3 Biotech (2023) 13:194
https://doi.org/10.1007/s13205-023-03610-2

ORIGINAL ARTICLE

Field and agroinoculation screening of national collection of urd bean (*Vigna mungo*) germplasm accessions for new sources of *mung bean yellow mosaic virus* (MYMV) resistance

V. Bindu Prathyusha[1] · E. Swathi[2] · D. Divya[1] · B. V. Bhaskar Reddy[3] · J. S. Bentur[1] · V. Celia Chalam[4] · D. P. Wankhede[4] · Kuldeep Singh[4,5] · K. Anitha[2]

Received: 6 April 2023 / Accepted: 3 May 2023 / Published online: 16 May 2023

Abstract

Yellow mosaic disease (YMD) is a major problem in Urd bean (*Vigna mungo* L.) in India, which causes huge yield losses. Breeding for wide spectrum and durable *Mungbean yellow mosaic virus* (MYMV) resistance and cultivating resistant cultivars is the most appropriate and effective approach. However, the task has become challenging with the report of at least two species of the virus, viz., *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV) and their recombinants; the existence of various isolates of these species with varied virulence and rapid mutations noted in the virus as well as in the whitefly vector population. Thus the present study was carried out to identify and characterize novel and diverse sources of YMV resistance and develop linked molecular markers for breeding durable and broadspectrum resistant urdbean cultivars against YMV. Towards this goal, we have screened 998 accessions of urdbean national collection of germplasm against YMD Hyderabad isolate both in a field under the natural level of disease incidence and through agro inoculation in the laboratory using viruliferous clones of the same isolate. Ten highly resistant accessions identified through repeated testing have been characterized in terms of reported linked markers. We attempted to see diversity among the ten resistant accessions reported here using earlier reported resistance-linked SCAR marker YMV1 and SSR CEDG180 marker. SCAR marker YMV1 did not amplify with any of the 10 accessions. But with CEDG180, results suggested that 10 accessions shortlisted through field and laboratory tests do not carry PU31 allele and this shows that it may be likely to carry novel gene(s). Further studies are needed to genetically characterize these new sources.

Keywords Blackgram · *Begomovirus* · Tolerance · Agroinoculation · Germplasm · Resistance

Introduction

Urdbean (*Vigna mungo* (L) Hepper), along with mungbean (*Vigna radiata* (L.) R. Wilczek), occupies third most important dietary legume position to provide protein requirement for most Indians (Gupta et al. 2021). During the year 2020–21, urdbean was grown in 4.70 million hectare area with a total production of 2.682 million tonnes at an average productivity of 618 kg/ha in India (Singh and Srivastava 2021). Relative low productivity noted above is mainly due to prevailing biotic stresses such as weeds, pests and diseases (NAAS 2016). Most important among these is the yellow mosaic disease (YMD) caused by the two types of Yellow mosaic viruses (YMV): *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV), transmitted by the whitefly, *Bemisia tabaci* (Genn.) (Naimuddin et al. 2016). Once injected into the

✉ K. Anitha
Kodaru.Anitha@icar.gov.in

1 Agri Biotech Foundation, Rajendranagar, Hyderabad, TG 500 030, India

2 ICAR-National Bureau of Plant Genetic Resources, Regional Station, Hyderabad, TG 500 030, India

3 Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Tirupati, AP 517 502, India

4 ICAR-National Bureau of Plant Genetic Resources, PUSA Campus, New Delhi 110 012, India

5 Present Address: Head, Gene Bank, ICRISAT, Patancheru, TG 502324, India

plant tissue, the virus multiplies rapidly causing yellow patches on the young leaves. These patches coalesce to turn the entire leaf yellow. As more and more leaves start turning yellow, affected plant loses its photosynthetic ability resulting in poor growth, reduced flowering and poor pod and seed formation (Kundu et al. 2022). Crop yield losses can be very high based on the time of disease incidence and its quick spread (Varma and Malathi 2003; Obaiah et al. 2013; Gupta et al. 2015). Managing the disease through vector control using insecticides is ineffective since even a very small population of whiteflies can also cause severe disease outbreak (Gilbertson et al. 2011). Cultural management through the selection of an appropriate time and season of sowing that would avoid disease build-up is effective but not totally reliable. Breeding for MYMV resistance and cultivating resistant cultivars is the most appropriate approach and was proven very effective (Younas et al. 2021).

Earlier efforts of breeding MYMV-resistant cultivars were based on field-identified resistance source and through the classical pedigree method of breeding (Gupta et al. 2021). However, the task of breeding urdbean varieties with wide spectrum and durable resistance has become challenging with the report of at least two species of the virus, viz., MYMV and MYMIV and their recombinants; the existence of various isolates of these species with varied virulence and rapid mutations noted in the virus as well as in the whitefly vector population. On the other hand, the development of the agroinoculation method for screening genotypes for virus resistance; the availability of molecular tools for the characterization of the virus and marker-assisted selection breeding has made the task achievable. There have been many recent reports of screening urdbean genotypes for resistance to YMV based on field screening under natural disease incidence levels (Iqbal et al. 2011; Obaiah et al. 2013; Mohan et al. 2014; Subedi et al. 2016; Bandi et al. 2018; Pavishna et al. 2019; Tamilzharasi et al. 2020) and through agroinoculation (Biswas and Varma 2012; Chaitanya et al. 2022). Biswas and Varma (2001) reported screening of genotypes against different variants of the virus both in field and greenhouse conditions. Bag et al. (2014) reported screening of 344 accessions of black gram germplasm from the National collection in three fields and one greenhouse tests. Relatively less effort is made in the genetic characterization of new sources of resistance (Sandhu et al. 1985; Khattak et al. 2000; Ammavasai et al. 2004; Dhole and Reddy 2012; Gupta et al. 2013). These studies suggest the involvement of major genes either dominant or recessive in nature. Despite these efforts, resistance genes have not been clearly designated and their allelic relationship was not worked out clearly (Verma and Singh 1986). Efforts to develop molecular markers linked to resistance gene helped in identifying closely linked markers like a SCAR marker YMV1 based on the ISSR811 amplified product sequence (Souframanien and Gopalkrishna 2006); another SCAR marker SCAR20 based on RAPD marker OPQ1 amplified product (Prasanthi et al. 2013); third SCAR marker based on RAPD marker OPB-07 linked to a recessive resistance gene in mungbean (Dhole and Reddy 2012) and SSR marker CEDG180 on linkage group 10 linked to a dominant resistance gene (Gupta et al. 2013; Vadivel et al. 2021; Subramaniyan et al. 2022). Recently, Satheesh et al. (2022) reported three SSR markers: CEDG141, CEDG008, CEDG264 to be linked to the recessive resistance gene in the genotype KKB14045. Based on these markers, at least two distinctly different groups of resistance sources have been recognised (Gupta et al. 2015). Mechanism of virus resistance has been investigated in urdbean (Maheswari et al. 2014; Ganguli et al. 2016; Jasrotia et al. 2017) and mungbean (Dasgupta et al. 2021). Though a generalized picture of the interaction of defense genes and their pathways is elucidated, the exact role of the R gene in the induction of defense is neither understood nor cloned so far.

It is thus imperative from the foregoing account that novel and diverse sources of YMV resistance need to be identified and characterized and linked molecular markers need to be developed for breeding durable and broad-spectrum resistant urdbean cultivars against YMV. Towards this goal, we have screened 998 accessions of urdbean national collection of germplasm against YMD Hyderabad isolate both in the field under the natural level of disease incidence and through agro inoculation in the laboratory using viruliferous clones of the same isolate. Ten highly resistant accessions identified through repeated testing have been characterized in terms of reported linked markers.

Materials and methods

Source of germplasm

A total of 998 accessions of urdbean germplasm was chosen from the germplasm pool available in the National Gene Bank, Indian Council of Agriculture (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 urdbean is grown. In addition, a core collection set consisting of 202 accessions was also included for evaluation in 2022. Two popular urdbean cultivars viz., PU31 (Resistant) and LBG685 (highly susceptible), were included as the checks. Besides, some of the accessions showing high levels of damage during 2020 and 2021 were also included during 2022 testing as additional susceptible checks.

Field evaluation

The germplasm evaluation trials were conducted on the experimental research farm of NBPGR, Rajendranagar, Hyderabad, during the summer season (Feb to April) of 2020, 2021 and 2022. In the preliminary screening during 2020, 998 germplasm accessions were evaluated against the virus following augmented block design (ABD) and those showing nil damage were further evaluated in the field during 2021 ($n=450$) and 2022 ($n=294$) along with suitable Resistant (R) and Susceptible (S) checks (Table1). Germplasm test accessions and checks were sown in 3 m rows with a 30 cm distance between rows and 15 cm between plants within rows. The crop was raised under recommended agronomic practices, except for insecticide application. To guarantee a uniform distribution of viral disease pressure across the experimental field, the infector row approach (Nene et al. 1972) was used. Susceptible variety (LBG685) was used as 'infector row' and the resistant variety (PU31) was maintained among the accessions as a resistant check. Further, the susceptible check was sown after every ten rows of the test germplasm accessions. Susceptible checks were also grown at the edges of full trial plots to provide the vector with a suitable virus source. Entries were scored for YMD incidence at weekly interval 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 1–9 standard arbitrary scale (Alice and Nadarajan 2007) and in agro inoculated plants the severity of disease incidence was scored on a 0–9 standard scale (Sudha et al. 2013) and also on percent plant damage (i.e., number of infected plants over total number of plants X 100). Highest damage score in time repeated observation was considered. Entries recording nil damage were retested during the subsequent test.

Table 1 Summary of Field Screening of urdbean germplasm accessions during 2020 through 2022

	2020	2021	2022
Total entries screened	998	450	294
Entries without YMV symptoms (R)	794	202	101
Entries with YMV symptoms (S)	200	248	192
Entries with < 20% YMV symptoms	168	107	36
Entries with > 20% YMV symptoms	32	141	156
Nil germination entries	4	0	1
Average plant damage (%) in S group in this test (mean ± SE)	3.0 ± 0.3	20.8 ± 1.43	47.2 ± 2.67

*Means in the row are significantly different (paired *t* test, $P<0.05$)

Source of virus isolate

YMD-infected leaf samples were collected from Rajendranagar, Hyderabad area during 2017–2018 and used to construct infectious full-length dimer clones for the Hyderabad-specific YMV isolate (Prathyusha et al. unpublished). This clone was used to screen the urdbean germplasm against YMV using the agro inocualtion approach in the laboratory. During field screening, infected leaf samples were collected periodically to validate prevailing virus isolate with our clone-specific primers.

Agroinoculation screening in laboratory

Construction of infectious full-length dimer clones of *Yellow mosaic virus* Urdbean genomic DNA obtained from YMV-infected samples from the Hyderabad region was used to isolate and clone virus DNA-A and DNA-B at the Institute of Frontier Technology, Acharya NG Ranga Agricultural University, Tirupati, Andhra Pradesh following the prescribed protocols (Chaitanya et al. 2022). Virus DNA was subjected to rolling circle amplification (RCA). Partial restriction digestion (*BamHI*) products of multimeric viral genomes synthesised by RCA with phi 29 DNA polymerase were used to construct viral dimers. In the previous study, the infectious viral dimer clone assembly was characterized to contain MYMIV-HYD-A based on amplification with full-length primers specific for MYMIV-A (Chattopadhyay et al. 2013) and for 1000 bp fragment specific to MYMV-A (Naimuddin et al. 2016) and MYMV-HYD-B based on primers specific to a 900 bp fragment of MYMV-B (Naimuddin et al. 2011) and primers specific to 2.7 Kb fragment of—MYMIV- B (Prathyusha et al. unpublished). After screening approximately 110 combinations of clones, a viruliferous combination was identified. These clones were used to screen urdbean accessions against YMV in the laboratory.

Artificial screening through agroinoculation

Full-length dimer clones were mobilized from *Escherichia coli*, strain *DH5α* to *Agrobacterium tumefaciens* strain *EHA105* using pCAMBIA 2301 as a helper plasmid in a triparental mating system with antibiotic selection markers of Rifampicin (20 mg/ml) and Kanamycin (50 mg/ml). *EHA105* cells containing either DNA-A or DNA-B constructs were grown on the media to an OD_{600} of 0.8 to 1.0 and then combined in equal proportions. Low-speed centrifugation (5000 rpm) was employed to extract bacterial cells, which were then resuspended in a small volume of Luria Bertani broth with 100um Acetosyringone and utilised for

inoculation. Agroinoculation was done through the sprouted seed method (Mandal et al. 1997).

Seeds from urdbean accessions along with those from PU31 (resistant check) and LBG685 (susceptible check) were surface sterilised and soaked in sterile water for 2–3 h before keeping at 37 ℃ overnight for germination. The seed coat of sprouted seeds was removed and pinpricked around the hypocotyl region with a fine syringe needle, and the seeds were immediately immersed in Agrobacterium cells containing an equal volume of both MYMIV DNA-A and MYMV DNA-B and seeds were incubated in agrobacterium culture for 2–3 h and then washed and sown in protrays with soil. Agroinoculated plants were maintained under controlled condition at 28 ℃ temperature and 80–90% relative humidity in a separate chamber. Symptom expression was monitored at regular intervals till 30 days after inoculation. For each test accession, 20–25 seeds were used for inoculation. Since some of the seeds failed to germinate further, the number of surviving seedlings at the time of scoring was considered as total plants tested. Only those tests with a minimum of ten plants were considered valid. Entries, showing nil damage or low damage (< 10%) were retested at least three times for validation of the reaction. In addition, entries recording nil damage in any of the three field tests were tested in three separate replications over time. Only those entries that recorded nil damage in three replications and in field tests were considered as resistant to YMV. The presence of the virus in the infected plants randomly selected was confirmed by PCR using virus gene-specific primers as mentioned above.

Molecular characterization of R lines

Ten accessions of urdbean germplasm that were confirmed with YMV resistance were tested for the presence of YMV following agroinoculation in symptom-free plants to know if a low titre of the virus could be detected in the plants. These lines were also tested with earlier reported MYMV resistance-linked markers CEDG180 (Gupta et al. 2013) and YMV1 (Gupta et al. 2015) to know if any of the accessions carried the reported gene/locus for resistance.

Quantification of viral load in the YMV infected plants

To quantify the viral DNA concentration in the YMV-infected plants, DNA was extracted from leaf samples collected from ten YMV-resistant germplasm accessions from field and agro-inoculated checks (PU31 (R) and LBG685 (S)) and a line ABFBG03 derived from the cross LBG685 X PU31. ABFBG03 is used as an additional check for viral load detection through PCR. PCR conditions were optimized at 25, 30 and 35 cycles to check the amplification of viral DNA using virus coat protein-specific marker.

Statistical analysis

Statistical analysis was performed with Microsoft Excel. Each numeric data point was calculated from at least three independent measurements and is represented as Mean ± S.E

Results

Field evaluation

In all, 998 accessions of urdbean genotypes were evaluated under field conditions during 2020 through mid-2022 (Table 1). These included about 998 accessions received during 2020 and 200 accessions of the core set received in 2021 from the National Gene Bank, NBPGR, New Delhi. During Summer 2020, the disease incidence was very low and only 27% of the accessions showed the incidence of yellow mosaic disease (YMD). However, during 2021 and 2022, disease incidence was 55% and 73%, respectively (Fig. 1). The summer season 2022 has been noted to be the most favourable for the YMD incidence in the Hyderabad

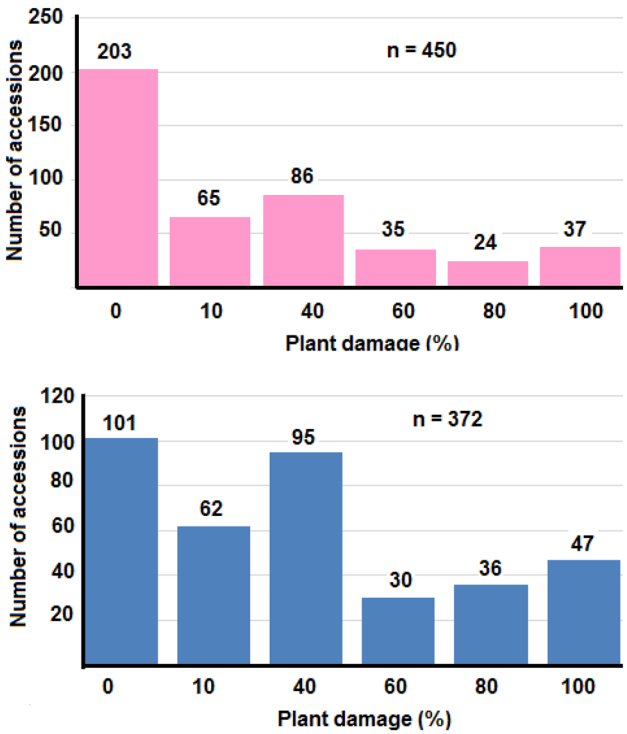

Fig. 1 Distribution of urdbean germplasm accessions under different levels of plant damage due to yellow mosaic disease in field screening during 2021 (top) and 2022 (bottom) on NBPGR, Hyderabad experimental farm

region. Also, in terms of average percent plant damage due to YMD in pre-identified S (Susceptible) group of entries during these two years was 20.8 ± 1.43 and 47.2 ± 2.67, respectively (Table 1). Latter was significantly higher than the former ($P < 0.05$). Thus, natural level of disease incidence was higher during 2022 than during 2021. Further, disease incidence levels, though moderate, were uniform across the experimental plots during both years (Fig. 2). Higher levels of disease were seen along the borders of the plot relative to the centre. In all, 26 accessions displayed nil damage during both years of testing. Besides, seven more accessions with nil damage during 2021 displayed less than 10% plant damage during 2022. These entries were further tested in the laboratory under agroinoculation.

Laboratory evaluation

During the period 2020 through mid-2022, a total of 1034 lines of urdbean germplasm accessions, including retesting lines, were evaluated in the laboratory through agro inoculation of the cloned Hyderabad isolate of the MYMV (Table 2). In preliminary tests, 813 accessions were evaluated along with the resistant (PU31) and susceptible (LBG685) checks. Of these, 439 tests could be considered valid with at least ten inoculated plants at the time of scoring. About 45% of these accessions displayed nil disease symptoms. All such accessions were retested in at least two more tests during different months. Only 31 of the accessions displayed nil plant damage in all three tests.

Table 2 Summary of lab Screening (agroinoculation) of urdbean germplasm accessions during 2020 through mid-2022

No. of inoculated plants at the time of scoring	>0	≥5	≥10	Replicated tests
Total entries screened	813	657	439	199
No. with nil damage	427	310	199	31
No. with 1–10% DP	49	49	49	5
No. with 11–40% DP	198	188	123	6
No. with 41–60% DP	63	58	36	2
No. with 61–80% DP	45	37	22	0
No. with 81–100% DP	31	15	10	0

Combined evaluation

Based on both the field and laboratory tests, 15 accessions of urdbean displayed consistent resistance reaction against MYMV in two field and three laboratory tests out of which 10 accessions were selected for molecular characterization (Table 3). Further, 17 entries displayed resistance to MYMV in 2021 field test and three lab tests. Another three entries had low plant damage of 10% or less. In addition, five more accessions displayed resistance in one field (2021) and three lab tests. Another set of 10 accessions recorded resistance against MYMV in one field test (2022) and one laboratory test. Performance of these entries is being confirmed with more laboratory tests.

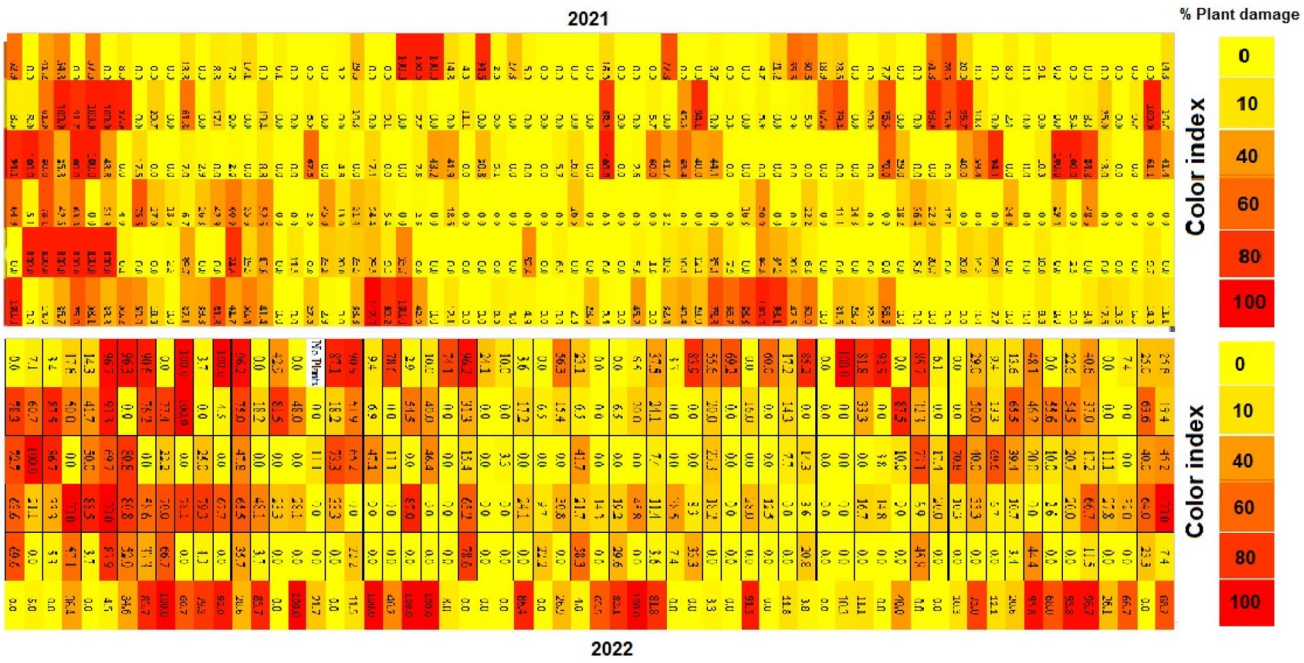

Fig. 2 Severity of yellow mosaic disease across experimental plot during 2021 (upper) and 2022 (lower) field screening of urdbean germplasm accessions on NBPGR, Hyderabad experimental farm

Table 3 List of urdbean germplasm accessions showing consistent resistance to MYMV in field and lab tests

S. No.	BG # (alternate id of the accession)	IC# Accession number	Field reaction (DP/TP)		Lab reaction DP/TP (Tests)
			2021	2022	
1	BG 1114	IC0472004	0/35	0/30	0/48 (3)
2	BG 1115	IC0485454	0/38	0/27	0/49 (3)
3	BG 1140	IC0485525	0/42	0/24	0/50 (3)
4	BG 1173	IC0485497	0/38	0/26	0/58 (3)
5	BG 1174	IC0485498	0/45	0/28	0/45 (3)
6	BG 1175	IC0485500	0/47	0/28	0/45 (3)
7	BG 1176	IC0485501	0/42	0/26	0/46 (3)
8	BG 1192	IC0421950	0/42	0/30	0/45 (3)
9	BG1198	IC0485410	0/29	4/18	0/48 (3)
10	BG 1200	IC0485414	0/35	0/23	0/52 (3)
	Checks				
	PU31	R Check	NT	0/182 (6)	0/134(3)
	IC0257489	R. Check		15/153 (6)	–
	IC0424616	S. Check		148/148 (6)	–
	LBG685	S. Check			119/141(3)
	IC0335131	S. Check			52/55(3)

DP Damaged Plants, *TP* Total plants, *NT* Not tested

Molecular characterization of the prevalent virus isolates

Infected leaf samples of urdbean genotypes collected during 2017–18 from the experimental fields in Rajendranagar, Hyderabad were the source for isolating, cloning and developing viruliferous agrobacterium strain. This virus isolate along with those isolated from field-infected leaf samples collected from the field during 2021–22 were characterized using MYMV and MYMIV0-specific primers (Naimuddin et al. 2016) to know the prevailing virus species in this area. Results (Fig. 3) suggested that the virus is a recombinant form of both MYMIV and MYMV. The samples of the virus from both agro inoculated infected leaf and field infect leaf amplified MYMIV DNA-A and MYMIV DNA-B and also MYMV DNA-B. These samples did not amplify MYMV DNA-A. Further, the test also confirmed that the field prevailing virus isolate and the one used for agro inoculation were the same all through the experimental duration.

Quantification of viral load using PCR

Semi-quantitative PCR assay was performed using a virus coat protein marker to analyze in which cycle of the PCR

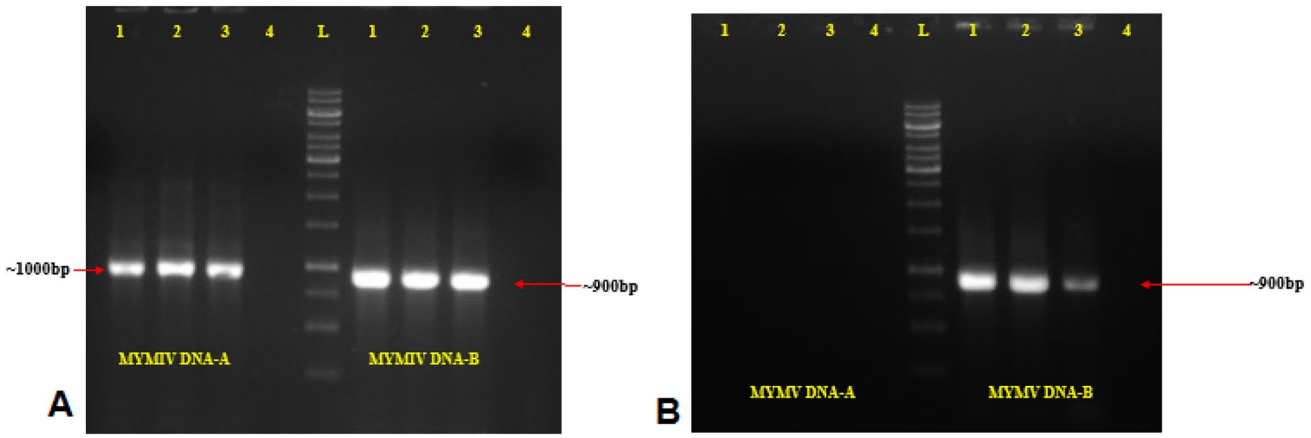

Fig. 3 Molecular Characterization of MYMV isolate at Hyderabad using primers specific to MYMIV DNA-A and DNA-B (**A**) and specific to MYMV DNA-A and DNA-B (**B**). Dominant markers specific to MYMV DNA-A did not amplify either with plasmid DNA (lane 1) or in agro-inoculated (lane 2) or field infected (lane 3) leaf samples; lane 4—negative control; L—marker ladder 1 kb

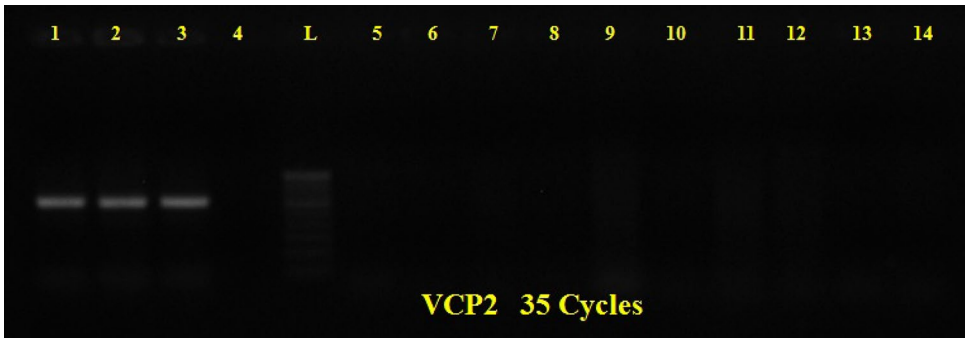

Fig. 4 PCR amplification of VCP2 in urd bean germplasm L -1 kb ladder; Lane 1–12: 1. PU31 AGI, 2. LBG685 AGI, 3. ABFBG03 AGI 4. Negative control 5. IC0472004, 6. IC0485454, 7. IC0485525, 8. IC0485497, 9. IC0485498, 10. IC0485500, 11. IC0485501, 12. IC0421950, 13. IC0485410, 14. IC0485414

Table 4 Screening of core set of urdbean germplasm against Yellow mosaic virus under natural field and laboratory conditions during 2022

Category	Field (Summer 2022)	Laboratory
Total number of accessions screened	202	192
Number of accessions without YMV symptoms (R)	52	44
Number of accessions with YMV symptoms (S)	149	148
Nil germination entries	1	0
Number of accessions without YMV symptoms in both field and laboratory	22	22
Number of accessions with YMV symptoms in both field and laboratory	121	121
Number of accessions without YMV symptoms in field and plants with > 20% YMV symptoms in laboratory	19	19
Number of accessions without YMV symptoms in laboratory and plants with > 50% YMV symptoms in field	12	12

R Resistant, *S* Susceptible

viral load can be detected by optimising PCR at 25, 30 and 35 cycles. In this test along with ten YMV-resistant genotypes from the field and agro inoculated checks (PU31 (R) and LBG685 (S)) and a line ABFBG03 derived from the cross LBG685XPU31were used. Experiment was designed by keeping the DNA concentration constant, and only the PCR cycles were varied. Agroinoculated samples PU31, LBG685 and ABFBG03 showed amplification even at 25 cycles and but the intensity of the band was very high at 35 cycles. Whereas the ten YMV-resistant genotypes collected from the field did not show any amplification even at 35 cycles of PCR. This shows that these ten YMV-resistant genotypes do not allow the multiplication of virus in them (Fig. 4).

Evaluation of core set of urdbean germplasm

A core set of urdbean germplasm has been constituted with 218 entries by NBPGR, New Delhi in consultation with the experts that represents diversity in the main collection. Of these 202 entries were evaluated in the field during the summer of 2022, and 52 entries recorded nil damage (Table 4). This core set is also being screened in the laboratory. Of 86 entries tested, 43 entries showed nil damage in the first round of testing. Based on these two tests, 21 entries could be picked up as promising in both field and lab tests (Table 5).

Table 5 Accessions of Core Set of urdbean germplasm recording nil damage against YMV in both field and laboratory tests during 2022

Core set #	IC #	Core set #	IC #
CS7	IC0519915	CS130	IC0530501
CS24	IC0140814	CS131	IC0530492
CS27	IC0557432	CS132	IC0530478
CS30	IC0279521	CS134	IC0530615
CS42	IC0250224	CS138	IC0250225
CS99	IC0330859	CS140	IC0485430
CS100	IC0001572	CS142	IC0427983
CS110	IC0326077	CS143	IC0472004
CS113	IC0616492	CS146	IC0485522
CS118	IC0530459	CS172	IC0519933
CS125	IC0485645	CS197	IC0472029

Molecular characterization of selected resistant genotypes

Using the reported SSR marker CEDG180 linked with YMV resistance, ten resistant accessions were genotyped to note the presence of resistance-specific allele in these. Results (Fig. 5) suggested the presence of three alleles. None of the accessions is carrying PU31 resistance-specific allele, this shows that they may be carrying the novel gene(s).

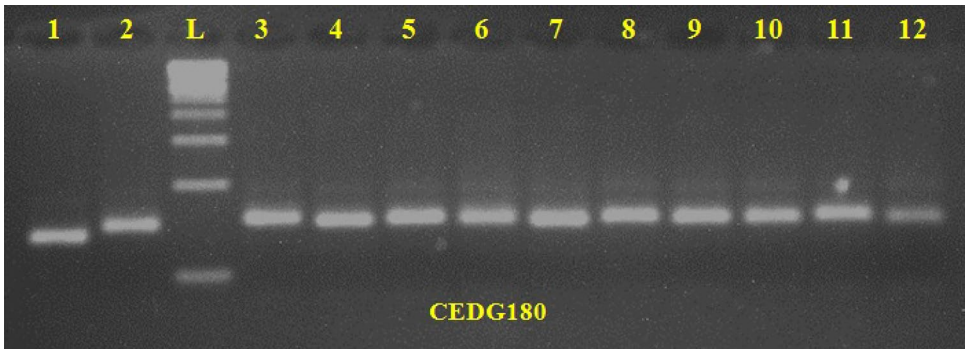

Fig. 5 PCR amplification of SSR marker CEDG180 in ten YMV resistant urdbean germplasm. L -100 bp ladder; Lane 1–12: 1. PU31, 2. LBG685 3. IC0472004, 4. IC0485454, 5. IC0485525, 6. IC0485497, 7. IC0485498, 8. IC0485500, 9. IC0485501, 10. IC0421950, 11. IC0485410, 12. IC0485414

Whereas the other marker YMV1 reported to be linked to YMV resistance, which was developed from SSR8111357 failed to amplify the 1357 bp marker fragment in all the urd bean genotypes used in this study.

Discussion

Yellow mosaic disease (YMD) of urdbean caused by MYMV or MYMIV and transmitted by the whitefly is the main production constraint in India and other urdbean-producing Asian countries. Development and cultivation of MYMV-resistant cultivars is a better choice than chemical control of the vector for the management of the disease and alleviating the yield losses due to it. Though significant achievement has been reported in breeding MYMV-resistant cultivars with the commercial release of more than 45 (Project Coordinator's Report, AICRP on MULLaRP, ICAR, IIPR, Kanpur. 2017–18 & 2020.) varieties over the past three decades, respite from the disease is short-lived. This is mainly due to, among other causes, the prevalence of at least two species of the virus, and possible recombinants of the two, and rapid mutations in both virus and vector populations. To address these issues, we need to identify genetically characterized new and diverse sources of resistance and molecular markers linked to such genes for possible pyramiding of the genes to develop durable and broad-spectrum resistance against YMV.

There have been several recent reports of field evaluation of urdbean genotypes for YMV resistance (Iqbal et al. 2011; Obaiah et al. 2013; Mohan et al. 2014; Subedi et al. 2016; Bandi et al. 2018; Pavishna et al. 2019; Tamilzharasi et al. 2020). In most of these studies, however, details of prevailing virus species or isolate were not precisely ascertained. It is generalized that MYMIV is prevalent in the northern, central and eastern regions of India, while MYMV is ubiquitous in the southern and western part of the country (Karthikeyan et al. 2014; Mishra et al. 2020; Gupta et al. 2021). However, a recent study based on whole genome sequence analysis (Chowdary et al. 2022) reported the occurrence of MYMIV with recombinant DNA-B component in southern peninsular India. Our results of molecular characterization using species-specific markers for both DNA-A and DNA-B also indicated virus isolate at Hyderabad to be a recombinant of MYMIV DNA-A and components of DNA-B of both MYMIV and MYMV. Exceptional study by Biswas and Verma (2001) has screened blackgram germplasm in both field and greenhouse through vector-mediated infection against several natural variants of MYMV. Bag et al. (2014) reported screening of 344 accessions of black gram germplasm from the National collection in three field tests and eight of the promising entries were also tested in one greenhouse tests followed by agroinoculation test. Four of the accessions reported promising in this work were not part of the present set. Recent report of Chaitanya et al. (2022) provides details of cloning the virus and screening of urdbean genotypes against different combinations of cloned DNA-A and DNA-B components through agroinoculation and in field. Our study is also based on both field and laboratory screening through agroinoculation with a characterized isolate of the virus. This is also a high-volume screening study covering more than half of the entire National Germplasm collection maintained at NBPGR, New Delhi.

The virus isolate used in the present study, though characterized by only one set of species-specific dominant markers, remained unchanged throughout the period of screening both in the field and laboratory tests. Hence, a good level of consistency between these two sets of screening is expected. Field screening is always best with non-uniform disease pressure and inadvertent seed mixture which may result in a large proportion of false positives. This limitation was overcome in this study by repeating the test at least for two consecutive years. Laboratory tests may also throw false positives due to improper practice of protocols or an unsuitable environment for disease development. This was minimised in our testing by conducting at least three tests during different months, each test with five replications having five plants in each. Further, a test was considered valid if at least ten plants survived at the time of disease scoring. Hence, out of 10 test entries that recorded nil plant damage in two field tests, all ten entries scored nil plant damage in all three laboratory tests. Reciprocally, of the ten entries scoring

nil plant damage in three laboratory tests only one accession (IC0485410) recorded 22% plant damage in field test during 2022. Such differences were also noted in the previous studies (Biswas and Verma 2001; Chaitanya et al. 2022).

A large number of YMV resistant genotypes has been reported in the above-listed field and laboratory screening studies. However, limited attempts have been made to note the inheritance of resistance and characterize resistance genes and note their allelic relationship with each other (Verma and Singh 1986). Recently, based on linked molecular markers, at least two distinctly different groups of resistance sources have been recognised by Gupta et al. (2015). Among several reported linked markers tested by them, two markers ISSR811 (and associated SCAR marker YMV1) and SSR marker CEDG180 were found distinguishing the resistance sources. We attempted to see diversity among the ten resistant accessions reported here using SCAR marker YMV1 and CEDG180 marker. SCAR marker YMV1 did not amplify with any of the 10 accessions. But with CEDG180, Results suggested that 10 accessions shortlisted through field and laboratory tests do not carry PU31 allele and this shows that may be likely to carry novel gene(s). Further studies are needed to genetically characterize these new sources.

Acknowledgements All authors hereby acknowledge the funding support received from the Department of Biotechnology (No. BT/AG/Network/Pulses-1/2017-18), Govt of India. Authors are grateful to the Director, ICAR-NBPGR for providing guidance and necessary facilities for carrying out the research. Coordination and support received from Dr Sunil Archak, Principal Scientist & Coordinator, Network Project-Minor Pulses, is duly acknowledged.

Data availability All the data pertaining to the present study are provided in the tables.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Alice D, Nadarajan N (2007) Screening techniques and assessment methods for disease resistance. Indian J Plant Prot 24:50–52

Ammavasai S, Phogat DS, Solanki IS (2004) Inheritance of resistance to *mungbean yellow mosaic virus* (MYMV) in green gram [*Vigna radiata* (L.) Wilczek]. Indian J Genet Plant Breed 64:146–146

Bag MK, Gautam NK, Prasad TV, Pandey S, Dutta M, 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

Bandi HRK, Rao KN, Krishna KV, Srinivasulu K (2018) Screening of blackgram (*Vigna mungo* L. Hepper) resistance to mungbean yellow mosaic virus under rice fallow situation. Bull Environ Pharmacol Life Sci. 7(1):125–128

Biswas KK, Varma A (2001) Evaluation of resistance in blackgram (*Phaseolus mungo*) to variants of mungbean yellow mosaic geminivirus. Indian J Agric Sci 71:215–218

Biswas KK, Varma A (2012) Agroinoculation: a method of screening germplasm resistance to mungbean yellow mosaic geminivirus. Indian Phytopathol 54:240–245

Chaithanya BH, Reddy BB, Prasanthi L, Devi RSJ, Manjula K, Naidu GM (2022) Construction of full-length dimer clones of yellow mosaic virus and screening of blackgram germplasm using agroinoculation. Legum Res 1:1–7

Chattopadhyay D, Yadav RK, Shukla RK (2013) Chimeric construct of mungbean yellow mosaic india virus (MYMIV) and its uses thereof. U.S. Patent 8,435,732

Chowdary VT, Kumar VM, Varma PK et al. (2022) New record of mungbean yellow mosaic India virus in Desmodiumlaxiflorum and association of bhendi yellow vein mosaic betasatellite in Abelmoschus moschatus in Andhra Pradesh, India.Virus Dis 33:119–121

Dasgupta U, Mishra GP, Dikshit HK, Mishra DC, Bosamia T, Roy A, Bhati J, Aski M, Kumar RR, Singh AK, Kumar A (2021) Comparative RNA-Seq analysis unfolds a complex regulatory network imparting yellow mosaic disease resistance in mungbean [*Vigna radiata* (L.) R. Wilczek]. PLoS ONE 16:e0244593

Dhole VJ, Reddy KS (2012) Genetic analysis of resistance to mungbean yellow mosaic virus in mungbean (*Vigna radiata*). Plant Breed 131:414–417

Ganguli S, Dey A, Banik R, Kundu A, Pal A (2016) Analyses of MYMIV-induced transcriptome in Vigna mungo as revealed by next generation sequencing. Genom Data 7:226–228

Gilbertson RL, Rojas M, Natwick E (2011) Development of integrated pest management (IPM) strategies for whitefly (*Bemisia tabaci*)-transmissible geminiviruses. In: Thompson WMO (ed) The whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) interaction with geminivirus-infected host plants. Springer, Dordrecht, pp 323–356

Gupta S, Gupta DS, Anjum TK, Pratap A, Kumar J (2013) Inheritance and molecular tagging of MYMIV resistance gene in blackgram (*Vigna mungo* Hepper). Euphytica 193:27–37

Gupta SK, Souframanien J, Reddy KS (2015) Validation of molecular markers linked with yellow mosaic virus disease resistance in diverse genetic background of black gram (*Vigna mungo*). Electron J Plant Breed 6:755–763

Gupta S, Das A, Pratap A, Gupta DS (2021) Urdbean. In: Pratap A, Gupta S (eds) The beans and the peas. Elsevier, Woodhead Publishing, New York, pp 33–54

Iqbal U, Iqbal SM, Afzal R, Jamal A, Farooq MA, Zahid A (2011) Screening of mungbean germplasm against *Mungbean yellow mosaic virus* (MYMV) under field conditions. Pak J Phytopathol 23:48–51

Jasrotia RS, Iquebal MA, Yadav PK, Kumar N, Jaiswal S, Angadi UB, Rai A, Kumar D (2017) Development of transcriptome-based web genomic resources of yellow mosaic disease in *Vigna mungo*. Physiol Mol Biol Plants 23:767–777

Karthikeyan A, Shobhana VG, Sudha M, Raveendran M, Senthil N, Pandiyan M, Nagarajan P (2014) Mungbean yellow mosaic virus (MYMV): a threat to green gram (Vigna radiata) production in Asia. Int J Pest Manag 60:314–324

Khattak GSS, Haq MA, Ashraf M, Elahi T (2000) Genetics of *Mungbean Yellow Mosaic Virus* (MYMV) in mungbean (*Vigna radiata* L.) Wilczek. J Genet Breed 54:237–243

Kundu A, Ganguli S, Pal A (2022) Genomic designing towards biotic stress resistance in mungbean and urdbean. In: Kole C

(ed) Genomic designing for biotic stress resistant pulse crops. Springer, Berlin, pp 381–414
Maheshwari R, Panigrahi G, Angappan K (2014) Molecular characterization of distinct YMV (Yellow mosaic virus) isolates affecting pulses in India with the aid of coat protein gene as a marker for identification. Mol Biol Rep 41:2635–2644
Mandal B, Varma A, Malathi VG (1997) Systemic infection of *Vigna mungo* using the cloned DNAs of the blackgram isolate of mungbean yellow mosaic geminivirus through agroinoculation and transmission of the progeny virus by whiteflies. J Phytopathol 145:505–510
Mishra GP, Dikshit HK, Ramesh SV, Tripathi K, Kumar RR, Aski M, Singh A, Roy A, Priti, Kumari N, Dasgupta U, Kumar A,Praveen S and Nair RM (2020) Yellow Mosaic Disease (YMD) of Mungbean (Vigna radiata (L.) Wilczek): Current Status andManagement Opportunities. Front Plant Sci 11:918. https://doi.org/10.3389/fpls.2020.00918
Mohan S, Sheeba A, Murugan E, Ibrahim SM (2014) Screening of mungbean germplasm for resistance to *Mungbean yellow mosaic virus* under natural condition. Indian J Sci Technol 7:891–896
NAAS (2016) Towards self-sufficiency of pulses in India. National Academy of Agricultural Sciences, New Delhi
Naimuddin K, Akram M, Pratap A (2011) First report of natural infection of mungbean yellow mosaic india virus in two wild species of Vigna. New Dis Rep 23:21
Naimuddin K, Akram M, Singh NP (2016) Yellow mosaic of mungbean and urdbean: current status and future strategies. J Food Legum 29:77–93
Nene YL (1972) A survey of viral diseases of pulse crops in Uttar Pradesh Research GB Pant University of Agriculture and Technology Pantnagar India. Bulletin 4:191
Obaiah S, Reddy BB, Reddy NPE, Prasad YS (2013) Screening of some blackgram (*Vigna mungo* (L.) Hepper) genotypes for resistance to yellow mosaic virus. Curr Biotica 7:96–100
Pavishna M, Kannan R, Arumugam Pillai M, Rajinimala N (2019) Screening of blackgram genotypes against mung bean yellow mosaic virus disease. J Pharmacogn Phytochem 8:4313–4318
Prasanthi L, Reddy BV, Geetha B, Jyothi R (2013) Molecular marker for screening yellow mosaic disease resistance in blackgram [*Vigna mungo* (L.) Hepper]. Electron J Plant Breed 4:1137–1141
Sandhu TS, Brar JS, Sandhu SS, Verma MM (1985) Inheritance of resistance to *Mungbean yellow mosaic virus* in green gram. J Res Punjab Agric Univ 22:607–611
Sathees N, Shoba D, Mani N, Saravanan S, Kumari MP, Pillai MA (2022) Tagging of SSR markers associated to yellow mosaic virus resistance in black gram (*Vigna mungo* (L.) Hepper). Euphytica 218:1–11
Singh AK, Srivastava PK (2021) Growth in area, production and productivity of urdbean in India: an empirical study. Himal J Agric 2:34–40
Souframanien J, Gopalakrishna T (2006) ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [*Vigna mu*ngo (L.) Hepper]. Plant Breed 125:619–622
Subedi S, Neupane S, Ghimire TN (2016) Screening of mungbean and black gram genotypes as sources of genetic resistance against Mungbean Yellow Mosaic Disease. J Agric Sci 14:148–155
Subramaniyan R, Narayana M, Krishnamoorthy I, Natarajan G, Gandhi K (2022) Novel and stable QTL regions conferring resistance to MYMV disease and its inheritance in blackgram (*Vigna mungo* (L.) Hepper). J Genet 101:1–7
Sudha M, Karthikeyan A, Nagarajan P, Raveendran M, Senthil N, Pandiyan M, Angappan K, Ramalingam J, Bharathi M, Rabindran R, Veluthambi K (2013) Screening of mungbean (*Vigna radiata*) germplasm for resistance to *Mungbean yellow mosaic virus* using Agroinoculation. Can J Plant Pathol 35:424–430
Tamilzharasi M, Vanniarajan C, Karthikeyan A, Souframanien J, Pillai MA, Meenakshisundram P (2020) Evaluation of urdbean (*Vigna m*ungo) genotypes for mungbean yellow mosaic virus resistance through phenotypic reaction and genotypic analysis. Legum Res Int J 43:728–734
Vadivel K, Manivannan N, Mahalingam A, Satya VK, Vanniarajan C, Ragul S (2021) Identification and validation of quantitative trait loci of mungbean yellow mosaic virus disease resistance in blackgram [*Vigna mung*o (L). Hepper]. Legum Res Int J 1:1–7
Varma A, Malathi VG (2003) Emerging geminivirus problems: a serious threat to crop production. Ann Appl Biol 142:145–164
Verma RPS, Singh DP (1986) The allelic relationship of genes giving resistance to mungbean yellow mosaic virus in blackgram. Theor Appl Genet 72:737–738
Younas M, Zou H, Laraib T, Rajpoot NA, Khan NA, Zaidi AA, Kachelo GA, Akhtar MW, Hayat S, Al-Sadi AM, Sayed S (2021) The impact of insecticides and plant extracts on the suppression of insect vector (*Bemisia tabac*i) of *Mungbean yellow mosaic virus* (MYMV). PLoS ONE 16:e0256449