

Original Research Article

Screening of Resistance Gene Analogue Marker(S) in Indian Bean [*Lablab purpureus* (L.) Sweet] against Yellow Mosaic Disease

ABSTRACT:

In this study, 104 genotypes of Indian beans were evaluated against the yellow mosaic disease in the field during Rabi 2021–2022, based on the disease rating scale (1–9). Out of the 104 genotypes of Indian beans, 33 genotypes showed resistance to the yellow mosaic disease, 14 genotypes showed moderate resistance, 17 genotypes showed moderate susceptibility, 29 genotypes showed susceptibility, and 11 genotypes showed highly susceptibility. The obtained sequence has a greatest identity of 95.05 percent with the full sequence of the dolichos yellow mosaic virus isolate AG segment DNA A with accession number MZ821026.1. As a result, the variant was given the accession number ON461886 and named dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA -A. In present investigation six resistant (P-19-169, V-19-15, V-19-35, GNIB-22, W-19-05, V-19-24) and six highly susceptible (P-19-176, W-19-60, P-19-127, V-19-11, W-19-58, V-19-106) Indian bean genotypes were screened by nine pairs of RGA primers. Seven pairs of RGA primers were successfully amplified as a result of this in all resistant genotypes, but not in all highly susceptible genotypes. The amplicons were obtained in seven pairs of RGA primers, where five pairs of RGA primers RGA1FCG & RGA1R, VMYR1F & VMYR1R, YR4F & YR4R, RGAIB3 & RGAIB4, RGAIB5 & RGAIB6 amplified single band size of approximately 450 bp, 450 bp, 450 bp, 450 bp and 1050 bp respectively in resistant genotypes, while only two pairs of RGA primers CYR1F & CYR1R and RGAIB1 & RGAIB2 were found with prominent band of approximately 950bp and 350bp. According to the data from the current investigation, seven pairs of RGA markers have proven successful in differentiating between the resistant and highly susceptible genotypes of Indian beans. These RGA markers can be employed in investigations mapping resistance genes and validating markers with long-lasting YMV resistance.

Comment [H1]: Abstract is length and author can consider removing of 1 or 2 statement made in abstract which is having less importance. Information on source of genotypes OR area/climatic condition in which it grows need to be given in abstract.

Keywords: Indian bean, DYMV, RGAs, YMD, begomovirus and BLAST

1. Introduction

Lablab purpureus (L.) Sweet, often known as the Indian bean, is a plant belonging to the *Fabaceae* subfamily Leguminosae with a variable number of chromosomes ($2n=20, 22, \text{ or } 24$). In the eighth century, it was brought to Africa from Southeast Asia where it had its inception [1]. The beans are naturally high in minerals including calcium, phosphorus, and iron as well as carbs, proteins, fats, and fiber [2]. Gujarat, Maharashtra, Tamil Nadu, and Karnataka are the principal bean-producing

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states in India. Karnataka makes a significant contribution, making up around 90% of the nation's area and production [3]. With an output of 0.89 million tonnes and a yield of 600 kg/ha, it covers 1.48 million hectares in Gujarat [4].

Legumes are extremely useful for regulating soil fertility, preventing soil erosion, and resolving nutritional imbalances in animals. More than 10 distinct diseases are caused by many pathogens that affect and infect the Indian bean crop. These diseases are brought on by viruses, nematodes, bacteria, and fungus. Fortunately, most diseases primarily affect local areas; however, a small number of diseases have an international impact. One of these is yellow mosaic disease, which has been linked to the destruction of numerous types of legumes across the country. It is generally believed that the same virus is to blame because the disease's symptoms are basically the same in all of the host plants. In India, a yellow mosaic of mungbean, urdbean, cowpea, and soybean results in a \$300 million annual loss [5]. However, in the past ten years, there has been a remarkable development in our understanding of the viruses responsible for YMD. Currently, four different viruses—Mungbean Yellow Mosaic India Virus (MYMIV), Mungbean Yellow Mosaic Virus (MYMV), Horse gramme Yellow Mosaic Virus (HYMV), and Dolichos Yellow Mosaic Virus (DYMV)—are known to cause Yellow Mosaic Disease (YMD) in various leguminous crops. These viruses are referred to as legume Yellow Mosaic Viruses (LYMVS). The production of the majority of the essential legume crops in the Indian subcontinent is currently severely hampered by YMD [6]. YMD has been reported in urdbean [7], mungbean [8], pigeon pea [7,9], horse gram [10] and French bean [11]. However, mungbean, urdbean, cowpea, moth bean, and soybean are the worst affected crops.

Yellow mosaic illness is brought on by the DYMV species of the family Geminiviridae genus of begomovirus. The majority of plants infected with ssDNA viruses, which are disseminated by the vector *Bemisia tabaci*, belong to the genus Begomovirus. The genomic components of begomoviruses are circular, single-stranded, encapsidated, and range in size from 18 to 30 nm. The bipartite genome of the begomovirus is made up of DNA A and DNA B. DNA B of the begomovirus codes for proteins facilitating intracellular and intercellular mobility, while DNA A of the virus codes for genes controlling replication and encapsulation. Yellow to bright yellow spots, vein clearing on leaves, and yellowing of the leaf are signs of the disease, which results in yield losses of 10% to 100%. Despite the serious damage that YMD causes, conventional breeding has been unable to produce a cultivar that is YMD resistant that is viable. According to a genetic analysis, RGAs are regularly connected to well-known disease resistance loci, where they typically exist in clusters [12]. According to genetic mapping studies of RGAs, more than 1% of the *A. thaliana* genome may correspond to the NBS type of R genes [13], and they co-segregate with resistance markers for fungi [14]. Furthermore, resistance-related quantitative trait loci have been linked to RGAs (QTL) [15,16]. Given the aforementioned information and the need to screen for resistance genes that confer resistance to the yellow mosaic virus, the current research to identify RGA markers connected with YMV resistance in Indian bean genotypes was conducted.

Materials and Methods

2.1 Experimental Material

Comment [H3]: Information on agro-climatic condition of selected location need to be given; Information on crop management practices also need to be given in short such as nutrient application rate, weed and sowing specification, etc.

A total of 104 Indian bean genotypes were growing during Rabi 2021-22. These 100 genotypes were grown along with 4 checks (resistant check GNIB-21, GNIB-22 and susceptible check V-19-154, W-19-58), where W-19-58 was spreader row in Augmented Block Design with 60 cm and 20 cm inter and intra row spacing, respectively. For growing a healthy crop, all advised agronomic procedures were used.

2.2 Field Screening

During the Rabi 2021–22 season, the experimental material was tested for its response to the yellow mosaic disease in a natural field setting. Since whiteflies are the cause of the virus in the field, no pesticides were used to keep the whitefly population in the experimental field from naturally declining. Whitefly activity and the emergence of disease signs in the crop were continuously observed.

Table1: Yellow mosaic disease rating scale used in Indian bean genotypes

Sr. No.	Rating scale	Percentage of plants foliage affected	Reaction
1	1	No visible symptoms (or) minute yellow specks covering 0.1–5.0% of the leaf area	Resistant (R)
2	3	Mottling of leaves covering 5.1–10.0% of the leaf area	Moderately resistant (MR)
3	5	Mottling and yellow discoloration of 10.1–25.0% of the leaf area	Moderately susceptible (MS)
4	7	Mottling and yellow discoloration of leaves on 25.1–50% of the leaf area	Susceptible (S)
5	9	Yellow mottling on more than 50% and up to 100% of the leaf area	Highly susceptible(HS)

After YMV incidence was detected in 80% of the plants in spreader rows, the test materials were scored. Screening of Indian bean genotypes for yellow mosaic disease (YMD) [17] was carried out using a 1–9 point grading system.

2.3 Amplification and Sequencing of Partial DNA-A Component of the Virus

The total genomic DNA was isolated from tender leaves of selected YMD infected sample of highly six susceptible and resistant Indian bean genotypes(P-19-169, V-19-15, V-19-35,GNIB-22, W-19-05,V-19-24, P-19-176, W-19-60, P-19-127, V-19-11, W-19-58, V-19-106) by modified CTAB method [18]. The integrity of genomic DNA isolated from various samples was examined. The crude genomic DNA was separated as per standard technique using agarose gel electrophoresis (0.8% agarose gel) and stained with ethidium bromide [19].

DNA purity was calculated using the OD 260/OD 280 ratio, which was then quantified. The YMV specific coat protein (CP) primers (–Table 1) designed using different coat protein sequences [GQ387509.1, DQ389148.1 (MYMIV), DQ389146.1 (MYMV), GU591170.1, KT282129.1 (DYMV)] and for the amplification of the coat protein gene of YMV, the Primer3 tool (<https://bioinfo.ut.ee/primer3-0.4.0/>) was employed as follows:

Table 2: 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	GTCATCGTGTGGTAAACGATT
10	DYMV4	Reverse	GGAGTGGGCTTACAAGAATGC

PCR reactions were performed under the previously mentioned parameters. It was performed in a 20µl reaction mixture containing 60 ng genomic DNA 2.5 µl, Master mix (2x) (Takara Bio) 10.0µl, each primer (forward and reverse) 2.0 µl and NFW 4.0 µl. The Biorad Thermal Cycler was used for the amplification, with the following settings: one cycle of pre-denaturation at 95°C for 7 minutes, 30-35 cycles each of denaturation at 95°C for 30 sec, annealing at 50–60°C for 45 sec, elongation for 30 sec at 72°C, and final elongation at 72°C for 10 minutes. The PCR was completed, and the reactions were then kept 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.

2.4 Phylogenetic analysis of the sequence and comparison with other *Begomoviruses*

Using the NCBI-BLSTN tool (<http://blast.ncbi.nlm.nih.gov>), database searches for begomovirus sequences, basic local alignments, and similarity index analysis were performed. The CLUSTALW(2) tool was used to perform multiple nucleotide (nt) sequence alignments for a portion of the viral isolate's DNA-A with other begomoviruses reported from India and around the world (<https://www.ebi.ac.uk/Tools/msa/clustalw2/>) [20]. The acquired sequence's ORFs were identified using the NCBI software's open reading frame finder (<https://www.ncbi.nlm.nih.gov/orffinder/>), and the coat protein sequence identities were confirmed using NCBI-BLASTP. The NCBI-CDD tool was also used to examine the sequence for the existence of conserved domains (<https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) [20,21]. Using online software MEGA version 7) (<https://www.megasoftware.net/>)[22] and the neighbor-joining method with 1000 bootstrap replications, a phylogenetic tree was created.

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

Nine pairs of the 18 RGA primers (Table 2) from the conserved region of the different classes of the soybean 'R' gene were employed in the current investigation. Three pairs of RGA primers were newly designed using the reported YMV resistant protein sequences (NM00137183.9 and EF091690.1) using the primer3 tool (<https://bioinfo.ut.ee/primer3-0.4.0/>) to amplified resistant gene like sequences from the genomic DNA by PCR. Six pairs of RGA primers were taken from the previously reported literature.

Screened nine pairs of RGA markers were present study polymorphic between six resistant and six highly susceptible Indian bean genotypes. 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 RGAIB1 & RGAIB2, RGAIB3 & RGAIB4, RGAIB5 & RGAIB6 were newly designed.

Table 3: List of RGA primers used in the present study

Sr. No.	Pairs	Primer	Sequences 5'-3'	Reference
1	1	RGA1FCG	AGTTTATAATTCGATTGCT	Kanazin, <i>et al.</i> 1996
2		RGA1R	ACTACGATTCAAGACGTCCT	Kanazin, <i>et al.</i> 1996
3	2	RGA8F	AGCGAGAGTTGTATTTAAG	Kanazin, <i>et al.</i> 1996
4		RGA8R	AGCCACTTTTGACAACCTGC	Kanazin, <i>et al.</i> 1996
5	3	RGA22F2	GGGTGGTTTGGGTAAGACCAC	Maiti <i>et al.</i> 2011
6		RGA24R2	TTCGCGGTGTGTGAAAAGTCT	Maiti <i>et al.</i> 2011
7	4	VMYR1F	AGTTTATAATTTGATTGCT	Basak <i>et al.</i> 2004
8		VMYR1R	ACTACGATTCAAGACGTCCT	Basak <i>et al.</i> 2004
9	5	YR4F	GGNAAGACGACACTCGCNTTA	Maiti <i>et al.</i> 2011
10		YR4R	GACGTCCTNGTAACNTTGATCA	Maiti <i>et al.</i> 2011
11	6	CYR1F	GGGTGGNTTGGGTAAGACCAC	Maiti <i>et al.</i> 2011
12		CYR1R	NTCGCGGTGNGTGAAAAGNCT	Maiti <i>et al.</i> 2011
13	7	RGAIB1	AGTATATAATTCAGTTGCTA	Newly designed
14		RGAIB2	ACGATTCCTGACTAGACTCA	Newly designed
15	8	RGAIB3	ATAGATTGCATTACTAG	Newly designed
16		RGAIB4	AGTGGTACGGTATTAGA	Newly designed
17	9	RGAIB5	TTATAAATCGAGTTGCTA	Newly designed
18		RGAIB6	CTACGATTCATGACTCATA	Newly designed

3. Results and Discussion

3.1 Field Screening

The outcomes of 104 Indian bean genotypes that were field-tested for the presence of yellow mosaic disease. Of the 104 genotypes of Indian beans, 33 were found to be resistant to YMD, 14 to be somewhat resistant, 17 to be moderately susceptible, 29 to be susceptible, and 11 to be very vulnerable (Figure 1, Figure 2, Table 3). Similar results were observed by [26,27,28,29] in Indian bean genotypes screened against DYMV.

Comment [H4]:

Some data need to give with statistical analysis of statistical significance [As most of the data is qualitative of not analyzed by following statistical procedure].

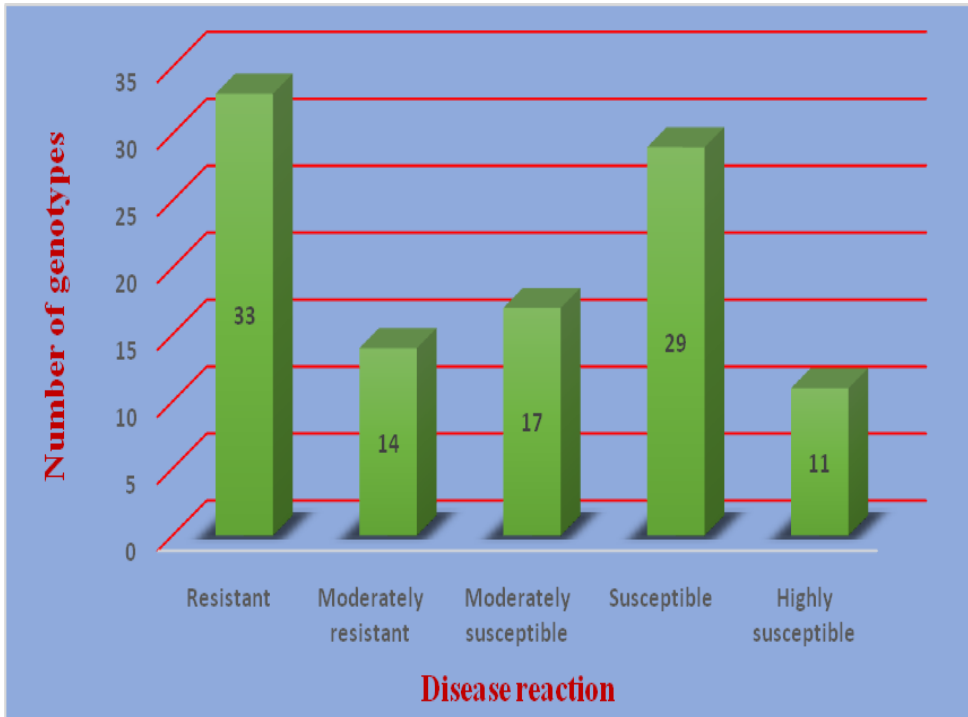


Fig. 1: Number of Indian bean genotypes categorized under different disease reactions against yellow mosaic disease



Indian bean genotypes showing typical symptoms of YMD



Indian bean genotypes showing resistant reaction to YMD

Fig. 2: Reaction of Indian bean genotypes against YMD in field condition

Table 4: Screening of Indian bean genotypes based on their reaction against yellow mosaic disease under field condition

Scale	Description	Reaction	Genotypes
1	No visible symptoms (or) minute yellow specks covering 0.1–5.0% of the leaf area	Resistant (R)	GNIB-22, P-19-193, W-19-63, W-19-65, V-19-185, V-19-03, P-19-261, P-19-66, P-19-192, P-19-50, P-19-193, P-19-94, W-19-132, V-19-35, V-19-131, W-19-05, P-19-98, P-19-07, V-19-17, V-19-111, W-19-63, W-19-90, V-19-162, V-19-24, V-19-171, V-19-15, V-19-140, W-19-30, P-19-25, P-19-169, V-19-211, V-19-63, GNBI-21
3	Mottling of leaves covering 5.1–10.0% of the leaf area	Moderately resistant (MR)	V-19-143, P-19-123, P-19-202, P-19-11, W-19-100, W-19-123, P-19-35, V-19-158, P-19-162, P-19-267, W-19-89, V-19-157, P-19-188, V-19-134
5	Mottling and yellow discoloration of 10.1–25.0% of the leaf area	Moderately susceptible (MS)	W-19-27, W-19-34, V-19-22, V-19-28, P-19-171, W-19-161, P-19-207, P-19-150, P-19-164, W-19-11, P-19-104, V-19-154, P-19-123, V-19-61, W-19-67, P-19-86, V-19-183
7	Mottling and yellow discoloration of leaves on 25.1–50% of the leaf area	Susceptible (S)	V-19-73, W-19-59, V-19-92, V-19-07, P-19-230, W-19-55, V-19-14, V-19-129, W-19-109, W-19-111, V-19-43, P-19-87, W-19-96, P-19-180, V-19-41, V-19-36, V-19-68, P-19-77, W-19-56, W-19-57, W-19-20, W-19-122, V-19-62, P-19-147, W-19-130, P-19-254, W-19-39, P-19-95, W-19-58
9	Yellow mottling on more than 50% and up to 100% of the leaf area	Highly susceptible (HS)	V-19-135, V-19-11, V-19-09, P-19-81, V-19-106, P-19-203, V-19-27, W-19-60, P-19-127, P-19-226, P-19-176

3.2 Amplification and Sequencing of Partial DNA A Component of the Yellow Mosaic Virus

DNA was isolated from tender YMD infected leaves of selected six highly susceptible and six resistant Indian bean genotypes (P-19-176, W-19-60, P-19-127, V-19-11, W-19-58, V-19-106, P-19-169, V-19-15, V-19-35, GNIB-22, W-19-05, V-19-24) by the CTAB method [18] with some modification. DNA quality was examined by putting it through a 0.8% Agarose gel. After DNA extraction, its quality and amount were assessed by measuring the DNA concentration with a Nano-drop. To achieve uniform findings, several DNA concentrations were ~~utilised~~utilized in the PCR process.

3.3 PCR amplification of partial DNA-A component

Certain primers (Table 2) were employed to amplify the partial coat protein region of the DNA-A component of the yellow mosaic virus (YMV) in order to complete the amplification of the YMVs partial nucleotide sequence. The amplicon of partial coat protein (CP) genes was confirmed by electrophoresis and ~ 450 bp bands were found to confirm the presence of partial CP gene in all

highly susceptible genotypes (P-19-176, W-19-60, P-19-127, V-19-11, W-19-58, V-19-106) and did not obtain amplification in resistance genotypes (P-19-169, V-19-15, V-19-35, GNIB-22, W-19-05, V-19-24). A DNA fragment of partial coat protein gene was amplified with coat protein primers DYMV1 and DYMV2 of the dolichos yellow mosaic virus at ~450 bp amplicon (Figure 3). Therefore, amplification of the partial coat protein gene fragment suggested that the YMD infection in Indian bean genotypes was due to dolichos yellow mosaic virus.

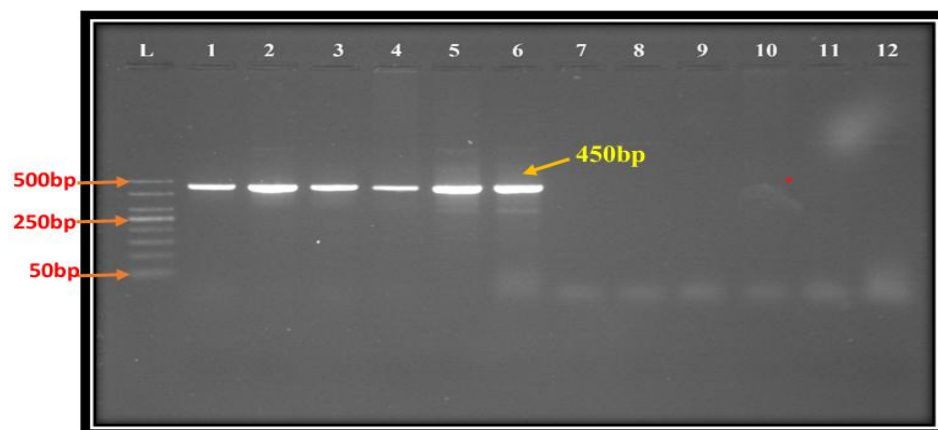


Fig 3: PCR amplification of the CP gene DNA-A of YMV using —DYMVF1 and DYMVR2 primer

L-50bp DNA ladder,		1-6 highly susceptible,	7-12 resistant Indian bean genotypes
1. P-19-176	4. V-19-11	7. P-19-169	10. GNIB-22
2. W-19-60	5. W-19-58	8. V-19-15	11. W-19-05
3. P-19-127	6. V-19-106	9. V-19-35	12. V-19-24

These outcomes are consistent with those of Singh et al. (2012), who isolated a ~750 bp partial CP gene of DNA-A segment from an Indian bean that had been infected with YMD. Using CP gene-specific primers with approximate amplicon sizes of 700 bp and 650 bp, it was possible to get the amplification of the coat protein gene of MYMV from several pulses [30]. Similarly, ~1300bp partial CP gene of DNA-A fragment from YMD infected mungbean [31]. Moreover, comparable research in mungbeans documented the use of particular primers to amplify the MYMV coat protein gene, which is around ~650 bp long [32]. Similarly, amplification of ~1000 bp coat protein gene of MYMV in mungbean [33].

3.4 Sequencing of partial DNA-A component of YMV:

Partial amplified CP gene fragment of DNA-A component was purified and sequenced in an automated DNA sequencer by the cycle sequencing method. The results revealed that the sequenced coat protein of YMV had a 432bp long nucleotide sequence. The results of the BLAST

(<http://www.ncbi.nih.gov/BLAST>) search, which was used to determine sequence homology, clearly showed that the coat protein gene sequence of the YMV matched previously reported isolates of the dolichos yellow mosaic virus from various geographical locations in the range of 95.05 to 90.69%. Based on sequence comparison, the YMD in Indian bean caused by YMV has been confirmed as dolichos yellow mosaic virus. Also, the sequence was uploaded using the Bankit submission facility of NCBI (<https://www.ncbi.nlm.nih.gov>) to GeneBank, NCBI, Bethesda, Maryland, USA. The sequence was assigned the accession number ON461886.1 by GeneBank and is available for public use (<https://www.ncbi.nlm.nih.gov>) (Appendix 1). Similar reported ~1300bp partial CP gene of DNA-A fragment from YMD infected mungbean. A fragment containing 1285 bp was obtained and later deposited to the GeneBank, NCBI (Accession No. JQ004982)[31]. Also [Similarly](#), reported amplification of ~1000 bp coat protein gene of MYMV in mungbean [33]. The exact length of the MYMV that was recovered was 889 bp, containing 257 deduced amino acids and 115 bp of pre-coat proteins at the 5' end of the coat protein.

3.5 Phylogenetic Analysis of the Sequence and Comparison with other *Begomoviruses* Multiple alignment and similarity index

Obtained results from the BLAST tool suggested that the sequenced virus showed the highest identity of 95.05% (Table 4) with dolichos yellow mosaic virus isolate AG segment DNA A, complete sequence with accession number MZ821026.1, MZ388546.1, GU591170.1. Followed by 94.70% and 94.58% sequence identity with accession number KJ481204.1 and AJ968370.1 correspondingly, while 93.98%, 93.86% sequence identity with accession number AM157412.1, KP784661.1. respectively. Other viruses which were closely related with accession number MT108190.1, AY309241.1, JX315325.1, MH795972.1 having 91.57%, 91.33%, 91.12%, 91.02% while accession number MK391940.1, HQ221570.1 having 90.82%, 90.69% sequence identity respectively. The 13 previously published coat protein gene sequences from other studies were downloaded from NCBI and compared to the present study's coat protein gene sequences for homology study and phylogenetic analysis. Using the CLUSTALW (2) tool (<https://www.ebi.ac.uk/Tools/msa/clustalw2/>), multiple sequence alignments were carried out for all 14 CP gene sequences in order to analyze the sequence homology. To find the conserved sequences among all the coat protein of DYMV sequences, multiple sequence alignments of the dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A, accession number ON461886.1, were performed. CLUSTALW_(2) produces biologically significant multiple sequence alignments of divergent sequences. It determines the best match for the chosen sequences and arranges them such that the software can show the identities, similarities, and differences, as well as the evolutionary links. 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 a tentative variant of DYMV (accession no MZ821026.1) [34]. Therefore, the proposed name of the variant as dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A, accession number ON461886.1.

Table 5: Per cent identities (nucleotide) between CP gene of DNA-A DYMV Accession number ON461886.1 with other reported dolichos yellow mosaic virus in NCBI database

Sr. No.	Virus name	Sequence identity (%)	Accession number	Geographical location
1	Dolichos yellow mosaic virus isolate AG segment DNA A, complete sequence	95.05	MZ821026.1	New delhi
2	Dolichos yellow mosaic virus isolate AG coat protein (AV1) and pre-coat protein (AV2) genes, partial cds; replication enhancer protein (AC3) and transcription activator protein (AC2) genes, complete cds; and replication initiation protein (AC1) gene, partial cds	95.05	MZ388546.1	New delhi
3	Dolichos yellow mosaic virus isolate DYMV-CpKn coat protein (AV1) gene, complete cds	95.05	GU591170.1	Uttar Pradesh
4	Dolichos yellow mosaic virus isolate DA segment DNA A, complete sequence	94.58	KJ481204.1	Uttar Pradesh
5	Dolichos yellow mosaic virus complete DNA-A segment, strain Mysore	94.70	AJ968370.1	Karnataka
6	Dolichos yellow mosaic virus DNA-A segment, complete sequence, isolate Bangalore-1	93.98	AM157412.1	Karnataka
7	Dolichos yellow mosaic virus isolate CBE1 segment DNA A, complete sequence	93.86	KP784661.1	Tamil Nadu
8	Dolichos yellow mosaic virus isolate BP-1 segment DNA-A, complete sequence	91.57	MT108190.1	Bangladesh
9	Dolichos yellow mosaic virus segment DNA-A, complete sequence	91.33	AY309241.1	New Delhi
10	Dolichos yellow mosaic virus clone JD18, complete genome	91.12	JX315325.1	New Delhi

11	Dolichos yellow mosaic virus isolate TN-TM1 DNA-A, complete sequence	91.02	MH795972.1	Tamil Nadu
12	Horsegram yellow mosaic virus isolate Hebbal 3 coat protein gene, complete cds	90.82	MK391940.1	Karnataka
13	Mungbean yellow mosaic India virus isolate West Bengal coat protein (AV1) gene, partial cds	90.69	HQ221570.1	West Bengal

3.6 Finding of Open Reading Frame (ORF):

The NCBI's ORF finding tool was used to further investigate the coat protein (AVI) gene (partial cds) sequence of the DYMV, and then NCBI-BLASTP was used to predict the coat protein sequence using the pfam database. Using the NCBI-CDD tool, the projected coat protein sequence of 104aa was further examined for the presence of a conserved domain (Figure 4). Using accession number Pfam00844 and an E-value of $5.05e-57$, the ORF from the coat protein gene sequence revealed the presence of a conserved domain as the geminivirus coat protein/nuclear export factor BR1 superfamily (Figure 5). Similar work was also carried out in the ToLCV coat protein sequence [21], Papaya Leaf curl virus coat protein [35] and Tomato leaf curl virus disease (ToLCVD) coat protein sequence [36].

3.7 Phylogenetic analysis of CP gene of DNA-ADYMV with other *begomoviruses*

To identify the conserved sequences throughout all of the coat proteins of DYMV sequences, multiple sequence alignments of the dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A, accession number ON461886.1, were performed. Also, using the Neighbour-Joining technique and 1,000 bootstrap replications, a phylogenetic tree was created. Two primary clusters could be seen in the phylogenetic tree constructed utilizing the incomplete coat protein (CP) genes of the dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A (Figure 6), Cluster A was further divided into two subclusters, with cluster A1 containing the dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA accession number ON461886.1 and cluster A2 containing the following sequences: MZ821026.1, MZ388546.1, GU591170.1, KJ481204.1, AJ968370.1, AM157412.1, KP784661.1, MT108190.1, AY309241.1, JX While cluster B was broken into two sub-clusters, cluster B1 and cluster B2 each contained HQ221570.1 and MK391940.1, respectively.

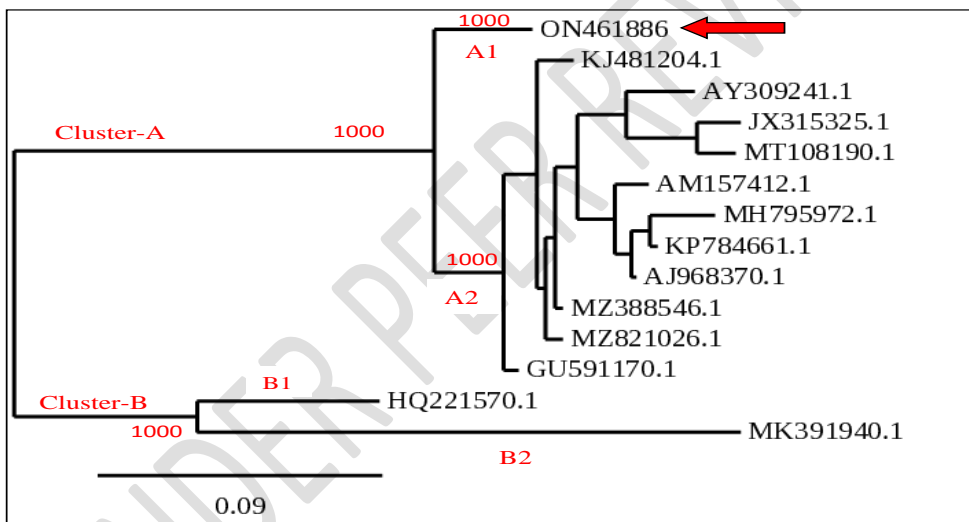


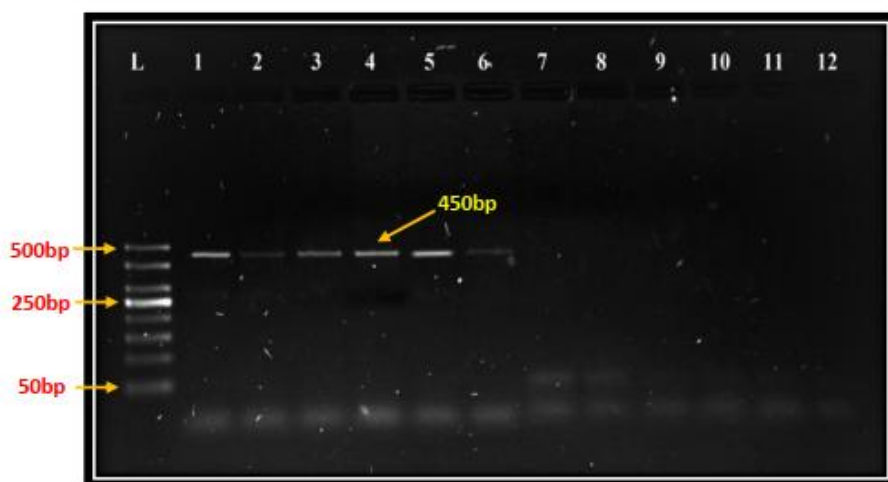
Fig. 6: Phylogenetic tree of sequences of coat protein gene of DYMV isolate(ON461886) and previously reported DYMV

Further, cluster A2 mostly comprised of the different DYMV strains which predominate in the different regions of India mainly Delhi, UP, Tamil Nadu, Karnataka, West Bengal, and worldwide United Kingdom, South Korea, while cluster A1 represented as dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A in Gujarat that conformed YMD infection as DYMV in Indian bean genotypes under field conditions during *Rabi* 2021-22. In the present study confinement of species of yellow mosaic disease infecting Indian bean genotypes in South Gujarat was due to dolichos yellow mosaic virus (DYMV). Similar phylogenetic analysis reported [31] mungbean against MYMV, dolichos bean against DYMV [37]. Also, similar work carried out by in mungbean against MYMV [38].

3.8 Screening of RGA Markers Associated with Resistance Gene

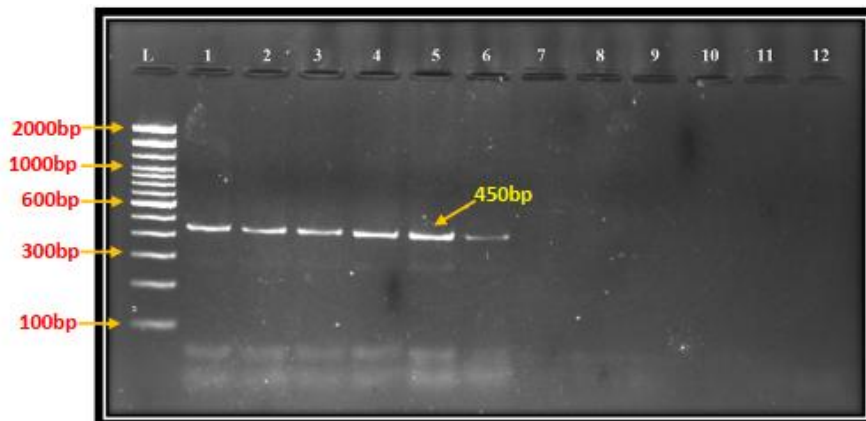
The present investigation showed that nine pairs of RGA primers were screened by using twelve Indian bean genotypes. For the RGA analysis, screening was carried out for nine pairs of RGA primers, using genomic DNA of twelve Indian bean genotypes. Out of this, seven pairs of primers RGA1FCG & RGA1R, VMYR1F & VMYR1R, YR4F & YR4R, CYR1F & CYR1R, RGAIB1 & RGAIB2, RGAIB3 & RGAIB4, RGAIB5 & RGAIB6 were showed the amplification in resistance genotypes, while two pairs of primers (RGA8F& RGA8R), (RGA22F2 & RGA24R2) did not amplified in resistance genotypes (Figure 7).

Out of seven pairs of RGA primers, five pairs of RGA primers RGA1FCG & RGA1R, VMYR1F & VMYR1R, YR4F & YR4R, RGAIB3 & RGAIB4, RGAIB5 & RGAIB6 were found single band of approximately 450bp, 450bp, 450bp and 1050bp, respectively in resistant genotypes, while only two pairs of RGA primers CYR1F & CYR1R and RGAIB1 & RGAIB2 were found with prominent band of approximately 950bp and 350bp, respectively. 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 Indian bean.



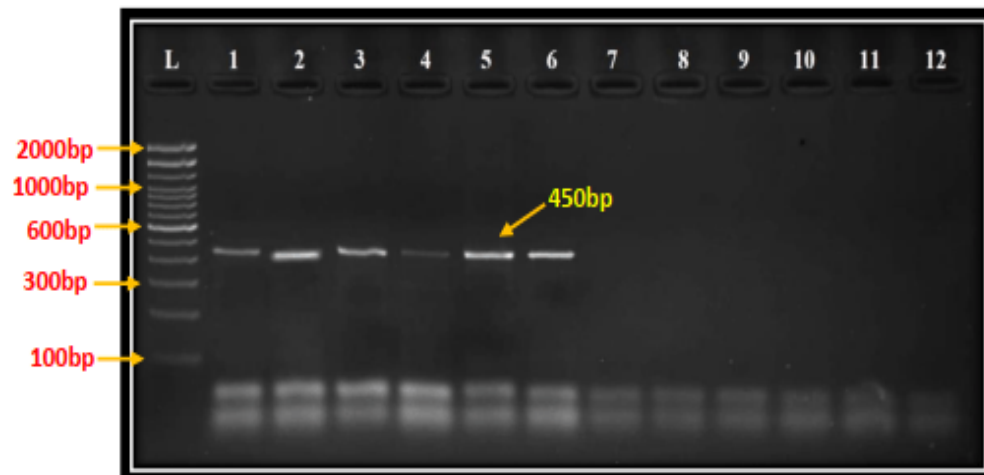
1) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair one (RGA1FCG & RGA1R)

L-50bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



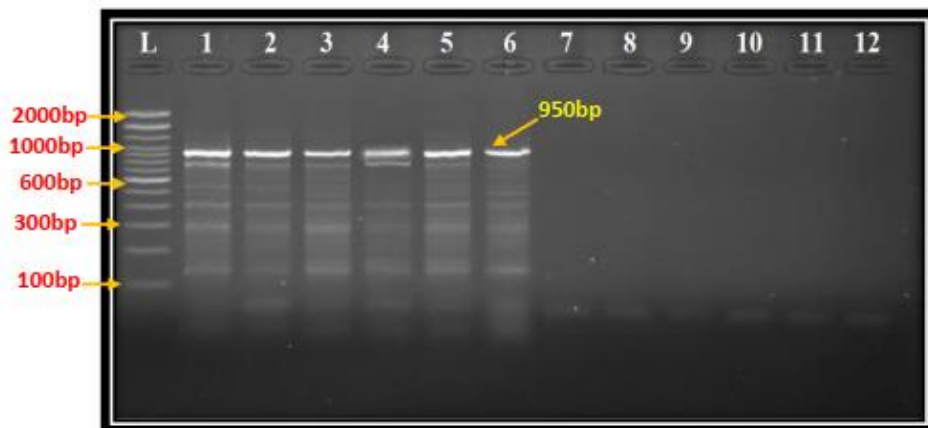
2) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair four (VMYR1F & VMYR1R)

L-100bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



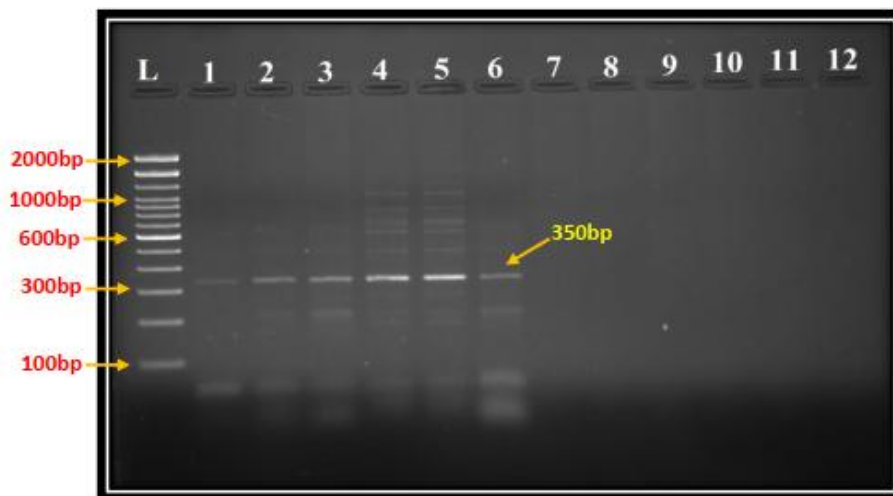
3) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair five (YR4F & YR4R)

L-100bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



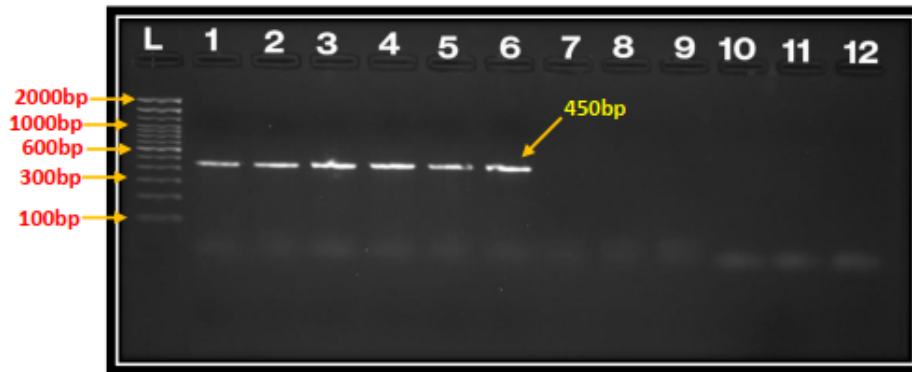
4) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair six (CYR1F & CYR1R)

L-100bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



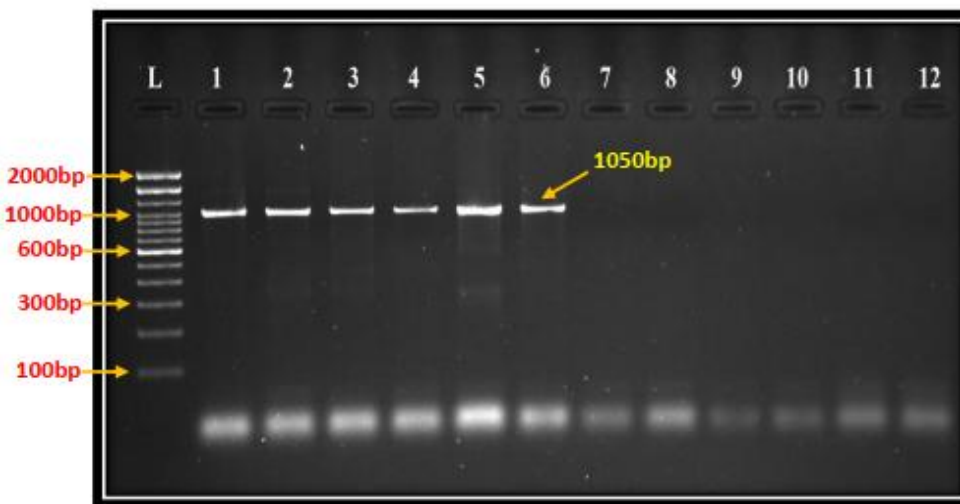
5) RGA profiling of resistant (1-6) and highly susceptible (7- 12) Indian bean genotypes by primer pair seven (RGAIB1 & RGAIB2)

L-100bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



6) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair eight (RGAIB3 & RGAIB4)

L-150bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106



7) RGA profiling of resistant (1-6) and highly susceptible (7-12) Indian bean genotypes by primer pair nine (RGAIB5 & RGAIB6)

L-100bp DNA ladder			
1. P-19-169	4. GNIB-22	7. P-19-176	10. V-19-11
2. V-19-15	5. W-19-05	8. W-19-60	11. W-19-58
3. V-19-35	6. V-19-24	9. P-19-127	12. V-19-106

Fig. 7: Potential seven RGA Markers Associated with YMD Resistance Gene in Indian bean genotypes

The results of the current studies are consistent with those of the [25] marker (VMYR1F & VMYR1R) amplified at 445bp in all of the tolerant lines of mungbean. Also, a YR4 amplified [24] 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. Similar results are reported primers RGA1 (RGA1FCG & 1R) and RGA8 (RGA8F & 8R) produced amplicon at 490-513bp and 387-390bp respectively [39]. Moreover, four RGA maker pairs demonstrated that all resistant lines contained consistent bands of size 450 bp, but susceptible GM4 did not [40]. Similarly reported primer pair RGA22F2 & RGA24R2 produced amplicon at 90bp in seven resistant genotypes [41]. Moreover, the same outcome was seen with the primer RGA pair 1F-CG/RGA 1R, which amplified a single 445 bp band in the resistant genotype but not in the susceptible genotype [42].

4. Conclusion:

In this study, based on the disease rating scale (1-9), A total of 104 Indian bean genotypes were screened against yellow mosaic disease, out of this 33 genotypes were found to have exhibit resistant, while 11 genotypes were found to have highly susceptible reaction to yellow mosaic disease. A DNA fragment of partial coat protein (CP) gene was amplified with coat protein (CP) primers DYMV1 and DYMV2 of the dolichos yellow mosaic virus at ~ 450 bp amplicon, while primers MYMIVF1 & MYMIVR2, MYMIVF3 & MYMIVR4, MYMVF5 & MYMVR6, DYMV3 & DYMV4 did not amplified. As a result, amplification of the incomplete coat protein gene fragment revealed that the dolichos yellow mosaic virus was responsible for the YMD infection in Indian bean genotypes. The purified and sequenced amplified fragment. Nucleotide sequences of 432 bp were found. Based on the sequence analysis, the proposed name of the variant as dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment DNA A, accession number ON461886.1. Results were obtained in seven pairs of RGA markers have been successful in differentiating between the resistant and highly susceptible genotypes. Because begomovirus pose a serious danger to the production of many economically significant crops, findings were obtained. As DYMV disease resistance qualities in Indian bean genotypes are improved, these RGA markers can be used effectively in MAS programmes. Applications for these RGA markers include linkage mapping, QTL mapping, cloning of plant resistance genes, and resistance breeding in genomics and bioinformatics.

Comment [H5]: The conclusion should be concise, address the objectives of study and based on the data presented in MS; Avoid writing conclusion as result section and also avoid giving general information in conclusion.

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Comment [H6]: Optimum.

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UNDER PEER REVIEW

Appendix 1

**Dolichos yellow mosaic virus isolate NAU-RA coat protein gene segment partial
DNA A sequence**

GenBank

Dolichos yellow mosaic virus isolate NAU-RA coat protein gene, partial cds

GenBank: ON461886.1

FASTA Graphics

Go to: (C)

LOCUS ON461886 419 bp DNA linear VRL 31-

AUG-2022

DEFINITION Dolichos yellow mosaic virus isolate NAU-RA coat protein gene,

partial cds.

ACCESSION ON461886

VERSION ON461886.1

KEYWORDS

SOURCE Dolichos yellow mosaic virus

ORGANISM Dolichos yellow mosaic virus

Viruses; Monodnaviria; Shotokuvirae; Cressdnaviricota; Repensiviricetes; Geplafuvirales; Geminiviridae;

Begomovirus.

REFERENCE 1 (bases 1 to 419)

AUTHORS Kalaria,R.K., Vakaliya,M.A.U., Modha,K.G. and Parekh,V.B.

TITLE Direct Submission

JOURNAL Submitted (07-MAY-2022) Bioinformatics Section, Aspee

Shakilam

Biotechnology Institute, NAU, Athwa Farm, Ghod Dod Road,

Surat,

Gujarat 395007, India

COMMENT ##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES

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ORIGIN

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