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RESEARCH ARTICLE

Identification of cowpea [*Vigna unguiculata* (L.) Walp.] germplasm accessions resistant to yellow mosaic disease

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Abstract

Yellow mosaic disease (YMD) of cowpea, predominantly caused by mungbean yellow mosaic India virus (MYMIV) and mungbean yellow mosaic virus (MYMV) is a major constraint to the production of cowpea (*Vigna unguiculata* L.) in India resulting in significant yield reductions. The present study extensively evaluated 1127 cowpea germplasm accessions from the National Gene Bank of India, New Delhi, at two hotspot locations (Hyderabad and Delhi) for their response to YMD. About 181 accessions showed no symptoms in field screening at both locations and were considered putatively resistant. A set of the best 100 out of 181 was selected for further confirmation of resistance to MYMIV through whitefly-mediated screening along with 40 accessions known to be susceptible to YMD. The results of whitefly-mediated transmission confirmed MYMIV resistance in 20 accessions with a disease score of zero. Further, the YMD-associated Begomovirus was characterized using rolling circle amplification coupled with sequencing of the full DNA-A genome of MYMIV, which shared an identity of 99.02% with the MYMIV isolate infecting cowpea in Pakistan. The 20 cowpea accessions identified as novel resistant sources to MYMIV in the present study could be used for mapping resistance genes and as donors in resistance breeding for yellow mosaic disease.

Keywords: Cowpea germplasm, evaluation, begomovirus, mungbean yellow mosaic India virus

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important multipurpose legumes. Its grains and green pods provide a valuable revenue source for resource-poor farmers in the developing world (Singh et al. 2002; Langyintuo et al. 2003). It is an important source of nutrition due to high protein content, palatability, and relative freedom from antimetabolites (Kareem and Taiwo 2007). In India, it is a minor legume cultivated over an area of 3.9 mha, grossing an annual production of 2.21 mt, primarily grown in arid and semi-arid Punjab, Haryana, Delhi, and Western Uttar Pradesh tracts. Cowpea production suffers losses due to virus infection ranging from 10 to 100% (Kareem and Taiwo 2007). More than 140 viruses are reported in cowpeas, of which 20 viruses are known to have widespread distribution (Thottappilly and Rossel 1992; Hema et al. 2014) and their infection often results in severe yield losses (Byoung et al. 2005). In India, cowpea is severely affected by golden mosaic disease (CGMD), and severe leaf curl diseases caused by different isolates of mungbean yellow mosaic India virus (MYMIV) and mungbean yellow mosaic virus (MYMV) (John 2008; Malathi et al. 2005; Rouhibakhsh et al. 2005; Surendranath et al. 2005; Varma and Reddy 1984). Viral diseases in legumes, including cowpeas, lead to losses of approximately $300 million per year (Varma and Malathi 2003).

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The Yellow Mosaic Disease (YMD) was first reported in mungbean in 1955 in the fields of Indian Agricultural Research Institute (IARI), Delhi (Nariani 1960). Begomoviruses (family *Geminiviridae*) are whitefly-transmitted viruses that cause YMD in different pulses, including cowpeas. The genus *Begomovirus* has characteristic twinned quasi-icosahedral particles (20 × 30 nm) that encapsidate mono- or bipartite-genome of circular single-stranded DNA of 2.7 kb length (Hanley-Bowdoin et al. 2013). They have coat proteins with specified amino acid sequences, which account for their vector-specific nature (Briddon and Markham 2000). Four distinct begomoviruses *viz*., mungbean yellow mosaic virus (MYMV), mungbean yellow mosaic India virus (MYMIV), horse gram yellow mosaic virus (HgYMV) and Dolichos yellow mosaic virus (DoYMV) have been identified to be associated with YMD, which are collectively known as YMV's (yellow mosaic viruses) (Qazi et al. 2007; Malathi and John 2008; Akram and Singh 2016). Among all the YMVs, *mungbean yellow mosaic India virus* and *mungbean yellow mosaic virus* are the most predominant viruses causing YMD in cowpea (Fauquet et al. 2003).

Although cowpea as a legume is severely affected by YMD, it has not been thoroughly investigated to identify confirmed sources of resistance to the predominant begomoviruses. This lack of investigations limits breeding efforts aimed at developing YMD resistant varieties. The present study was, therefore, conducted to identify virus associated with YMD of cowpea under North Indian conditions using molecular markers and doing a comprehensive analysis of cowpea gene pool to identify sources of resistance based on field and artificial screening.

Materials and methods

Source of germplasm

A total of 1127 accessions of cowpea germplasm were taken from the germplasm pool available in the National Gene Bank of ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. These germplasm accessions were originally collected from various agro-climatic zones of India where cowpea is grown. They were selected based on preliminary evaluation screening data of yellow mosaic disease at the ICAR-NBPGR Hyderabad Regional Station. Popular cowpea cultivars, namely, Pusa Komal, C-152, KM-5 were used as highly susceptible checks and were included as the infector rows, whereas PL4 was used as a resistant check.

Screening of cowpea germplasm for YMD resistance under field and controlled environment by whitefly transmission

The preliminary screening of 3350 cowpea germplasm accessions was done during *kharif* 2021 in augmented block design (ABD). Based on the preliminary evaluation results, the cowpea germplasm showing either no disease or resistant score were further advanced for directed screening against YMD. A sub-set of 1127 cowpea germplasm was grown along with PL-4 and susceptible checks during the summer season (February to April, 2022) and *kharif* 2022 at the experimental research farm of ICAR-NBPGR Regional Station, Hyderabad and ICAR-NBPGR, Issapur farm, Delhi, respectively. Germplasm test accessions and checks were planted in 1 m rows with a 30 cm distance between rows and 15 cm between plants within rows

Virus inoculum was augmented by using the infector rows of susceptible varieties for efficient screening against YMD as per the method described by Nene et al. (1972). Susceptible checks were used as infector rows after every eight rows of the test germplasm accessions as done earlier (Nair et al. 2017; Sai et al. 2017; Habib et al. 2007), while PL-4 was replicated among the accessions as a resistant check. Further, the susceptible checks were also grown at the edges of full trial plots to provide the vector with a suitable virus source. The germplasm lines were scored for YMD reaction at weekly intervals with initiation of symptoms in the field until the crop matured completely. The severity of disease incidence in naturally infected plants in the field was scored on a 1–4 standard arbitrary scale (Bag et al. 2014; Akhtar et al. 2009) and the coefficient of infection (CI) and Area Under the Disease Progress Curve (AUDPC) was calculated for the field data (Campbell and Madden 1990). Out of 181 resistant accessions from both locations, 100 were selected based on their phenotypic and genotypic diversity and supplemented with 40 susceptible germplasm accessions from both locations relying on the availability of GBS data for further studies.

Source and maintenance of MYMIV and whitefly transmission

The viral inoculum of MYMIV for whitefly transmission was collected from naturally infected cowpea plants in an experimental area of IARI field (hot spot for MYMIV) and inoculum was maintained in the greenhouse on susceptible genotype KM-5. The virus in the source inoculum was characterized as MYMIV.

All 140 cowpea genotypes were inoculated with MYMIV using the whitefly *B. tabaci* as a vector during the summer season of 2023 following Shivaprasad et al. (2006). The aviruliferous culture of whiteflies were raised on cucumber plants in a whitefly rearing chamber at the Advanced Centre for Plant Virology, Division of Plant Pathology, ICAR-IARI, New Delhi. These were subjected to 24h acquisition access period (AAP) on MYMIV-infected cowpea plants (Malathi and John 2009) followed by a 48h inoculation access period (IAP) on 4-5 week-old cowpea test plants under fine mesh cages. A total of 10 plants per test entry (10 plants per cage) were inoculated using 100 viruliferous whiteflies per pot (Govindan et al. 2014). Data were recorded on the percentage of disease transmission and average disease

severity 60 days after inoculation following the rating scale as per Bag et al. (2014).

PCR confirmation of MYMIV

DNA from leaf samples of cowpea germplasm accessions (both field and artificial screening experiments) was extracted using the DNeasy Plant Mini Kit (Qiagen, Germany). PCR was performed in a 20 µL reaction volume containing 1µl of genomic DNA (~100 ng), 5 µL of 10X reaction buffer, 1.5 mM $MgCl_2$, 0.2 to 0.5 µM each MYMIV DNA specific primer pairs, 0.2 mM dNTPs and 10U of Taq DNA polymerase (Promega, USA) and finally water was added to make up the final volume (Roja et al. 1993). The thermal cycling profile consisted of an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 58°C and extension at 72°C for 1-minute, followed by the final extension at 72°C for 10 minutes. The PCR products were electrophoresed in a 2% agarose gel in 1X TAE buffer (pH 8.0) at 70 V. To estimate the amplicon size, 100 bp DNA molecular ladder was used (GBiosciences, USA) and electrophoresis was conducted for 1.5 h. The amplicons were observed under UVlamp in a gel-documentation system (Analytikjena).

Virus source and sequencing of viral genome

The cowpea germplasm accessions exhibiting typical YMD symptoms were collected from the experimental field at the Issapur farm area during *kharif* 2022 for molecular characterization of associated Begomovirus. Total genomic DNA from the leaf samples was extracted using DNeasy Plant Mini Kit (Qiagen, Germany). The full-length viral genome was amplified through rolling circle amplification (RCA) (Qiagen, Germany) (Packialakshmi et al. 2010). Aliquots of the RCA product were digested with different restriction endonucleases (*BamH*I, *Kpn*I, *EcoR*I, *Hind*III and *Sac*I) to identify single cutting enzymes that produce linear products of ~2.7kb nucleotide length. The full-length DNA A (produced using the restriction enzyme *BamH*I) and DNA B (produced using the restriction enzyme *Bgl*I) were gel purified (Gel extraction kit, Qiagen, Germany) and sequenced bi-directionally (Xcelris Genomics, India).

Rolling circle amplification

Rolling Circle Amplification (RCA) was employed to amplify the complete circular single-stranded DNA genome of Begomovirus associated with YMD of cowpea (Packialakshmi et al. 2010). The reaction mixture consisted of 500 ng of DNA, 4 µl of a 10x reaction buffer, 2 µL of exo-resistant random hexamer primers (ThermoFisher Scientific, USA), 3 µL of 10 mM dNTPs, and double-distilled water, culminating in a final volume of 24 µl. The mixture was initially denatured at 94°C for 5 minutes, followed by centrifugation. It was then allowed to cool down to room temperature for 5 minutes before the addition of 4 µL of pyrophosphatase and 1.2 µL of phi29 DNA polymerase. The reaction mixture was incubated at 30°C for 19 hours in a heating block and subsequently inactivated at 65°C for 10 minutes. After digestion with a specific restriction enzyme, the amplified RCA product was assessed on a 1% agarose gel.

A group of restriction enzymes were selected based on the restriction analysis of known begomovirus species. The RCA-amplified concatemers were cleaved using a predetermined set of restriction enzymes (*BamH*I, *Kpn*I, *Hind*III, *EcoR*I, *Sal*I). Following this, 3 µL of the RCA product, equivalent to 300 to 400 ng of DNA, was digested with 1-µL (10 U) of the designated restriction enzymes and was subsequently subjected to analysis on a 1% agarose gel.

Cloning of linearized RCA product, sequencing and sequence analysis

A linear genomic segment, produced through restriction enzyme digestion of the RCA products, was excised from the gel and purified using the Wizard SV Gel and PCR Clean-Up system (Promega, USA). The linear monomers, representing the complete begomovirus genome after specific restriction enzyme digestion, were then inserted into alkaline phosphatase-treated pUC18 vectors. These vectors were previously prepared using the same enzymes. The ligation reaction mixture was incubated overnight at 4°C, followed by transformation into competent cells of *E. coli*DH5α strain. Positive white colonies were subjected to plasmid isolation using the Gene JET Plasmid Miniprep Kit (Thermo Scientific, USA). The extracted plasmid was subsequently subjected to digestion using specific restriction enzymes to confirm the success of the cloning. Finally, positive clones were sequenced at the Sequencing Facility, South Campus, Delhi University, using M13 forward and reverse primers, followed by search for corresponding sequences through BLAST analysis in NCBI (http://ncbi.nlm.nih.gov/BLAST).

Sequence data were assembled and analyzed using Bioedit (V7.2) (Hall et al. 1999). The identity and homology of the sequences were first evaluated using the BLASTN program on NCBI. The full-length sequence of MYMIV DNA A of Delhi isolate (further referred to as the Delhi isolate) was deposited in the NCBI GenBank.

Per cent nucleotide identity and phylogenetic analysis

Pairwise percent nucleotide identity of DNA A of different ORFs was obtained using the software package SDTv1.2. The DNA A of Delhi isolate was compared with other reported DNA A sequences available in NCBI GenBank. DNA sequences were aligned using the Clustal algorithm of MEGA11 (Kumar et al. 2016). Phylogenetic inference was drawn on the matrices of aligned sequences with 1000 bootstrap replicates following the neighbor-joining phylogeny of MEGA11.

Results

Field screening for YMD

A set of 1127 accessions of cowpea genotypes were evaluated under field conditions during 2022 at two hotspot locations viz., Hyderabad during summer and New Delhi during *kharif* seasons. In the summer of 2022, the disease incidence was low at 34.8% with 807 accessions (71.60%) showing the symptoms of YMD. The grouping of accession based on disease reaction, along with the disease progress curve and coefficient of infection, was presented in (Table 1). However, disease incidence in *kharif* 2022 was higher at 40.3%, with 946 accessions (83.93%) exhibiting incidence of YMD. The range of AUDPC of the accessions within the resistant class was found to be lower (0-51) than those belonging to the susceptible class (16.5–358.5). The correlation between CI and AUDPC values was 0.78 ($P < 0.002$). The grouping of accession based on disease reaction, along with the disease progress curve and coefficient of infection, was provided in Table 2. The *kharif* season is the most favorable for the YMD incidence at the Issapur farm area (similar to Delhi conditions). Additionally, regarding the average percent plant damage due to YMD, susceptible KM-5 showed the highest percentage of disease incidence at 92.6 and 86% during the summer and *kharif* seasons, respectively. Significant differences were observed between the genotype and test and the results of the analysis of variance for individual locations. A total of 181 accessions out of 1127 showed no symptoms (severity and PDI = 0) of YMD in both locations and no disease was observed in the resistant check, while the susceptible checks showed a susceptible reaction (disease score of 3–4). The viral infection was further confirmed by PCR using begomovirus-specific primers, which showed the presence of the virus in symptomatic plants while being absent in asymptomatic ones (score 0) (Figs. 1 and 2).

Table 1. Grouping of cowpea germplasm accessions based on their reaction to yellow mosaic disease at Hyderabad location (preliminary screening)

Disease reaction	Total accessions	PDI range (Mean)[a]	CI range (Mean)[a]	AUDPC (Mean)[a]
Highly Resistant (HR)	321	0-12.5 (0.1)	0-3.1 (0.1)	0-51 (0.2)
Resistant (R)	69	9.1-35.7 (27.6)	4.2-8.9 (7.2)	1-153.5 (67.5)
Moderately Resistant (MR)	232	15.4-70 (39.7)	9.1-18.8 (13.9)	6-230.5 (69.5)
Moderately Resistant (MR)	332	20-100 (43.4)	19.2-38.5 (28.3)	6-288 (78)
Susceptible (S)	157	40-100 (58.3)	40-66.7 (52.1)	16.5-358.5 (86.2)
Highly Susceptible (HS)	16	75-100 (94.7)	75-100 (85.3)	26-358.5 (144.8)
Susceptible checks		22.2-100 (75.8)	8.3-100 (46.9)	2.5-100 (188)

PDI, Per cent disease incidence; CI, Co-efficient of infection; AUDPC, Area under disease progress curve; (Mean)[a], mean values of all the accessions in respective category given in parenthesis.

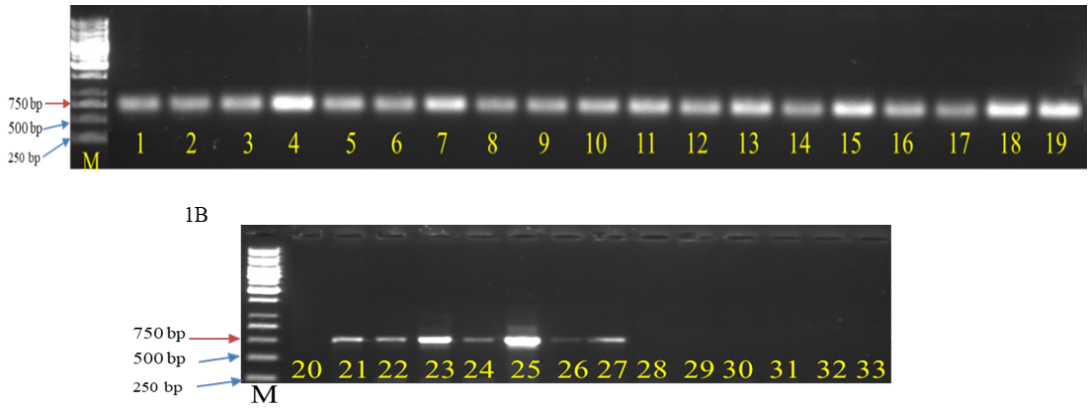

Fig. 1. PCR screening of suspected leaf samples collected from Hyderabad field using MYMIV DNA A specific primers
M-1kb marker, Lane 1: EC107123, Lane 2: EC241035A, Lane 3: EC472253, Lane 4: IC201082, Lane 5: IC419506, Lane 6: IC606582, Lane 7: EC107124, Lane 8: EC244144, Lane 9: IC202807, Lane 10: IC426811, Lane 11: IC606259, Lane 12: IC91430, Lane 13: EC240940, Lane 14: EC36571, Lane 15: IC140237, Lane 16: IC278672, Lane 17: IC519824, Lane 18: IC-91458, Lane 19: EC107181, Lane 20: PL-4 (R), Lane 21: PK (S1), Lane 22: C-152 (S2), Lane 23: KM-5(S3), Lane 24: EC243928, Lane 25: IC257446, Lane 26: IC446715, Lane 27: IC606360, Lane 28: EC-240858, Lane 29: EC724681, Lane 30: EC725110, Lane 31: IC402159, Lane 32: IC590842, Lane 33: IC97806 (Lane 20 and 28-33 – resistant)

Whitefly-mediated screening for YMD

The screening of 140 cowpea germplasm (100 resistant and 40 susceptible) using whitefly-mediated inoculation of MYMIV identified 20 accessions as highly resistant/immune (score of 0) and did not exhibit any symptoms up to 4 weeks post-inoculation. Fifteen were graded as highly resistant (score 1), 19 as resistant (score 2), 19 as moderately resistant (score 3), 40 as susceptible (score 4) and 27 were highly susceptible (score 5) (Fig. 3A). Area under disease progress curve (AUDPC) for whitefly transmitted cowpea germplasm accessions. The first disease symptom on susceptible genotypes started appearing as minute yellow spots after 10-11 days post-whitefly-mediated transmission. Most of these spots were yellow and green patches with irregular margins. Complete necrosis followed by chlorosis was observed after 9-10 days of disease symptom initiation and plants did not show any recovery. Screening with MYMIV-specific primers confirmed the absence of MYMIV in 20 accessions showing no symptoms (Figs. 4A and 4B, Table 3).

Genome characterization of mungbean yellow mosaic India virus-associated with YMD of cowpea

The prevalent Begomovirus associated with YMD in the hotspot location of North India (Delhi) was selected for characterization, as it was used for whitefly-mediated artificial screening. The full genome of the Begomovirus associated with YMD of cowpea under North Indian conditions (Delhi region) was amplified by RCA and linear concatemers restriction digested with *BamH*1 were cloned and sequences were obtained. The assembled complete genome sequence of DNA-A of characterized isolate (MYMIV-CP.DL.IND) showed highest similarity of 99.02% with mungbean yellow mosaic India virus (MYMIV) cowpea isolate from Pakistan (AY269990), followed by 98% similarity with MYMIV isolates samples from black gram and soybean in Pakistan. The maximum likelihood phylogenetic analysis confirmed the phylogenetic placement of MYMIV-CP.DL.IND in a distinct cluster along with the MYMIV isolates of cowpea, black gram and soybean reported from Pakistan (Fig. 5). This confirmed the predominant association of a distinct MYMIV isolate with the YMD of cowpea under North Indian conditions.

Discussion

Yellow mosaic disease caused by whitefly-transmitted begomoviruses such as MYMIV (prevalent in North India) and MYMY (prevalent in South India), poses a significant threat to cowpea cultivation. Despite its widespread occurrence in Indian cowpea belts, authentic sources of resistance have not been identified in the cowpea gene pool. In the present study, we undertook extensive evaluation of 1127 cowpea accessions available in the National Gene Bank of ICAR-NBPGR, New Delhi, to identify the sources of resistance, which is a major prerequisite for cowpea improvement programs.

YMD is prevalent in both North and South Indian belts; therefore, in the present study, we undertook screening of germplasm at both the hotspot locations. The results indicated the dynamic nature of YMD incidence in cowpea, emphasizing the need to continuously monitor and evaluate germplasm resistance. The present study identified a significant increase in YMD incidence from 34.8% to 40.3% at two locations with the *kharif* showing the highest YMD incidence. This observation suggests the prevalence season of YMD and the congenital conditions for disease spread under North Indian environmental conditions. Several previous studies in India have also documented the occurrence and severity of YMD in different regions. Kumar et al. (2018) conducted a survey in five districts of Rajasthan for yellow mosaic disease and identified differential disease severity and whitefly populations in different areas.

Earlier studies highlighted the significance of the robust field and artificial screening for the identification of sources of resistance for their effective utilization in breeding programmes. An extensive evaluation of 119 accessions, comprising 99 wild *Vigna* accessions from 19 different species, 18 cultivated genotypes of *Vigna*, and two *Phaseolus* genotypes, led to the identification of confirmed sources

Table 2. Grouping of cowpea germplasm accessions based on their reaction to yellow mosaic disease at Delhi location (preliminary screening)

Disease reaction	Total accessions	PDI range (Mean)[a]	CI range (Mean)[a]	AUDPC (Mean)[a]
Highly Resistant (HR)	184	0-15.4 (0.2)	0-3.8 (0.1)	0-51.5 (0.508)
Resistant (R)	80	9.1-35-7 (27.3)	4.2-8.9 (7.1)	0.5-153.5 (69.05)
Moderately Resistant (MR)	269	9.1-75 (39.4)	9.1-18.8 (13.9)	6-278.5 (75.78)
Moderately Susceptible (MS)	384	20-100 (44.6)	19.2-38.5 (28.5)	6-527 (85.86)
Susceptible (S)	190	40-100 (61.6)	40-66.7 (51.9)	16-493 (94.05)
Highly Susceptible (HS)	20	71.4-100 (91.3)	71.4-100 (83.8)	30-491.5 (148.7)
Susceptible checks		50-100 (83.9)	13-100 (60)	18.5-528.5 (234.6)

PDI = Percent disease incidence; CI = Co-efficient of infection; AUDPC = Area under disease progress curve; (Mean)[a] = Mean value of all the accessions in respective category given in parenthesis.

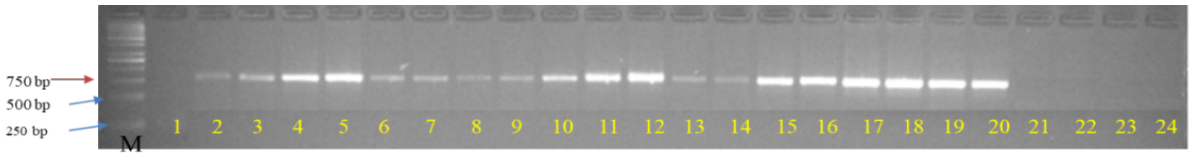

Fig. 2. PCR screening of suspected leaf samples collected from Delhi field using MYMV specific primers
M-1 kb marker, Lane 1: PL4 (R), Lane 2: PK (S1), Lane 3: C152(S2), Lane 4: KM5(S3), Lane 5: EC240911, Lane 6: EC724363, Lane 7: IC259107, Lane 8: IC625401, Lane 9: EC149430, Lane 10: EC724258, Lane 11: IC259093, Lane 12: IC91505, Lane 13: EC241020, Lane 14: IC201095, Lane 15: IC282031, Lane 16: IC610398, Lane 17: EC243987, Lane 18: IC209164, Lane 19: IC326999, Lane 20: IC-91474, Lane 21: EC107124, Lane 22: EC16973, Lane 23: EC240636, Lane 24: EC4232. (Lane 1 and 21-24 – resistant)

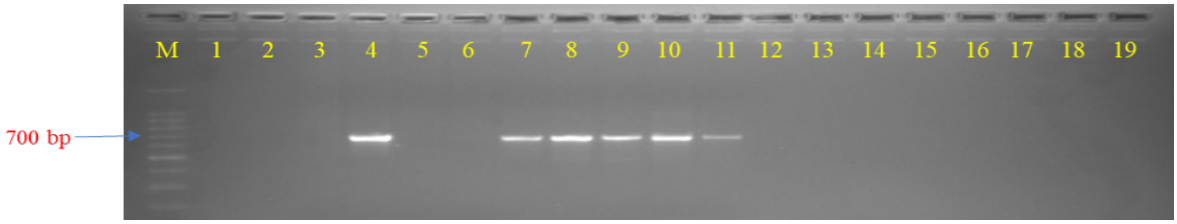

Fig. 3. PCR screening of suspected leaf samples collected from glass house using MYMIV DNA A specific primers
M-100bp marker, Lane 1: EC98661, Lane 2: EC107128. Lane 3: EC109493-1047-1, Lane 4: IC202803, Lane 5: EC149242-A, Lane 6: EC240636, Lane 7: IC214836, Lane 8: IC397252, lane 9: S1, Lane 10: S2, Lane 11: S3, Lane 12: R, Lane 13: EC244059, Lane 14: EC244063, Lane 15: IC326793, Lane 16: IC329764, Lane 17: IC338877, Lane 18: IC412901, Lane 19: IC426824

Table 3. Whitefly transmission of MYMIV in selected field-resistant accessions of cowpea germplasm

Severity	Disease response category	Number of accessions*
0	Immune (I)	20 (14.3)
1	Highly Resistant (HR)	15 (10.7)
2	Resistant (R)	19 (13.6)
3	Tolerant (T)	19 (13.6)
4	Susceptible (S)	40 (28.6)
5	Highly Susceptible (HS)	27 (19.3)

*Value in parenthesis indicates percentages.

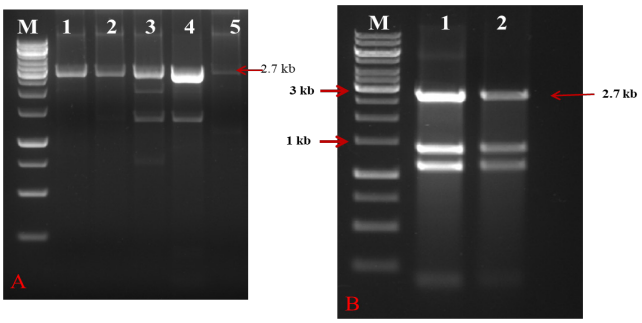

Fig. 4. Cloning of MYMIV DNA A by RCA. (A). RCA of YMD infected cowpea, followed by restriction digestion and cloning: Lane M: 1 kb marker, lane 1: BamHI, lane 2: KpnI, lane 3: EcoRI, lane 4: HindIII, lane 5: SacI. (B). Verification of clones using restriction digestion. Restriction digestion with BamHI and BglI. Lane M: 1Kb marker, lane 1: colony 1, lane 2: colony 2

Table 4. Contrasting genotypes for disease susceptibility

Resistant accessions (acc.)		Highly susceptible (acc.)
EC98661	IC412901	EC107145
EC107128	IC426824	EC241004
EC109493-1047-1	IC437181	EC243999
EC149242-A	IC569092	EC4381
EC240636	IC257829	EC97167
EC244059	IC605356	IC342702
EC244063	IC278035	IC397252
IC326793	IC311929	IC39905
IC329764	IC331708	IC45415
IC338877	IC334368	IC471926

of resistance and their use in the breeding programmes (Kumari et al. 2021b). The present study highlighted that in the *kharif* season, when YMD incidence is highest, it coincides with the cultivation of other legumes such as mungbean, urdbean, soybean, and pigeonpea, which are also susceptible to YMD. This co-cultivation may have contributed to increased inoculum build-up and, consequently, higher disease incidence. Thus, this season can be considered ideal for YMD field screening and research on disease management strategies.

Artificial screening using whitefly-mediated methods has commonly been used to assess germplasm resistance against begomoviruses (Akhtar et al. 2011). This approach provides valuable insights into the resistance mechanisms and potential sources of resistance within the germplasm. Based on the combined score of YMD disease at both the hotspot locations, 181 accessions with no visible disease symptoms (disease score and PDI of 0) were putatively

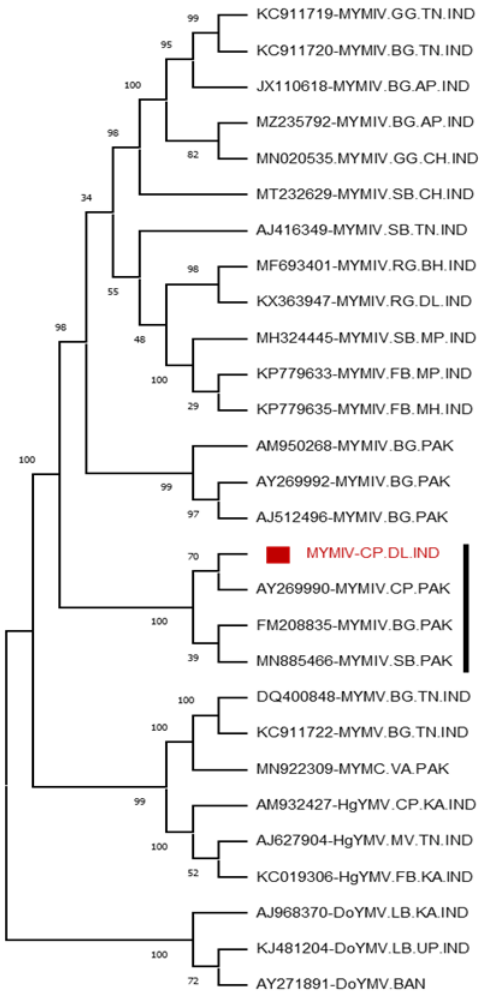

Fig. 5. The phylogenetic analysis of MYMIV DNA with other MYMIV isolates

resistant. In the present study, we undertook the artificial screening of selected genotypes against the MYMIV prevalent in North India conditions. The artificial screening for the MYMIV using whitefly-mediated inoculation of 140 cowpea germplasm genotypes further enriched the understanding of resistance. Based on the combined screening of field and whitefly-mediated screening, 20 accessions were identified as confirmed sources of resistance to MYMIV with score of 0 (Table 3). Contrastingly, 10 accessions displayed high susceptibility with a disease severity score of 4 (Table 3). The genotypes sorted into highly susceptible and highly resistant categories can be utilized as parents for studying heritability and the nature of genes involved in controlling the resistance to YMD. Once the markers linked to the trait are confirmed, they could be employed in marker-assisted backcross breeding, facilitating introgression breeding to develop improved varieties resistant to MYMIV.

The genotypes have been categorized based on the differential response of hosts to MYMIV through whitefly transmission in selected field-resistant accessions of cowpea germplasm (Table 4). The temporal aspect of symptom appearance and progression was noteworthy, with susceptible genotypes exhibiting symptoms relatively early after transmission, followed by rapid necrosis and chlorosis. This cascade of symptoms is a characteristic feature of begomovirus infections. Importantly, the presence of MYMIV-specific amplification in both field-symptomatic genotypes and artificially inoculated genotypes adds another layer of confirmation to the viral infection status. This conclusion is supported by several prior studies (Chaithanya et al. 2020; Deepa et al. 2019; Tsai et al. 2013) and confirms the viral infection, thereby supporting the reliability of field observations.

The consistency between field observations and controlled inoculation experiments provides a more comprehensive understanding of cowpea-MYMIV interactions. The molecular characterization of MYMIV in this study aligns with previous research efforts. The present study also characterized the isolate of Begomovirus prevalent in Delhi and confirmed the previous findings of its presence and prevalence in Pakistan (Ilyas et al. 2010).This suggests that the predominant MYMIV isolate might have been distributed over a large geographical area and spread by whitefly vectors. Genetic diversity studies are essential for monitoring the evolution of Begomovirus and developing effective strategies for their management. The present study motivates comprehensive insights into the dynamics of YMD incidence in cowpea emphasizing the importance of continuous monitoring and the identification of potential sources of resistance. The combination of field observations and controlled inoculation experiments enhances the understanding of cowpea-MYMIV interactions and lays a foundation for future research, particularly in gene mapping and resistance breeding. These findings will facilitate the development of effective management strategies and the selection of resistant genotypes for sustainable cowpea cultivation.

Authors' contribution

Conceptualization of research (VCC, KT, SKS, VM); Designing of the experiments (KT, AG, DPW); Contribution of experimental materials (KT, SKS, NG, SKA); Execution of field/ lab experiments and data collection (KT, KK, BP, DD, DDD, DT); Analysis of data and interpretation (SKS, KK, DD, DT); Preparation of the manuscript (GJA, SKS, VCC, KK).

References

Akhtar K.P., Kitsanachandee R., Srinives P., Abbas G., Asghar M.J., Shah T.M., Atta B.M., Chatchawankanphanich O., Sarwar G., Ahmad M. and Sarwar N. 2009. Field evaluation of mungbean recombinant inbred lines against mungbean yellow mosaic disease using new disease scale in Thailand. Plant Pathol. J., **25**(4): 422-428.

Akhtar K.P., Sarwar G., Abbas G., Asghar M.J., Sarwar N. and Shah T.M. 2011. Screening of mungbean germplasm against

Mungbean yellow mosaic India virus and its vector *Bemisia tabaci*. Crop Prot., **30**: 1202-1209.

Bag M.K., Gautam, N.K., Prasad, T.V., Pandey, S., Dutta, M. and Roy, A. 2014. Evaluation of an Indian collection of black gram germplasm and identification of resistance sources to Mungbean yellow mosaic virus. Crop Prot., **61**: 92-101.

Briddon, R.W. and Markham, P.G. 2000. Cotton leaf curl virus disease. Virus Res., **71**(1-2): 151-159.

Byoung-Cheorl K., Inhwa Y. and Molly M. J. 2005. Genetics of Plant Virus Resistance. Annu. Rev. Phytopathol., **43**: 581–621.

Campbell C. L. and Madden L.V. 1990. Introduction to Plant Disease Epidemiology. Wiley-Interscience, New York.

Deepa H., Govindappa M.R., Naganur P. and Shankarappa K.S. 2019. Detection of Mungbean yellow mosaic virus in greengram through Rolling Circle Amplification. J. Exp. Zool., **22**(1): 425-428.

Govindan K., Nagarajan P. and Angappan K. 2014. Molecular studies on transmission of mung bean yellow mosaic virus (MYMV) by *Bemisia tabaci* Genn. in mungbean. Afr J. Agril. Res., **9**(38): 2874–2879. doi: 10.5897/AJAR2013.7237

Habib S., Shad N., Javaid A. and Iqbal U. 2007. Screening of mungbean germplasm for resistance/tolerance against yellow mosaic disease. Mycopath., **5**(2): 89–94

Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acid S. 1999; **41**: 95-98.

Hanley-Bowdoin L., Bejarano E.R., Robertson D. and Mansoor S. 2013. Geminiviruses: masters at redirecting and reprogramming plant processes. Nat. Rev. Microbiol., **11**(11): 777-788.

Hema M., Sreenivasulu P., Patil B.L., Kumar P.L. and Reddy D.V. 2014. Tropical food legumes: virus diseases of economic importance and their control. Adv. Virus Res., **90**: 431-505. Academic Press.

Ilyas M., Qazi J. Mansoor S. and Briddon R.W. 2010. Genetic diversity and phylogeography of begomoviruses infecting legumes. Pakistan. J. Gen. Virol., **91**(8): 2091-2101.

John P., Sivalingam P.N., Haq Q.M.I., Kumar N. and Mishra A. 2008. Cowpea golden mosaic disease in Gujarat is caused by a Mungbean yellow mosaic India virus isolate with a DNA-BDNA-B variant. Arch. Virol., **153**: 1359–1365.

Kareem K.T. and Taiwo M.A. 2007. Interactions of viruses in cowpea: effects on growth and yield parameters. Virol. J., **4**: 15. https://doi.org/10.1186/1743-422X-4-15 PMID: 17286870

Kumar S., Stecher G. and Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol., **33**(7): 1870-1874

Langyintuo A.S., Lowenberg-DeBoer J., Faye M., Lambert D and Ibro G. 2003. Cowpea supply and demand in West and Central Africa. Field Crops Res., **82**: 215–231.

Malathi V. C. and John P. 2009. Mungbean yellow mosaic viruses. In: Desk encyclopaedia of plant and fungal virology. (EdS. Van M. Regenmortal and B. Mahy). London: Academic press, 217–226.

Malathi V.G., Surendranath B., Naghma A. and Roy A. 2005. Adaptation to new hosts shown by the cloned components of Mungbean yellow mosaic India virus causing cowpea golden mosaic in northern India. Can. J. Plant Pathol., **27**: 439–447.

Malathi V.G. and John P. 2008. Mungbean Yellow Mosaic Viruses, Desk Encyclopedia of Plant and Fungal Virology in Encyclopaedia of Virology. Elsevier Ltd, Amsterdam, Netherlands: 364-71.

Naimuddin M.A. and Singh N.P. 2016. Yellow mosaic of mungbean and urdbean: Current status and future strategies. J. Food Legumes, **29**: 77-93.

Nair R.M., Götz M., Winter S., Giri R.R., Boddepalli V.N., Sirari A., Bains T.S., Taggar G.K., Dikshit H.K., Aski M. and Boopathi M. 2017. Identification of mungbean lines with tolerance or resistance to yellow mosaic in fields in India where different begomovirus species and different *Bemisia tabaci* cryptic species predominate. Eur. J. Plant Pathol., **149**(2): 349–365. doi: 10.1007/s10658-017-1187-8

Nariani T.K. 1960. Yellow mosaic of Mung (*Phaseolus aureus* L.). Indian Phytopathol., **13**: 24-29.

Nene Y.L., Srivatsava S.K. and Naresh. J.S. 1972. Evaluation of black gram green gram varieties and germplasm for resistance to yellow mosaic virus of green gram. Indian J. agric. Sci., **42**: 251-254.

Omomowo O.I. and Babalola O.O. 2021. Constraints and prospects of improving cowpea productivity to ensure food, nutritional security and environmental sustainability. Front. Plant Sci., **12**: 751-731.

Qazi J., Ilyas M., Mansoor S. and Briddon R.W. 2007. Legume yellow mosaic viruses: genetically isolated begomoviruses. Mol. Plant Pathol., **8**(4): 343-348.

Rojas Maria R. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis., **77**(4): 340.

Rouhibakhsh A. and Malathi V.G. 2005. 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.

Sai C.B., Nagarajan P., Raveendran M., Rabindran R., Kannan Bapu J.R. and Senthil N. 2017. Understanding the inheritance of mungbean yellow mosaic virus (MYMV) resistance in mungbean (*Vigna radiata* L. Wilczek). Mol. Breed., **37**: 1-15. doi: 10.1007/s11032-017-0650-8

Shivaprasad P.V., Thillaichidambaram P., Balaji V. and Veluthambi K. 2006. Expression of full-length and truncated Rep genes from Mungbean Yellow Mosaic Virus-Vigna inhibits viral replication in transgenic tobacco. Virus Genes, **33**: 365–374. doi: 10.1007/s11262-006-0077-5

Singh B.B., Ehlers J.D., Sharma B. and Freire Filho F.R. 2002. Recent progress in cowpea breeding. In: Proc. World Cowpea Conference, III. Challenges and opportunities for enhancing sustainable cowpea production (Eds. C.A. Fatokun, S.A. Tarawali, B.B. Singh, P.M. Kormawa and M. Tamo). International Institute of Tropical Agriculture (IITA), Ibadan: 22-40.

Surendranath B., Usharani K.S., Nagma A., Victoria A.K. and Malathi V.G. 2005. Absence of interaction of genomic components and complementation between Mungbean yellow mosaic India virus isolates in cowpea. Arch. Virol., **150**(9): 1833–1844. https://doi.org/10.1007/s00705-005-0542-9 PMID: 15931466

Thottappilly G. and Rossel H.W. 1992. Virus diseases of cowpea in tropical Africa. Int. J. Pest Manag., **38**: 337–348.

Varma A. and Malathi V.G. 2003. Emerging geminivirus problems: a serious threat to crop production. Ann. Appl. Biol., **142**: 145–164.

Varma A. and Reddy D.R. 1984. Golden and green mosaics-two new diseases of cowpea in Northern India. Indian Phytopathol., **37**: 409.