*Original Research Article*

# Resistant Gene Analogue Marker(S) Screening against Yellow Mosaic Disease in Mungbean [*Vigna Radiata* (L.) Wilczek]


**ABSTRACT**

**Using the disease grading system, 100 genotypes of mugbeans were tested in the field for yellow mosaic disease resistance during Rabi 2021-2022. The yellow mosaic disease was resistant to 49 of the 100 mugbean genotypes, Moderately resistant to 22, Moderately susceptible to 11, susceptible 3, and Highlysusceptible to 15 of the genotypes. According to BLAST tool results, the sequenced virus has a 98.94% similarity to the entire coding sequence of the MYMIV-Mb02 coat protein (AV1) gene from the mungbean yellow mosaic India virus strain, accession number GQ387502.1. Consequently, the variant was given the accession number ON622515.1 and named mungbean yellow mosaic India virus isolates NAU-RJ coat protein gene segment. Six resistant (40 C, NMS-21-01, NMS-21-06, NMS-21-22, NMS-21-49, NMS-21-95) and six highly susceptible (GM 4, NMS-21-23, NMS-21-24, NMS-21-40, NMS-21-68, NMS-21-69), were studied in the current study. Nine pairs of RGA primers were used to screen six resistant and six susceptible genotypes of mungbean. This resulted in effective amplification of five pairs of RGA primers in all resistant genotypes, but not in all highly susceptible genotypes. According to the findings of this investigation, five pairs of RGA markers may successfully discriminate between the severely sensitive and resistant mugbean genotypes. These RGA markers can be used for mapping resistance gene and marker validation study because of their long-lasting YMV resistance.**



**Keywords:** Mugbean, MIYMV, RGAs, YMD, begomovirus and BLAST

## 1. INTRODUCTION

One of India's most significant short-duration pulse crops and a superior source of high-quality protein is the mungbean, often known as green gramme or [*Vigna radiata* (L.) Wilczek]. Other names for it include moong, mung, and golden bean [1]. It has a 2n=2x=22 chromosome number and its genome size is predicted to be 579 Mb [2]. It is a self-fertilizing pulse crop that ranks third in production behind chickpea and pigeon pea [3]. Mungbean is frequently farmed in South China, Indonesia, Malaysia, Thailand, Laos, Cambodia, Pakistan, Bangladesh, Sri Lanka, and India. India's only state having the largest agriculture and productivity is Rajasthan. According to the fourth advanced estimate (2019–20), India is the world's greatest producer of green gramme with an annual production of 2460 thousand tonnes, up from 2455.37 thousand tonnes with a productivity of 516 kg/ha [4]. Numerous biotic (viruses, fungus, bacterial pathogens, and insects) and abiotic (salinity, drought, temperature, water logging, etc.) stressors are the main yield-limiting factors. The mungbean yellow mosaic virus, which causes yellow mosaic disease (YMD), is the primary threat to large economic losses in the Indian subcontinent [5].Yellow mosaic disease is the most severe viral illness brought on by the yellow mosaic virus (YMD). The countries of Bangladesh, Pakistan, Sri Lanka, and India are where the disease is most prevalent. The causal agents of yellow mosaic illness are a variety of Geminiviruses from the family Geminiviridae and genus Begomovirus, which are transmitted by whiteflies (YMD) [6]. The transmission and behaviour of whiteflies are influenced by host genotypes, vector biotypes, and growing circumstances. The yellow mosaic virus is one of the most pervasive and dangerous viruses of mungbeans (YMV). Among its principal hosts are the common bean, urdbean, soybean, cowpea, and bean. It results in a significant drop in output and a decline in seed quality. Little, irregular yellow spots and patches first emerge in the veins of YMD-affected leaves, and gradually spread until the leaves are entirely yellow. The four most prevalent kinds of YMD are the Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Horsegram yellow mosaic virus (HYMV), and Dolichos yellow mosaic virus (DYMV) [7]..MYMV and MYMIV are the two main pathogens that cause YMD in the Indian mungbean. MYMV in South and West India and MYMIV in Northern and Central India cause yellow mosaic disease [8]..The Mungbean Yellow Mosaic India Virus is the most prevalent virus causing cowpea yellow mosaic disease in the state, according to DNA studies (MYMIV). The yellow mosaic disease in moth beans was also connected to MYMIV for the first time in Pakistan [7].

The discovery of resistant donors is a difficult task because of the quick evolution of novel YMD isolates, the intricacy of the resistance mechanism, and the lack of a reliable screening method for assessing the resistance of current types. Thus, any molecular strategy that can lead to the identification of YMD-resistant genotypes is needed by molecular breeders. Plant disease resistance gene analogue (RGA) markers were created and utilised to map resistance genes based on the conserved sequence of known RGAs. Leucine-rich repeats (LRR) and nucleotide binding sites are the most prominent functional domains in the majority of disease resistance genes (R-genes) found in plants (NBS). RGAs have also been connected to quantitative trait loci relevant to resistance (QTL) [9,10,11]. In view of the previous data and the requirement for a screening of resistance genes providing resistance to the yellow mosaic virus, the current study was conducted to identify RGA indicators associated to YMV resistance in mugnbean genotypes.

## 2. MATERIAL AND METHODS

After There were 100 distinct mugbean genotypes growing in Rabi 2021–2022. These 100 genotypes were planted alongside 4 checks (the resistant check 40C and GM6, the susceptible check GM4 & GM7). In the augmented block design, GM4 was employed as a spreader row with inter and intra row spacings of 60 cm and 15 cm, respectively. The advised agronomic methods for cultivating a strong crop were used.

### 2.1. Field Screening

The experimental material's reaction to the yellow mosaic illness was examined in a real-world field situation over the Rabi 2021–22 seasons. Since whiteflies are the local source of the virus, no insecticides were used to stop the experimental field's whitefly population from naturally declining. Whitefly activity and the beginning of disease symptoms in the crop were continuously monitored.

Since YMV incidence was discovered in 80% of the plants in spreader rows, the test materials were graded. Yellow mosaic disease (YMD) [12] screening of mugbean genotypes was done using a 1–9 point grading scale.

### 2.2 Component of the virus's partial DNA was amplified and sequenced

Six highly susceptible and resistant mugbean genotypes (GM 4, NMS-21-23, NMS-21-24, NMS-21-40, NMS-21-68, NMS-21-69,40 C, NMS-21-01, NMS-21-06, NMS-21-22, NMS-21-49, NMS-21-95) were chosen, and the whole genomic DNA was extracted from tender leaves using a modified CTAB method (Doyle & Doyle, 1987). The quality of genomic DNA extracted from different samples was checked. The rough genomic DNA was separated as per standard technique using agarose gel electrophoresis (0.8% agarose gel) and stained with ethidium bromide [12].

The OD 260/OD 280 ratio was used to determine DNA purity, which was then measured. Primer3 (https://bioinfo.ut.ee/primer3-0.4.0/) and primers specific for the YMV coat protein (CP) [GQ387509.1, DQ389148.1 (MYMIV), DQ389146.1 (MYMV), GU591170.1, and KT282129.1 (DYMV)] were used to amplify the YMV coat protein gene.

**Table 1: List of coat protein primers used in the present study**

| Sr. No. | Primer | Primer direction | Sequences 5'-3' |
|---|---|---|---|
| 1 | MYMIVF1 | Forward | CCAAAGCGGACCTTCGATA |
| 2 | MYMIVR2 | Reverse | AACGATTCACCATGGCTTGT |
| 3 | MYMIVF3 | Forward | GACCTTCCCGAATCACTGC |
| 4 | MYMIVR4 | Reverse | AACGATTCACCATGGCTTGT |
| 5 | MYMVF5 | Forward | GTGGACACTCTGAACCCAGTA |
| 6 | MYMVR6 | Reverse | AACGATTCACCATGGCTTGT |
| 7 | DYMV1 | Forward | GTAGAGCATGGACCAATCGT |
| 8 | DYMV2 | Reverse | ACGCATATTGACCTCCGGT |
| 9 | DYMV3 | Forward | GCTCATCGTGTTGGTAAACGATT |
| 10 | DYMV4 | Reverse | GGAGTGGGCTTACAAGAATGC |

PCR reactions were conducted under the previously mentioned conditions. A 20 µl reaction mixture containing 100 ng of genomic DNA, 2.5 µl of Master mix (2x) (NEB), 10.0 µl of each primer (forward and reverse), and 4.0 µl of NFW was used for the experiment. The Thermal Cycler was used to perform the amplification; it was programmed to perform one cycle of pre-denaturation at 95°C for 10 mins, 30-35 cycles of denaturation at 95°C for 30 sec, annealing at 50–60°C for 45 sec, and elongation for 30 sec at 72°C, and final elongation at 72°C for 10 minutes. After the PCR was finished, the reactions were maintained at 4°C. Gel electrophoresis at 80 volts for 90 minutes with 1 X TAE buffer in 1.8% agarose gel containing 5 µl (1 mg/ml) ethidium bromide was used to resolve amplified products.

### 2.3 Phylogenetic analysis of the sequence and comparison with other *Begomoviruses*

Database searches for begomovirus sequences, simple local alignments, and similarity index analysis were carried out using the NCBI-BLSTN programme (http://blast.ncbi.nlm.nih.gov). Multiple nucleotide (nt) sequence alignments were performed for a section of the viral isolate's DNA-A with other begomoviruses reported from India and other countries using the CLUSTALW(2) programme(https://www.ebi.ac.uk/Tools/msa/clustalw2/) [15]. The obtained sequence's ORFs were located using the NCBI software's open reading frame finder (https://www.ncbi.nlm.nih.gov/orffinder/), and the coat protein sequences were then confirmed using NCBI-BLASTP. Also, the sequence was checked for the presence of conserved domains using the NCBI-CDD tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) [15,16]. A phylogenetic tree was produced using online software (MEGA version 7; http://www.megasoftware.net/) [17] and the neighbor-joining method with 1000 bootstrap replications.

### 2.4 Identification of RGA Marker(S) associated with Resistance Gene:

In the current study, nine pairs of the 18 RGA primers (Table 2) from the conserved area of the various classes of the soybean 'R' gene were used. Using the primer3 tool (https://bioinfo.ut.ee/primer3-0.4.0/), three pairs of RGA primers were freshly created using the reported YMV resistant protein sequences (NM00137183.9 and EF091690.1) to amplify resistant gene-like sequences from the genomic DNA by PCR. We selected six sets of RGA primers from the previously published literature.

Studying polymorphism between six resistant and six highly vulnerable genotypes of mugbean, nine pairs of RGA markers were found. Out of this, six pairs of primers RGA1FCG & RGA1R, VMYR1F & VMYR1R, YR4F & YR4R, CYR1F & CYR1R, RGA8F & RGA8R, RGA22F2 & RGA24R2 were obtained from previously reported literature and three pairs primers RGAMB1 & RGAMB2, RGAMB 3 & RGAMB 4, RGAMB 5 & RGAMB 6 were newly designed.

**Table 2: List of RGA primers used in the present study**

| *Sr. No.* | *Pairs* | *Primer* | *Sequences 5'-3'* | *Reference* |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 | 1 | *RGA1FCG* | *AGTTTATAATTCGATTGCT* | [18] |
| 2 | | *RGA1R* | *ACTACGATTCAAGACGTCCT* | [18] |
| 3 | 2 | *RGA8F* | *AGCGAGAGTTGTATTTAAG* | [18] |
| 4 | | *RGA8R* | *AGCCACTTTTGACAACTGC* | [18] |
| 5 | 3 | *RGA22F2* | *GGGTGGTTTGGGTAAGACCAC* | [19] |
| 6 | | *RGA24R2* | *TTCGCGGTGTGTGAAAAGTCT* | [19] |
| 7 | 4 | *VMYR1F* | *AGTTTATAATTTGATTGCT* | [20] |
| 8 | | *VMYR1R* | *ACTACGATTCAAGACGTCCT* | [20] |
| 9 | 5 | *YR4F* | *GGNAAGACGACACTCGCNTTA* | [20] |
| 10 | | *YR4R* | *GACGTCCTNGTAACNTTGATCA* | [20] |
| 11 | 6 | *CYR1F* | *GGGTGGNTTGGGTAAGACCAC* | [20] |
| 12 | | *CYR1R* | *NTCGCGGTGNGTGAAAAGNCT* | [20] |
| 13 | 7 | *RGAMB1* | *AGTATATAATTCAGTTGCTA* | *Newly designed* |
| 14 | | *RGAMB2* | *ACGATTCCTGACTAGACTCA* | *Newly designed* |
| 15 | 8 | *RGAMB3* | *ATAGATTGCATTACTAG* | *Newly designed* |
| 16 | | *RGAMB4* | *AGTGGTACGGTATTAGA* | *Newly designed* |
| 17 | 9 | *RGAMB5* | *TTATAAATCGAGTTGCTA* | *Newly designed* |
| 18 | | *RGAMB6* | *CTACGATTCATGACTCATA* | *Newly designed* |

## 3. RESULTS AND DISCUSSION

### 3.1 Field Screening

The results of 100 mungbean genotypes that were subjected to yellow mosaic disease resistance testing in the field. Of the 100 genotypes of Mungbean evaluated, 49 were found to be resistant to YMD, 22 to be only marginally resistant, 11 to be somewhat susceptible, 3 to be vulnerable, and 15 to be severely susceptible (Fig. 1,Fig. 2,Table 3). Similar outcomes were seen when mugbean genotypes were tested for MIMIV. Similar outcomes in mungbean genotypes tested for MYMIV were also reported [21,22,23].

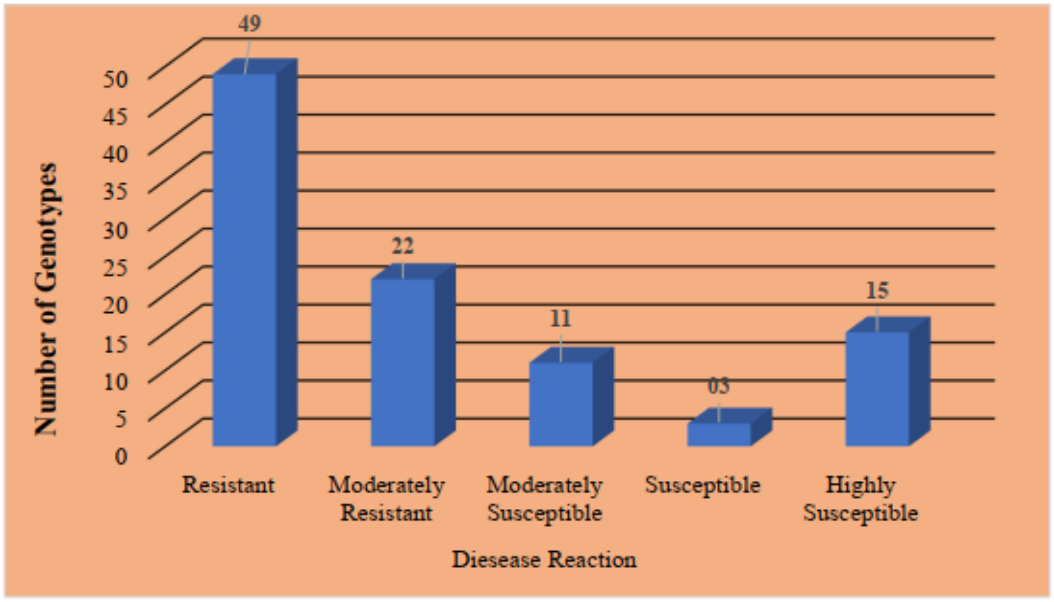



**Fig 1: Number of mugbean genotypes categorized under different disease reactions against yellow mosaic disease**

**Mungbean genotypes showing typical symptoms of YMD**

**Mungbean genotypes showing resistant reaction to YMD**

**Fig 2: Mugbean genotypes' response to YMD under field conditions**

**Table 3: Screening of Mugbean genotypes based on their reaction against yellow mosaic disease under field condition**

| Scale | Reaction | Genotypes |
|---|---|---|
| 1 | Resistant (R) | NMS-21-01,NMS-21-04,NMS-21 53, NMS-21-94, NMS-21-02, NMS-21-05, NMS-21-06, NMS-21-07, NMS-21-08, NMS-21-10,NMS-21-11, NMS-21-12, NMS-21-14, NMS-21-15, NMS-21-20, NMS-21-22, NMS-21-28, NMS-21-32, NMS-21-33, NMS-21-34, NMS-21-35,NMS-21-36, NMS-21-38, NMS-21-42, NMS-21-44, NMS-21-46, NMS-21-50, NMS-21-52, NMS-21-54, NMS-21-55, NMS-21-59, NMS-21-61,NMS-21-63, NMS-21-64, NMS-21-71, NMS-21-72, NMS-21-73, NMS-21-74,NMS-21-76,NMS-21-78,NMS-21-80,NMS-21-82 ,NMS-21-85, NMS-21-86,NMS-21-87, NMS-21-92,NMS-21-95,NMS-21-96, 40C. |
| 3 | Moderately resistant (MR) | NMS-21-03,NMS-21- 09, NMS-21-13,NMS-21- 21,NMS-21-27,NMS-21- 29, NMS-21- 30, NMS-21- 31, NMS-21- 37, NMS-21-56, NMS-21- 57,NMS-21- 60, NMS-21- 62, NMS-21- 65, NMS-21-75, NMS-21- 77, NMS-21- 79,NMS-21- 81, NMS-21-83,NMS-21-91, NMS-21-93,GM-6. |
| 5 | Moderately susceptible (MS) | NMS-21- 17, NMS-21- 18, NMS-21- 19, NMS-21- 25, NMS-21- 26, NMS- 21- 39, NMS-21- 45, NMS-21- 47, NMS-21-51, NMS-21-58, GM-7. |
| 7 | Susceptible (S) | NMS-21-16, NMS-21-41, NMS-21-43. |
| *9* | *Highly susceptible (HS)* | NMS-21-23, NMS-21-24, NMS-21-40, NMS-21-48, NMS-21-49, NMS-21-66, NMS-21-67, NMS-21-68, NMS-21-69, NMS-21-70, NMS-21-84, NMS-21-88, NMS-21-89, NMS-21-90, GM-4. |

### 3.2 Amplification and sequencing of a Yellow Mosaic Virus component's partial DNA

The CTAB method [14] with some modifications was used to isolate DNA from tender YMD infected leaves of six highly susceptible and resistant mugbean bean genotypes (GM 4, NMS-21-23, NMS-21-24, NMS-21-40, NMS-21-68, NMS-21-69,40 C, NMS-21-01, NMS-21-06, NMS-21-22, NMS-21-49, NMS-21-95). The DNA's quality was evaluated

using a 0.8% Agarose gel. After DNA extraction, its quantity and purity were evaluated using a Nano-drop to measure DNA concentration. Many DNA concentrations were used in the PCR process to provide uniform results.

### 3.3 PCR amplification of partial DNA-A component

To complete the amplification of the YMV's partial nucleotide sequence, specific primers (Table 1) were used to amplify the partial coat protein region of the DNA-A component of the yellow mosaic virus (YMV). The gene amplicons for partial coat protein (CP) were verified by electrophoresis. All highly susceptible genotypes (GM 4, NMS-21-23, NMS-21-24, NMS-21-40, NMS-21-68, NMS-21-69) included partial CP gene amplicons, but resistant genotypes did not (40 C, NMS-21-01, NMS-21-06, NMS-21-22, NMS-21-49, NMS-21-95 ). The mungbean yellow mosaic India's coat protein primers MYMIVF1 and MYMIV2 were used to amplify a DNA fragment of the partial coat protein gene at a size of around ~620 bp (Fig. 3). The amplified partial coat protein gene fragment suggested that the mugbean yellow mosaic India virus was the origin of the YMD infection in mugbean genotypes.

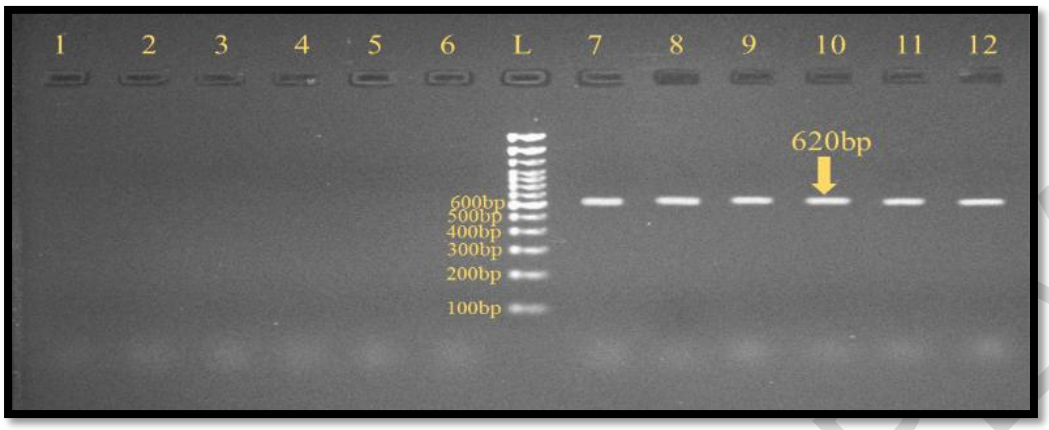



**Fig 3: PCR amplification of the CP gene DNA-A of YMV using MYMIVF1 and MYMIVR2 primer**

| ***L-100bp DNA ladder, 1-6 highly susceptible, 7-12 resistant mugbeangenotypes*** | | | |
|---|---|---|---|
| ***1.GM 4*** | ***4. NMS-21-40*** | ***7.40 C*** | ***10.NMS-21-22*** |
| ***2.NMS-21-23*** | ***5. NMS-21-68*** | ***8.NMS-21-01*** | ***11.NMS-21-49*** |
| ***3.NMS-21-24*** | ***6. NMS-21-69*** | ***9.NMS-21-06*** | ***12.NMS-21-95*** |

These outcomes are in line with the coat protein gene of MYMV, which was amplified from multiple pulses using primers that were specific for the CP gene and had estimated amplicons of 700 bp and 650 bp, respectively [24].. Similarly, ~1300bp partial CP gene of DNA-A fragment from YMD infected mungbean[25]. Moreover, similar research in mungbean demonstrated the use of particular primers to amplify the 650 bp coat protein gene of MYMV [26]. Similarly, amplification of ~1000bp coat protein gene of MYMV in mungbean[27].

### 3.4 Sequencing of partial DNA-A component of YMV:

The cycle sequencing method was used to purify and sequence the DNA-A component's partially amplified CP gene fragment. The outcomes showed that the nucleotide sequence of the YMV coat protein was 574 bp long. The coat protein gene sequence of the YMV was clearly found to match previously reported isolates of the mungbean yellow

mosaic India virus from various geographical locations in the range of 98.05 to 98.94%, according to the results of the BLAST (http://www.ncbi.nih.gov/BLAST) search, which was used to identify sequence homology. The YMD in mungbean caused by the YMV has been identified as the mungbean yellow mosaic India virus based on sequence comparison. Further, the sequence has been submitted to GeneBank, NCBI, Bethesda, Maryland, USA using the Bankit submission tool of NCBI (https://www.ncbi.nlm.nih.gov). The sequence was given the accession number ON622515.1 by GeneBank, which is accessible at https://www.ncbi.nlm.nih.gov (Appendix 1). Similar research also revealed a DNA-A fragment containing a partial CP gene of 1300 bp from mungbean infected with YMD[22].A 1285 bp fragment was obtained and later submitted to the NCBI's GeneBank (Accession number JQ004982). Also Similarly, amplification of ~1000bp coat protein gene of MYMV in mungbean. Furthermore, the true length of the MYMV was 889 bp, including 257 deduced amino acids, a 115 bp pre-coat protein at the 5' end, and a 774 bp core coat protein [28].

### 3.5 Phylogenetic analysis of the sequence and comparison with other Begomoviruses Multiple alignment and similarity index

The sequenced virus's BLAST results revealed a 98.94% identity to the entire cds of the mungbean yellow mosaic India virus isolate MYMIVMb02 coat protein (AV1) gene with accession number GQ387502.1 (Table 4). Accession numbers MZ235792.1, MT232629.1, AM950268.1, FM208844.1, GQ387508.1, EU523045.1, and AF416742.1 have been followed up to 98.05% sequence identity. For homology and phylogeny analysis, the coat protein gene sequences from the current study were compared to the 47 previously reported coat protein gene sequences that were acquired from NCBI. Multiple sequence alignments were performed for each of the 48 CP gene sequences using the CLUSTALW (2) tool (https://www.ebi.ac.uk/Tools/msa/clustalw2/) in order to examine the sequence homology. In order to identify the conserved sequences among all the coat protein sequences of MYMIV, multiple sequence alignment of the NAU-RJ coat protein gene segment DNA A, accession number ON622515.1, was performed. CUSTALW(2) produces biologically significant multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences and lines them up so that the identities, similarities and differences can be seen using this software and evolutionary relationships can be observed. As per the recent trends in the nomenclature and demarcation of species, strains and variants of the species, it is concluded that the sequenced virus reported as tentative variant of MYMIV (accession no GQ387502.1). Therefore, the proposed name of the variant mungbean yellow mosaic India virus isolates NAU-RJ coat protein gene segment DNA A, accession number ON622515.1[29].

**Table 4: Per cent identities (nucleotide) between CP gene of DNA-A MYMIV accession number ON622515.1 with other reported mungbean yellow mosaic India virus in NCBI database**

| ***Sr. No.*** | ***Virus name*** | ***Sequence identity (%)*** | ***Accession number*** | ***Geographical location*** |
|---|---|---|---|---|
| *1* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-Mb02 coatprotein (AV1) gene, complete cds* | *98.94* | *GQ387502.1* | *Uttar Pradesh* |
| *2* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-SBOsegment DNA-A, completesequence* | *98.76* | *MF693401.1* | *Bihar* |
| *3* | *Mungbean yellow mosaic India virus transcription activation protein and replication enhancerprotein genes, partial cds; andreplication associated protein,AC4, pre-coat protein, coatprotein, and AC5 genes, complete cds* | *98.76* | *KP677496.1* | *Andhra Pradesh* |
| *4* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-Ub02 coatprotein (AV1) gene, complete cds* | *98.76* | *GQ387507.1* | *Uttar Pradesh* |
| *5* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-Mb01 coatprotein (AV1) gene, complete cds* | *98.76* | *GQ387501.1* | *Uttar Pradesh* |

| | | | | |
|---|---|---|---|---|
| *6* | *Mungbean yellow mosaic Indiavirus segment DNA A, completesequence* | *98.76* | *DQ389153.1* | *New Delhi* |
| *7* | *Mungbean yellow mosaic Indiavirus isolate Krishna segmentDNA-A pre-coat protein (AV2)gene, partial cds; and coat protein(AV1) gene, complete cds* | *98.58* | *MT270285.1* | *Andhra Pradesh* |
| *8* | *Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate: Sehore-1* | *98.93* | *LC271560.1* | *Madhya Pradesh* |
| *9* | *Mungbean yellow mosaic Indiavirus coat protein (CP) gene,partial cds* | *98.58* | *KX655577.1* | *Bihar* |
| *10* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-DES-APcoat protein (AV1) gene, partialcds* | *98.58* | *MZ475998.1* | *Andhra Pradesh* |
| *11* | *Mungbean yellow mosaic Indiavirus segment A, completesequence, isolate Palampur* | *98.58* | *FN794200.1* | *Himachal Pradesh* |
| *12* | *Mungbean yellow mosaic Indiavirus genomic DNA, segmentDNA-A, complete sequence,isolate: Bhopal* | *98.40* | *LC271792.1* | *Madhya Pradesh* |
| *13* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-ABEL-APcoat protein (AV1) gene, partialcds* | *98.40* | *MZ475997.1* | *Andhra Pradesh* |
| *14* | *Mungbean yellow mosaic Indiavirus isolate MYMIV-GN-APcoat protein (AV1) gene, partialcds* | *98.40* | *MZ475994.1* | *Andhra Pradesh* |
| *15* | *Mungbean yellow mosaic Indiavirus segment DNA-A, completesequence* | *98.40* | *JX110618.1* | *Andhra Pradesh* |
| *16* | *Mungbean yellow mosaic Indiavirus isolate Guntur pre-coatprotein (AV2) gene, partial cds;and coat protein (AV1) gene,complete cds* | *98.40* | *JN181003.1* | *Andhra Pradesh* |
| *17* | *Mungbean yellow mosaic Indiavirus, segment DNA-A, completesequence, clone MI9* | *98.40* | *FM208842.1* | *Pakistan* |
| *18* | *Mungbean yellow mosaic Indiavirus - [Mungbean Pakistan]segment A, complete genome* | *98.40* | *AY269992.1* | *Pakistan* |
| *19* | *Legume yellow mosaic viruscomplete genomic DNA-A,isolate 14* | *98.40* | *AJ512495.1* | *Pakistan* |
| *20* | *Mungbean yellow mosaic Indiavirus-[SoybeanTN] completegenome* | *98.40* | *AJ416349.1* | *Tamil Nadu* |
| *21* | *Soybean yellow mosaic virus partial av1 gene for coat protein* | *98.40* | *AJ315667.1* | *Tamil Nadu* |
| *22* | *Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate* | *98.57* | *LC271563.1* | *Madhya Pradesh* |
| *23* | *Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate* | *98.57* | *LC271561.1* | *Madhya Pradesh* |

| | | | | |
|---|---|---|---|---|
| 24 | Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate | 98.57 | LC271558.1 | Madhya Pradesh |
| 25 | Mungbean yellow mosaic Indiavirus isolate MRBA-2 segmentDNA-A, complete sequence | 98.23 | MN020535.1 | Chhattisgarh |
| 26 | Mungbean yellow mosaic Indiavirus coat protein (CP) gene,complete cds | 98.23 | KX655579.1 | Bihar |
| 27 | Mungbean yellow mosaic Indiavirus coat protein (CP) gene,complete cds | 98.23 | KX655576.1 | Bihar |
| 28 | Mungbean yellow mosaic Indiavirus coat protein (CP) gene,complete cds | 98.23 | KX655575.1 | Bihar |
| 29 | Mungbean yellow mosaic Indiavirus isolatesIN:ND:Pigeonpea:13 segment DNA-A, complete sequence | 98.23 | KX363947.1 | Bihar |
| 30 | Mungbean yellow mosaic Indiavirus genomic DNA, segmentDNA-A, complete sequence,isolate | 98.23 | LC271790.1 | Madhya Pradesh |
| 31 | Mungbean yellow mosaic Indiavirus isolates 30-07-2014 coatprotein (AC1) gene, complete cds | 98.23 | KX575866.1 | Bihar |
| 32 | Mungbean yellow mosaic Indiavirus isolate MJS1 segmentDNA-A, complete sequence | 98.23 | KR052025.1 | Uttar Pradesh |
| 33 | Mungbean yellow mosaic Indiavirus clone CA3 segment DNAA, complete sequence | 98.23 | KC911720.1 | Tamil Nadu |
| 34 | Mungbean yellow mosaic Indiavirus clone CA4 segment DNAA, complete sequence | 98.23 | KC911719.1 | Tamil Nadu |
| 35 | Mungbean yellow mosaic Indiavirus, segment DNA-A, completesequence, clone MI2 | 98.23 | FM208846.1 | Pakistan |
| 36 | Mungbean yellow mosaic Indiavirus isolate MYMIV-Ub05 coatprotein (AV1) gene, complete cds | 98.23 | GQ387510.1 | Uttar Pradesh |
| 37 | Mungbean yellow mosaic Indiavirus pre-coat protein (AV2) andcoat protein (AV1) genes,complete cds | 98.23 | DQ389151.1 | Panjab |
| 38 | Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate | 98.39 | LC271565.1 | Madhya Pradesh |
| 39 | Mungbean yellow mosaic Indiavirus AV1 gene for coat protein,partial cds, isolate | 98.39 | LC271562.1 | Andhra Pradesh |
| 40 | Mungbean yellow mosaic Indiavirus isolate MVMIV-BG-BPTVTC segment DNA-A, completesequence | 98.05 | MZ235792.1 | Andhra Pradesh |
| 41 | Mungbean yellow mosaic Indiavirus clone SB-Bam-NIBSMsegment DNA-A, completesequence | 98.05 | MT232629.1 | Chhattisgarh |
| 42 | Mungbean yellow mosaic Indiavirus segment DNA A, completesequence, clone MI15 | 98.05 | AM950268.1 | Pakistan |
| 43 | Mungbean yellow mosaic Indiavirus, segment DNA-A, completesequence, clone MI12 | 98.05 | FM208844.1 | Pakistan |
| 44 | Mungbean yellow mosaic Indiavirus isolate MYMIV-Ub04 coatprotein (AV1) gene, complete cds | 98.05 | GQ387509.1 | Uttar Pradesh |
| 45 | Mungbean yellow mosaic Indiavirus isolate MYMIV-Ub03 coatprotein (AV1) gene, complete cds | 98.05 | GQ387508.1 | Uttar Pradesh |
| 46 | Mungbean yellow mosaic Indiavirus | 98.05 | EU523045.1 | Delhi |

| | | | | |
|---|---|---|---|---|
| | *segment DNA A, completesequence* | | | |
| *47* | *Mungbean yellow mosaic Indiavirus-[Mungbean] DNA A,complete sequence* | *98.05* | *AF416742.1* | *Delhi* |

### 3.6 Finding of Open Reading Frame (ORF) :

The coat protein (AVI) gene (partial cds) sequence of MYMIV was further examined using the NCBI ORF finding tool, and the coat protein sequence was subsequently predicted using the pfam database by NCBI BLASTP. The 187aa projected coat protein sequence was further investigated for the presence of a conserved domain using the NCBI-CDD tool (Fig 4). An ORF from the coat protein gene sequence with the accession number Pfam00844 and an E-value of 6.40e-69 confirmed the presence of a conserved domain as the geminivirus coat protein/nuclear export factor BR1 superfamily (Fig 5). Similar work was also carried out in ToLCV coat protein sequence [16], Papaya Leaf curl virus coat protein [29] and Tomato leaf curl virus disease (ToLCVD) coat protein [30].

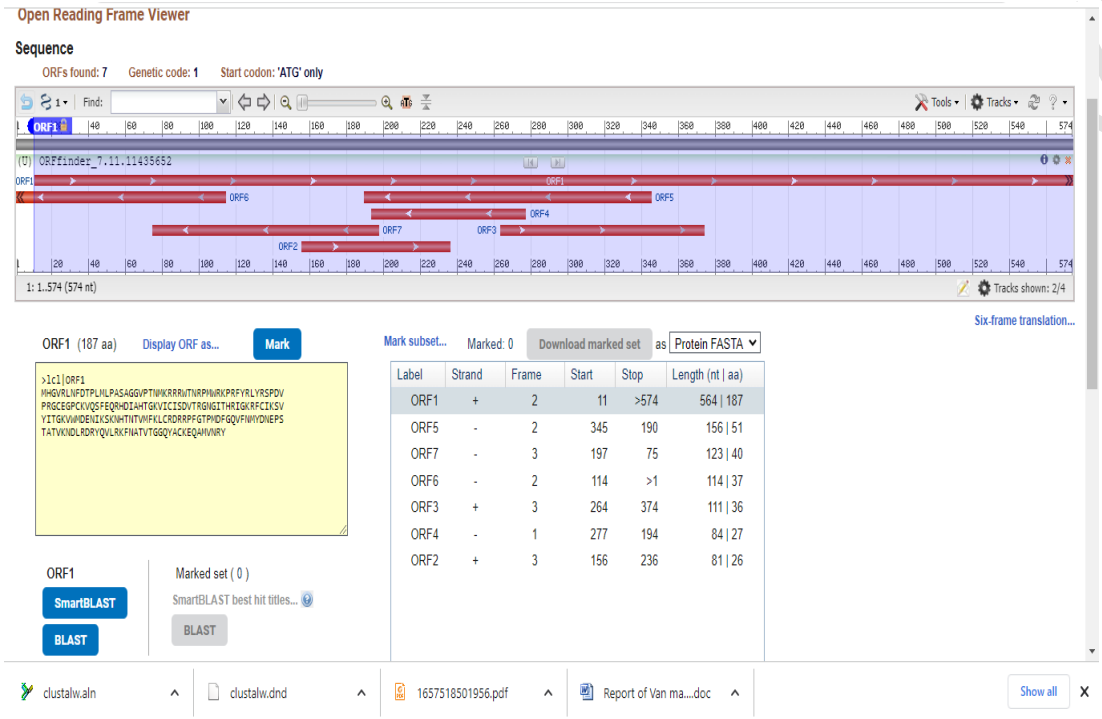



**Fig. 4: Predicted amino acid sequence from mungbean yellow mosaic India isolate NAU-RJ coat protein gene segment DNA A**


**Fig 5: Prediction of conserved domain mungbean yellow mosaic India virusisolate NAU-RJ coat protein gene segment DNA A**

### 3.7 Phylogenetic analysis of CP gene of DNA-A MYMIV with other *Begomoviruses*

In order to identify the conserved sequences across all of the coat protein of MYMIV sequences, several sequence alignments of the mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene segment DNA A, accession number ON622515.1.1, were performed. Also, using the Neighbour-Joining technique and 1,000 bootstrap replications, a phylogenetic tree was created. The phylogenetic tree built using the partial coat protein genes of mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene segment DNA A showed two main clusters (Fig 6)(Appendix-1), Further cluster A divided into two subdivisions as cluster A1 and A2. Further, a subdivision of cluster A2 divided into A2a included Mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene segment DNA A with the accession number ON622515.1 while subcluster A2b included AJ512495.1, FM208842.1, FM208846.1, FM208844.1, AY269992.1, AM950268.1, GQ387510.1, GQ387509.1, LC271563.1 followed by cluster A2c including GQ387507.1. While cluster B divided into two subdivision as cluster B1 included FN794200.1, AF416742.1, KX655579.1, KX655576.1, KX655575.1, KX575866.1, JX110618.1, KC911719.1, MZ475998.1, JN181003.1, KP677496.1, MN020535.1, MZ235792.1, MZ475994.1, MT270285.1, MZ475997.1, GQ387501.1, LC271560.1, KX655577.1, MT232629.1, GQ387508.1, while sub cluster B2 included LC271792.1, LC271561.1, LC27565.1, LC271562.1, LC271558.1, AJ416349.1, AJ315667.1, DQ389151.1. Further, Cluster A2b and A2c mostly comprised of the MYMIV strains which predominates in the different regions of India mainly Delhi, Madhya Pradesh and worldwide Pakistan. While cluster A2a represented as mungbean yellow mosaic virus isolate NAU-RJ coat protein gene segment DNA A in Gujarat that conformed YMD infection as MYMIV in mungbean genotype under field condition during Summer 2021.In the present study confinement of species of yellow mosaic disease infecting mungbean genotypes in South Gujarat was due to mungbean yellow mosaic India virus (MYMIV).Similar phylogenic analysis reported in black gram against MYMIV[30], in mungbean against MYMV[31]. Also, similar work carried out in mungbean against MYMV [32].

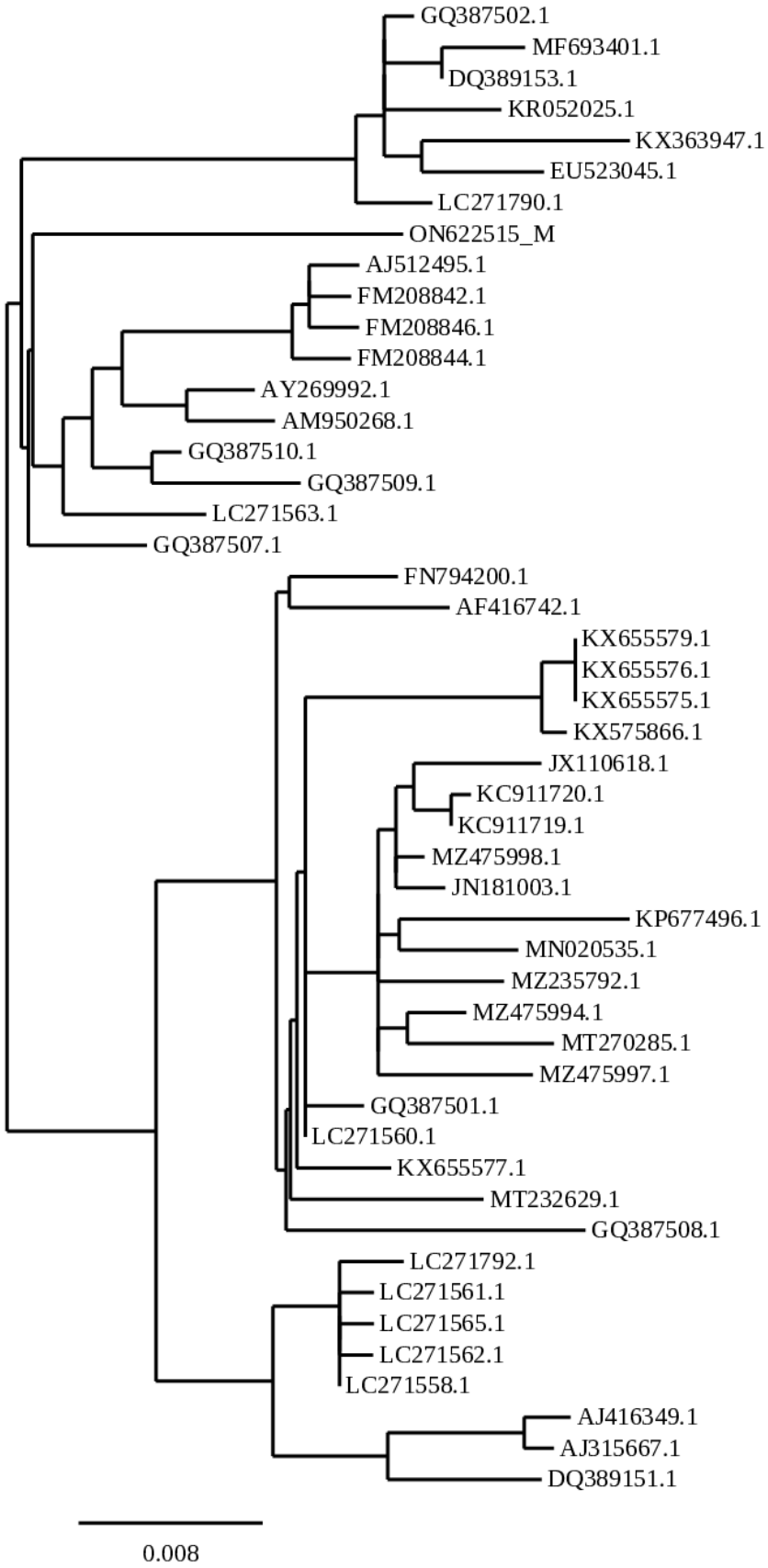
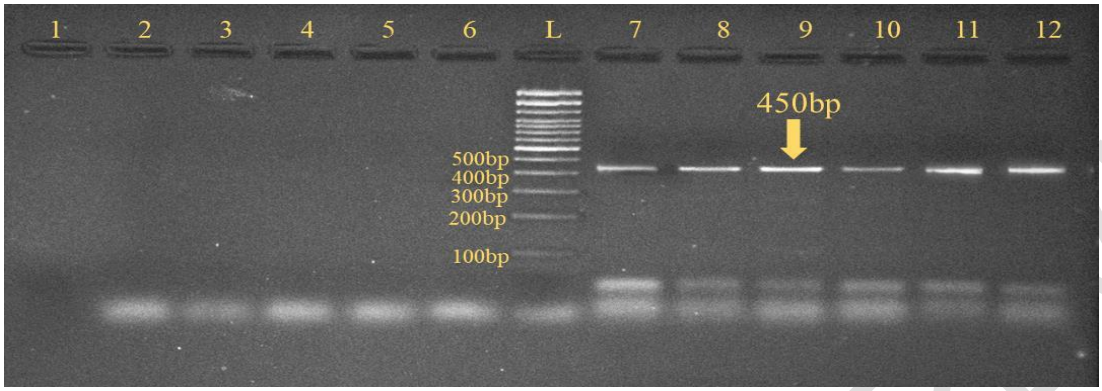



**Fig 6: Phylogenetic tree of sequences of coat protein (CP) gene of MYMIVisolate (ON622515.1) and previously reported MYMIV**

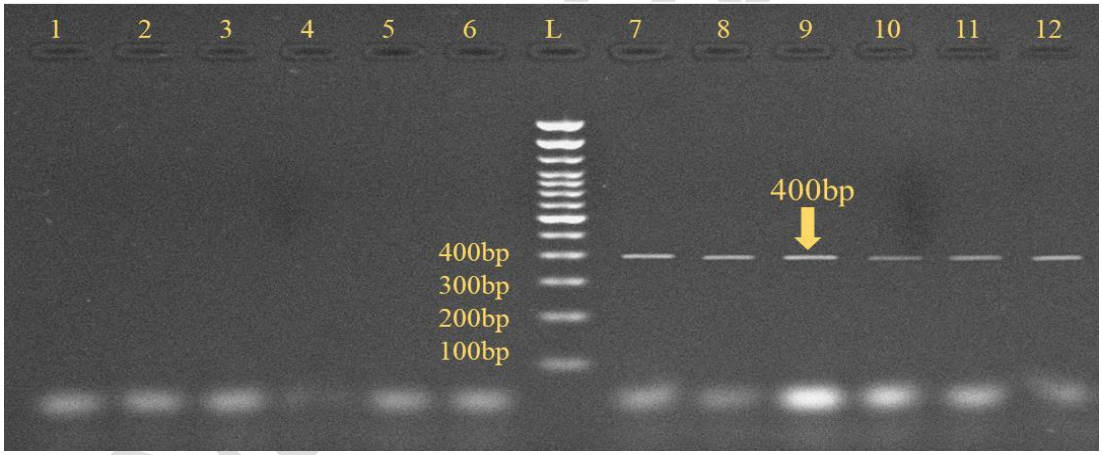
### 3.8 Screening of RGA markers associated with resistance gene

The present investigation showed that nine pairs of RGA primers were screened by using twelve mugbean genotypes. For the RGA analysis, screening was carried out for nine pairs of RGA primers, using genomic DNA of twelve mugbean genotypes. Out of this, five pairs of primers RGA1FCG &RGA1R, RGA22F2 & RGA24R2, VMYR1F & VMYR1R, RGA8F& RGA8R and RGAMB1 & RGAMB2 were showed the amplification in resistance genotypes, while four pairs of primers (YR4F & YR4R, CYR1F & CYR1R, RGAMB3 & RGAMB4 and RGAMB5 & RGAMB6) did not amplified in resistance genotypes (Fig 7).



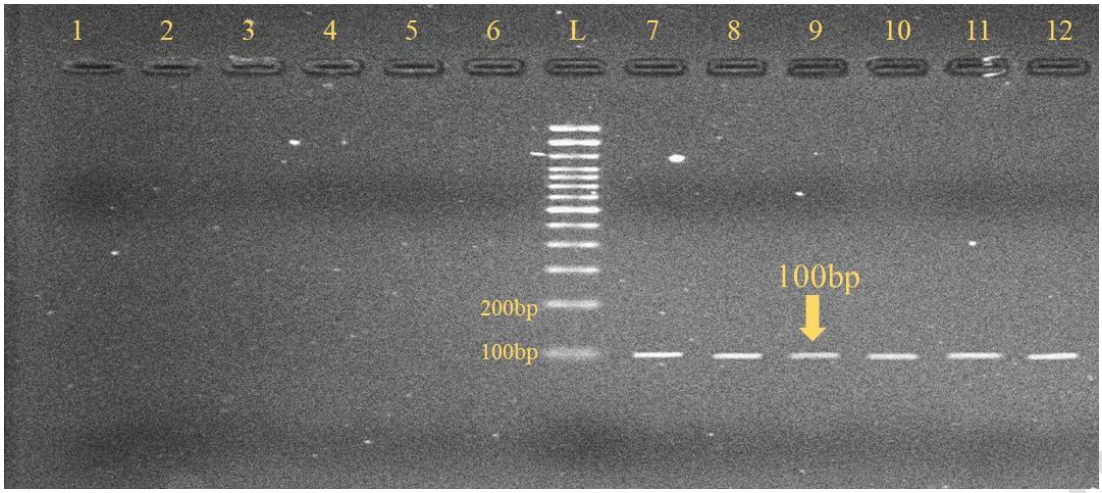

**1) RGA profiling of highly susceptible (1-6) and resistant (7-12) mungbean genotypes by primer pair one RGA1FCG and RGA1R**

| ***L-100bp DNA ladder*** | | | |
|---|---|---|---|
| ***1.GM 4*** | ***4. NMS-21-40*** | ***7.40 C*** | ***10.NMS-21-22*** |
| ***2.NMS-21-23*** | ***5. NMS-21-68*** | ***8.NMS-21-01*** | ***11.NMS-21-49*** |
| ***3.NMS-21-24*** | ***6. NMS-21-69*** | ***9.NMS-21-06*** | ***12.NMS-21-95*** |



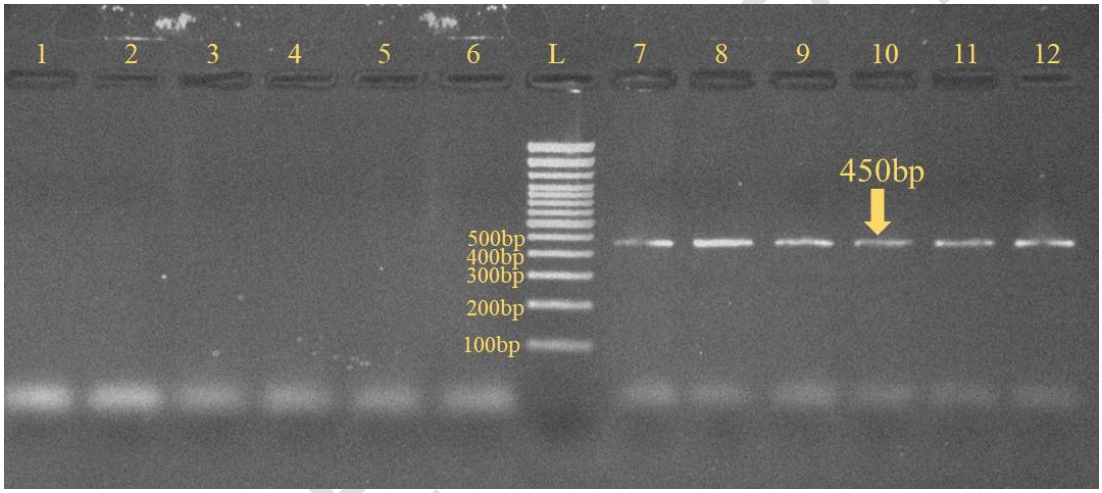

**2) RGA profiling of highly susceptible (1-6) and resistant (7-12) mungbean genotypes by primer pair two RGA8F and RGA8R**

| ***L-100bp DNA ladder*** | | | |
|---|---|---|---|
| ***1.GM 4*** | ***4. NMS-21-40*** | ***7.40 C*** | ***10.NMS-21-22*** |
| ***2.NMS-21-23*** | ***5. NMS-21-68*** | ***8.NMS-21-01*** | ***11.NMS-21-49*** |
| ***3.NMS-21-24*** | ***6. NMS-21-69*** | ***9.NMS-21-06*** | ***12.NMS-21-95*** |



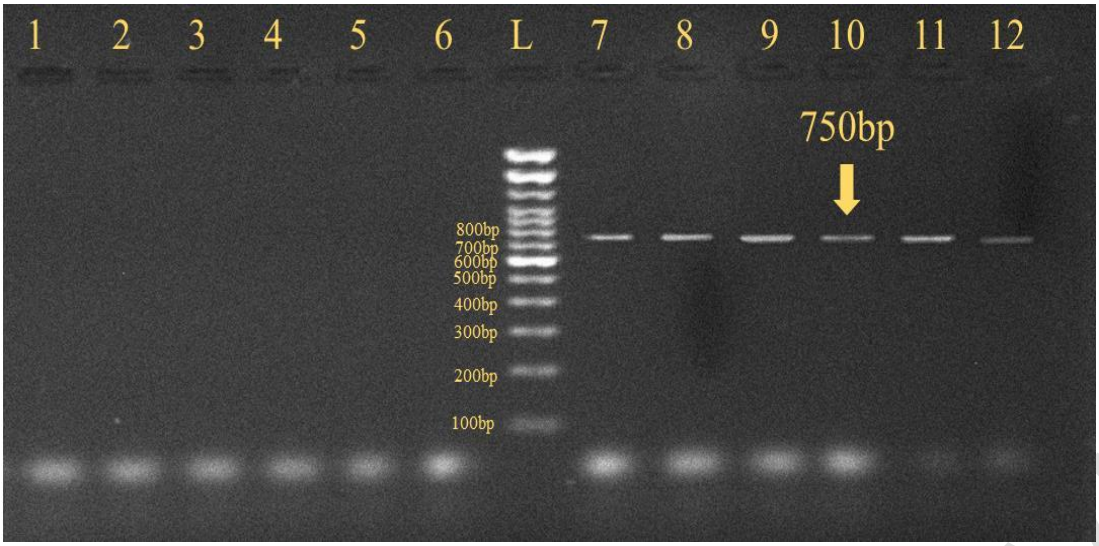

**3) RGA profiling of highly susceptible (1-6) and resistant (7-12) mungbean genotypes by primer pair three RGA22F2 and RGA22R2**

| *L-100bp DNA ladder* | | | |
|---|---|---|---|
| *1.GM 4* | *4. NMS-21-40* | *7.40 C* | *10.NMS-21-22* |
| *2.NMS-21-23* | *5. NMS-21-68* | *8.NMS-21-01* | *11.NMS-21-49* |
| *3.NMS-21-24* | *6. NMS-21-69* | *9.NMS-21-06* | *12.NMS-21-95* |




**4) RGA profiling of highly susceptible (1-6) and resistant (7-12) mungbean genotypes by primer pair four VMYR1F and VMYR1R**

| *L-100bp DNA ladder* | | | |
|---|---|---|---|
| *1.GM 4* | *4. NMS-21-40* | *7.40 C* | *10.NMS-21-22* |
| *2.NMS-21-23* | *5. NMS-21-68* | *8.NMS-21-01* | *11.NMS-21-49* |
| *3.NMS-21-24* | *6. NMS-21-69* | *9.NMS-21-06* | *12.NMS-21-95* |




**5) RGA profiling of highly susceptible (1-6) and resistant (7-12) mungbean genotypes by primer pair seven RGAMB1 and RGAMB2**

| ***L-100bp DNA ladder*** | | | |
|---|---|---|---|
| ***1.GM 4*** | ***4. NMS-21-40*** | ***7.40 C*** | ***10.NMS-21-22*** |
| ***2.NMS-21-23*** | ***5. NMS-21-68*** | ***8.NMS-21-01*** | ***11.NMS-21-49*** |
| ***3.NMS-21-24*** | ***6. NMS-21-69*** | ***9.NMS-21-06*** | ***12.NMS-21-95*** |

**Fig. 7: Potential seven RGA Markers Associated with YMD Resistance Gene in mugbean genotypes**

Out of five pairs of RGA primers, five pairs of RGA primers RGA1FCG &RGA1R, RGA8F& RGA8R, RGA22F2 & RGA24R2, VMYR1F & VMYR1R, and RGAMB1 & RGAMB2 were found single band of approximately 450bp, 400bp, 100bp, 450bp and 750bp, respectively in resistant genotypes. The amplification observed in resistant genotypes while absent in highly susceptible genotypes indicated that these markers were associated with the gene controlling YMD resistance in mugbean. The results of the current studies, which were conducted on markers (VMYR1F & VMYR1R) amplified at 445bp in all of the tolerant lines of mungbean, are consistent with those previously described [20]. Also, a YR4 amplified one such polymorphic fragment of 456bp while a CYR1 amplified another polymorphic fragment of 1236bp, present in tolerant lines, but absent in the susceptible cultivar [19]. Similar results are also reported primers RGA1 (RGA1FCG & 1R) and RGA8 (RGA8F & 8R) produced amplicon at 490-513bp and 387-390bp respectively [31]. Further, four RGA makers pairs showed the presence of consistent bands of size ~450bp in all resistant lines while absent in susceptible GM 4[32]. A pair RGA22F2 & RGA24R2 produced amplicon at 90bp in seven resistant genotypes[33]. Moreover, identical results were published, except in the resistant genotype, just one primer RGA pair 1F-CG/RGA 1R amplified a single 445 bp band while being lacking in the susceptible genotype [34,35].

## 4. CONCLUSION

A total of 100 mugbean genotypes were tested in this study for resistance to yellow mosaic disease using a disease grading system (1–9). Of these, 33 genotypes showed resistance, while 11 genotypes showed very vulnerable reactions. The mungbean yellow mosaic India virus' coat protein primers MYMIVF1 and MYMIVR2 amplified a DNA fragment of the partial coat protein gene at a size of around 620 bp, whereas primers MYMIVF3 & MYMIVR4, MYMVF5 & MYMVR6, and DYMV1 & DYMV2, DYMV3 & DYMV4 did not. The amplified partial coat protein gene fragment suggested that the mungbean yellow mosaic India virus was the source of the YMD infection in a number of mungbean genotypes. The segment that has been amplified and purified. A 574 bp long nucleotide sequence was found. The variation, also known as the mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene segment, has been assigned the accession number ON622515.1. The genotypes of particularly susceptible and resistant individuals have been successfully distinguished using data from five pairs of RGA markers because begomoviruses pose a substantial threat to the production of many commercially important crops. Additionally, full-length gene isolation and expression analysis open the door to pinpointing the molecular mechanism behind resistance. The present understanding of RGA markers linked with MYMIV resistance may be useful for mungbean improvement programs that use marker-assisted selection to confer

mosaic resistance in high yielding cultivars and also for expression analysis to determine its activity during pathogen interaction and will decipher the molecular mechanism involved in resistance.

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**Appendix 1**

**Mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene segment partial DNA A sequence**

# Mungbean yellow mosaic India virus isolate NAU-RJ coat protein gene, partial cds

GenBank: ON622515.1

FASTA Graphics

Go to:

```
LOCUS       ON622515                553 bp    DNA     linear   VRL 31-AUG-2022
DEFINITION  Mungbean yellow mosaic India virus isolate NAU-RJ coat protein
            gene, partial cds.
ACCESSION   ON622515
VERSION     ON622515.1
KEYWORDS    .
SOURCE      Mungbean yellow mosaic India virus
  ORGANISM  Mungbean yellow mosaic India virus
            Viruses; Monodnaviria; Shotokuvirae; Cressdnaviricota;
            Repensiviricetes; Geplafuvirales; Geminiviridae; Begomovirus.
REFERENCE   1  (bases 1 to 553)
  AUTHORS   Kalaria,R.K., Bhabhor,J.M., Modha,K.G. and Suthar,K.P.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-MAY-2022) Bioinformatics Section, Aspee Shakilam
            Biotechnology Institute, NAU, Athwa Farm, Ghod Dod Road, Surat,
            Gujarat 395007, India
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