellite which have single ORF βC1 and has crucial role in pathogenicity [7]. Several studies have been made on the involvement of βC1 in host defence signaling pathways and gene silencing for effective infection [7]. In previous studies, agro-inoculation of *Radish leaf curl virus* along with associated betasatellite in Radish plants and *Chilli leaf curl virus* along with associated betasatellite in pepper plants have signified the role of betasatellite in symptom development [29, 30]. Similarly, our experiment also signified the role of betasatellite in symptoms severity and as well as increased in DNA-A titer with nearly three folds, when co-inoculated with DNA-A in comparison to alone DNA-A (Fig 6 & 7) on *N. benthamiana* plants.

However, when all three molecules (pMbA+pMbB+pMbβ) were agro-inoculated together in *N. benthamiana* we observed early emergence of symptoms and nearly one or three folds higher viral titer of DNA-A in inoculated plants in comparison to pMbA+pMbβ or pMbA+pMbB respectively. Thus, it seems that there has been sharp competition between DNA-B and DNA-β for DNA-A as both are dependent on DNA-A for replication. Accordingly, movement protein and nuclear shuttle protein of DNA-B and the βC1 of betasatellite might have provided favorable conditions to DNA-A so that, DNA-A could spread faster in plant body, resulted into early emergence of symptom and high titer of DNA-A. Similar, results were also found in previous study when DNA-A and DNA-B of *Tomato leaf curl New Delhi virus* agro-inoculated along with Cotton leaf curl betasatellite on tomato [31] and bipartite *Rhynchosia yellow mosaic virus* in association with a betasatellite causing dwarf mosaic disease in French bean [32].

**Conclusion:** This is the unique combination of non-helper bipartite MYVMIV with betasatellite causing mosaic disease in soybean crop, suggesting evolution of tripartite nature of begomovirus. This study will help to

explore pathogenecity of begomoviruses and develop resistant variety of soybean against MYVMIV.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Author contribution statement**

BKY and AKS conceived and designed research. BKY and S conducted experiments. BKY, AKS, SP and RKJ analysed the data. BKY and AKS wrote the manuscript. SP and AKS corrected and improved the manuscript. All authors read and approved the manuscript

## REFERENCES

1. Zerbini F.M., *et al,* ICTV virus taxonomy profile: Geminiviridae, J. Gen. Virol, 98:131-133, (2017).
2. Brown J.K., *et al.,* Virus taxonomy-ninth report of the International Committee on Taxonomy of Viruses. King, AMQ, Lefkowitz, E, Adams, M J, Carstens, E B, Eds 351373 (2012).
3. Fauquet C.M., *et al.,* Geminivirus strain demarcation and nomenclature. Arch Virol, 153:783-821, (2008).
4. Laufs J., *et al.*, Geminivirus replication: genetic and biochemical characterization of Rep protein function, a review. Biochimie, 77:765-773, (1995).
5. Noueiry A.O., Lucas W.J. and Gilbertson R.L., Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport, Cell,76:925-932, (1994).
6. Briddon R.W., *et al.,* Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses, Virology, 312:106-121, (2003).
7. Cui X., *et al.,* A begomovirus DNAβ-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus, J Virol,79: 10764-10775,(2005).
8. Liu K., Chemistry and nutritional value of soybean components. In *Soybeans*, Springer, Boston, M A, 25-113, (1997).
9. USDA, Economics Research Service, 2015.
10. Economic Survey of India 2017; GoI, Ministry of finance.
11. Naimuddin K., Akram M., and Sanjeev G., Identification of Mungbean yellow mosaic India virus infecting Vigna mungo var. silvestris L, Phytopathol. Mediterr, 50:94–100, (2011).
12. Pierce W.H., The identification of certain viruses affecting leguminous plants, J Agric Res, 51:1017-1039, (1935).
13. Nariani T.K., Yellow mosaic of Mung (*Phaseolus aureus* L.), Indian Phytopath,13 (1960).
14. Varma A., Dhar A.K., and Mandal B., MYMV transmission and control in India. In: Mungbean Yellow Mosaic Disease (S.K. Green, D. Kim, ed.), Asian Vegetable Research and Development Centre, Taipei, Taiwan, 8–27, (1992).
15. Varma A., and Malathi V.G., Emerging geminivirus problems: a serious threat to crop production, Ann. Appl. Biol, 142:145–164, (2003).
16. Wrather J.A., *et al.,* Soybean disease loss estimates for the top 10 soybean producing countries in 1994, PlantDis, 81:107–110, (1997).
17. Dellaporta S.L., Wood J., and Hicks J.B., A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19–21, (1983).
18. Wyatt S.D., and Brown J.K., Detection of subgroup II geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology 86:1288–93, (1996).
19. Briddon R.W., *et al.,* Universal primers for the PCR-mediated amplification of DNA-β: A molecule associated with some monopartite begomoviruses, Mol. Bio-Technol, 20:315–318, (2002).
20. Varma A., MYMV transmission and its control in India. In Mungbean yellow mosaic disease: proceedings of an international workshop, Bangkok 1991. AVRDC, (1992).
21. Usharani K.S., *et al., Yellow mosaic virus* infecting soybean in northern India is distinct from the species infecting soybean in southern and western India, Curr. Sci, 845-850, (2004).
22. Fontes E.P., *et al.,* Interaction between a geminivirus replication protein and origin DNA is essential for viral replication, J BiolChem, 269:8459-8465, (1994).
23. Lazarowitz S.G., Geminivirus: genome structure and gene function, Crit Rev Plant Sci, 11:327-349, (1992).
24. Usharani K.S., *et al.,* Infectivity analysis of a soybean isolate of *Mungbean yellow mosaic India virus* by agroinoculation, JGPP, 71:230-237, (2005).
25. Banerjee A., *et al.,* Molecular evidence of an isolate of *Mungbean yellow mosaic India virus* with a recombinant DNA B component occurring on mungbean from mid-hills of Meghalaya, Indi, VirusDisease, 29:68-74, (2018).
26. Rathore S., *et al.,* A new begomovirus species in association with betasatellite causing tomato leaf curl disease in Gandhinagar, India, Plant Dis, 98:428-428, (2014).
27. Sivalingam P.N., and Varma A., Role of betasatellite in the pathogenesis of a bipartite begomovirus affecting tomato in India, Arch Virol, 157:1081-1092, (2012).
28. Rouhibakhsh A., and Malathi V.G., Severe leaf curl disease of cowpea–a new disease of cowpea in northern India caused by *Mungbean yellow mosaic India virus* and a satellite DNA β, Plant Pathol, 54:259-259, (2005).
29. Singh A.K., Chattopadhyay B., and Chakraborty S., Biology and interactions of two distinct monopartite begomoviruses and betasatellites associated with radish leaf curl disease in India, Virol J, 9:43, (2012).

30. Kumar R.V., *et al.,* Complexity of begomovirus and betasatellite populations associated with chilli leaf curl disease in India. J. Gen. Virol, 96: 3143-3158, (2015).
31. Jyothsna P., *et al.,* Infection of *Tomato leaf curl New Delhi virus* (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites, Appl Microbiol Biotechnol. 97:5457-5471, (2013).
32. Bhatt B.S., *et al.,* Dwarf Mosaic Disease of French Bean in India Caused by *Rhynchosia yellow mosaic virus* in Association with a Betasatellite. Plant Dis, 99: 1290, (2015).

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