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Identification of BCMV resistant germplasm in mungbean (*Vigna radiata*) using serological and molecular diagnostics

D D DEEPIKA[1], KULDEEP TRIPATHI[2], ABHISHEK G J[1], NEETA SINGH[2], RUCHI SHARMA[2], VAISHALI DUTTA SHARMA[2], A K MAURYA[2] and V CELIA CHALAM[2]*

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

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Mungbean [*Vigna radiata* (L.) Wilczek] of family Fabaceae, chromosome number 2n=2x=22 is a vital pulse crop of India, and consists of vast diversity. Virus diseases are significant constraints among biotic stresses for successful pulses production, e.g. bean common mosaic virus (BCMV) regularly occurs on major pulses in tropical and sub-tropical countries (Loebenstein and Thottappilly 2013). It is a ubiquitous seed-borne infective agent of common bean and further spreads through vector aphids, and belongs to the genus *Potyvirus*, family *Potyviridae*. In India, yield loss of up to 84% in mungbean has been documented by Sastry *et al.* (1992). Germplasm has provided novel genes since the dawn of crop improvement programme. Currently, host plant resistance is the best, reliable and economic practice of disease and insect management (Mukeshimana *et al.* 2005, Tripathi *et al.* 2012, Worrall *et al.* 2019).

The present study was carried out during 2018–19 at Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi and field experiments at research farm of ICAR-NBPGR and ICAR-IARI, New Delhi is an overlap of monsoon-influenced humid subtropical and semi-arid climate. Based on passport data, 65 mungbean accessions, two susceptible checks (K851 and ML267) and one resistant check (LGG460) were procured from National Genebank, ICAR-NBPGR, New Delhi. These accessions were screened under natural and artificial conditions.

During rainy (*kharif*) season 2018, a total of 65 mungbean accessions and resistant and susceptible checks were grown in augmented block design. Observations were recorded every day and per cent disease incidence (PDI) was calculated. Same accessions along with checks were grown in pots in three replications under controlled conditions. The seedlings were mechanically inoculated with sap at second trifoliate leaf stage and were observed for symptoms development every day. Different symptoms and disease incidence were recorded two and three weeks post-inoculation and PDI was calculated. Disease scoring was done using a 0–5 scale adopted from Reddy *et al.* (2001). Natural and artificially screened mungbean leaf samples were subjected to electron microscopic detection (JEOL JEM 1011, JEOL Ltd.) by following the leaf dip method (Chalam 2008). Direct Antigen Coating-Enzyme-linked Immunosorbent Assay (DAC-ELISA) is conducted using Agdia® ELISA kit according to manufacturer instructions to detect BCMV in the mungbean accessions (65) grown under natural conditions and artificially inoculated plants. Also, it was used to detect BCMV in the seed coat and embryo of 65 accessions along with two susceptible checks and one resistant check.

Healthy and symptomatic plant leaves from both natural and artificial screening, were subjected to molecular detection using RT-PCR. The total RNA isolation was done using RNeasy® Plant Mini Kit (QIAGEN® kit catalogue Nos.74903 and 74904). Total RNA was taken for reverse transcription. A Thermo Scientific Verso cDNA Synthesis kit was used. The cDNA obtained was subjected to PCR amplification using forward 5' CAA CAC TCC GCC AGA TCA AG 3' and reverse primers 5' GCT GCC TTC ATC TGT GCT AC 3' of product size 205 bp as per the method followed by Manjunatha *et al.* 2017.

A total of 65 accessions were screened under natural and artificial disease incidence along with two susceptible and one resistant check. Under field screening, the aphid vectors were not observed and disease incidence was low during *kharif* 2018, and susceptible checks (K 851 and ML 267) showed disease incidence ranging from 10–16%. Out of 65 accessions, only one accession IC0148387 had mosaic symptoms with 12% disease incidence indicating that it is resistant to BCMV and the remaining 64 accessions showed no symptoms. Accession IC0148387 leaves observation under EM revealed the flexuous particles of size 821 nm, and no such particles were observed for remaining

[1]ICAR-Indian Agricultural Research Institute, New Delhi; [2]ICAR-National Bureau of Plant Genetic Resources, New Delhi. *Corresponding author email: mailcelia@gmail.com

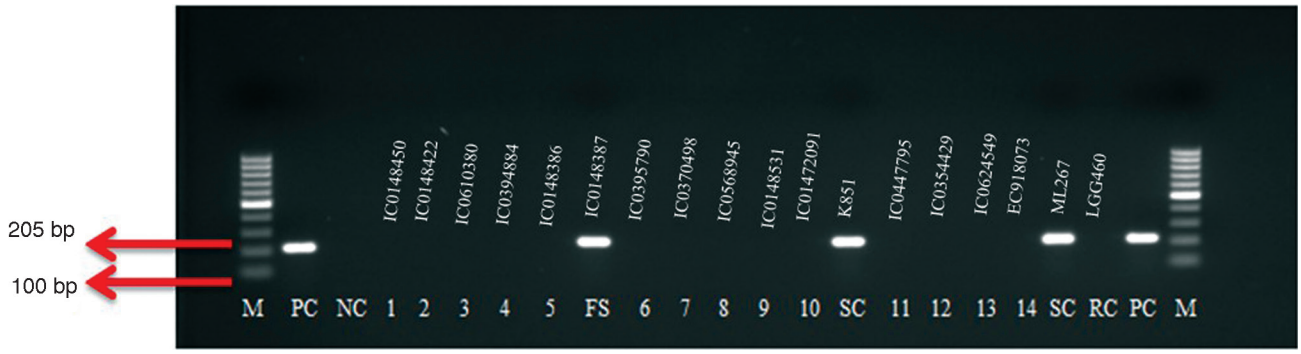


Fig 1 Gel image showing PCR amplification of BCMV in leaf samples collected from plants grown under field conditions. M, Marker; PC, Positive control; NC, Negative control; RC, Resistant check; 1–14, Germplasm and; SC, Susceptible check.

accessions. ELISA results for 65 accessions showed that only one accession was ELISA positive, and the remaining accessions (64) were ELISA negative. RT-PCR detection of BCMV revealed the presence of bands in the infected samples, and no such bands were observed in the healthy samples (Fig 1).

The same set of accessions was artificially inoculated at the 2nd trifoliate leaf stage, and inoculated plants started to express symptoms 10–14 days post-inoculation. Out of 65 accessions, one accession IC0148525 showed seed transmission of 16.67%. Artificially inoculated plants produced mosaic, vein clearing, vein banding, leaf rolling, puckering and reduced leaf size. These symptoms are typical to the BCMV, and various workers observed similar symptoms (Chand *et al.* 2002, Hamid 2016, Manjunatha *et al.* 2017). The PDI under artificial inoculation conditions ranged from 0–83.33% (Table 1). Accession IC0148525 and IC568946 recorded the highest disease incidence of 83.33% each. Chand *et al.* (2002) identified 29 accessions resistant to BCMV in the core collection of mungbean germplasm. Hamid (2016) evaluated 85 french bean accessions against BCMV in a controlled glasshouse and identified 17 resistant genotypes to the test virus strains.

Serological detection of BCMV using DAC-ELISA for accessions collected after artificial inoculation showed that out of 65 accessions, 15 accessions showed positive reaction indicating the presence of BCMV and 50 accessions showed negative reaction indicating the absence of BCMV. Both the susceptible checks were positive, and the resistant check was negative to the ELISA test. Feng *et al.* (2019) used the ELISA test to detect BCMV in *Phaseolus lunatus* while studying the systemic infection of BCMV in lima bean plants. ELISA has been used to detect several viruses, including BCMV in germplasm of mungbean, french bean

Table 1 Grouping of mungbean accessions based on the reaction against BCMV after artificial inoculation

Scale	Description	Reaction	No. of accessions	Accessions
0	No plants showing symptoms	Immune	50	IC0418452, IC0418454, IC0418469, IC0418510, IC0539814, IC0148391, IC0148392, IC0148422, IC0148432, IC0148465, IC0610380, IC0394728, IC0394884, IC0394907, IC0148386, IC0103862, IC0103873, IC0395790, IC0395832, IC0370497, IC0370498, IC0370532, IC0370713, IC0370714, IC0065871, IC0256884, IC0373199, IC0568945, IC0148530, IC0148531, IC0148535, IC0472087, IC0472089, IC0472091, IC0472092, IC0392343, IC0424633, IC0447795, IC0354429, IC0413410, IC0624549, IC118998, IC396705, EC918077, EC918071, EC918079, EC918076, EC400174, EC918074, EC918073 and control
1	1–10% plants showing symptoms	Highly resistant	-	LGG 460
2	11–25% plants showing symptoms	Resistant	4	IC0148387, IC0103833, IC0447793 and IC0447794
3	26–40% plants showing symptoms	Moderately resistant	2	IC0148388, IC0148528
4	41–60% plants showing symptoms	Susceptible	3	IC0062200, IC0000149, IC0472088
5	>60% plants showing symptoms	Highly Susceptible	6	IC0394901, IC0148389, IC0148525, IC0413355, IC568946, EC918080 and K851 and ML267

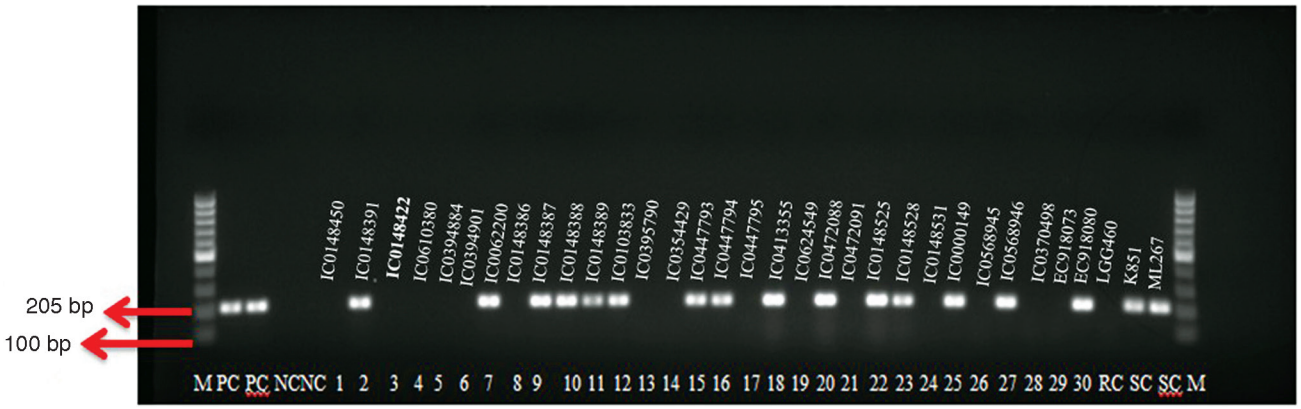


Fig 2 Gel image showing PCR amplification of BCMV in leaf samples after artificial inoculation. M, Marker; PC, Positive control; NC, Negative control; RC, Resistant check; SC, Suceptible check and; 1–30, Germplasm.

and soybean imported into the country (Chalam *et al.* 2005, Chalam and Khetarpal 2008, Chalam and Maurya 2018). Molecular detection of BCMV using RT-PCR was carried out. A total of 15 symptomatic and 15 healthy samples from artificially inoculated mungbean accessions were subjected to RT-PCR. Amplified products were analyzed by 1.2% agarose gel electrophoresis. Bands of 205 bp were observed in symptomatic samples indicating the presence of BCMV, and in healthy samples, no such bands were observed (Fig 2). Wani *et al.* (2017) used SSR markers to identify BCMV resistant genotypes in common bean and out of 132 genotypes identified eight resistant genotypes. Manjunatha *et al.* (2017) and Feng *et al.* (2019) also observed similar results.

DAC-ELISA testing of embryo and seed coat of mungbean germplasm for the presence of BCMV revealed that out of 65 mungbean accessions 6 and 27 showed ELISA positive results respectively and 59 and 38 accessions respectively were ELISA negative indicating the absence of BCMV. Provvidenti and Cobb (1975) while explaining the seed transmission nature of BCMV, mentioned that the virus is generally located in the embryo and perpetuate through seeds for next generation. Thus, seeds are a source of initial infection and even after prolonged storage of infected seeds up to 30 years, the infectivity of BCMV is not appreciably affected. Susceptible checks K851 and ML267 showed a positive reaction, and resistant check LGG460 showed negative reactions in both the cases. BCMV in the seed coat is inactivated during seed maturation. Thus, despite the presence of the virus in the seed coat, it does not transmit in the seedlings raised by infected seed.

The results of seed transmission under controlled conditions, testing of seed coat and embryo, screening in the field and after artificial inoculation followed by ELISA and RT-PCR testing of BCMV in mungbean germplasm were pooled. It reveals that out of 50 accessions found immune after artificial inoculation followed by ELISA and RT-PCR testing, two accessions of an embryo and 19 accessions of seed coat were BCMV positive, indicating these 21 accessions were also not immune to BCMV. Thus, out of 65 accessions tested, 29 accessions were found to be immune to BCMV (IC0418452, IC0418454, IC0418469, IC0418510, IC0539814, IC0148392, IC0610380, IC0394728, IC0394884, IC0394907, IC0103862, IC0103873, IC0395790, IC0392343, IC0424633, IC0472087, IC0472089, IC0472091, IC0472092, IC0568945, IC0370497, IC0370532, IC0370713, IC0370714, EC918071, EC918079, EC918076, EC918074 and EC918073). The accessions from Andaman & Nicobar Island, Arunachal Pradesh, Assam, Bangladesh, Kerala and Rajasthan showed no symptoms. They were found resistant to BCMV, and accessions from Andhra Pradesh, Bihar, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Maharashtra and Odisha showed the varied amount of susceptibility to the BCMV. Thus, identified accessions will best serve as source of resistance genes in crop improvement programme. However, these accessions need to be tested under disease hotspots and further evaluated under multilocation trials.

SUMMARY

Diverse germplasm of mungbean serves as a source of resistant gene, screening of germplasm for BCMV resistance will provide resistance source. So, in 2018–2019 a total of 65 diverse mungbean accessions along with susceptible and resistant checks were screened against BCMV under natural conditions in the field and artificial inoculation under controlled conditions. Transmission electron microscopy, per cent disease incidence, serological Direct antigen coating-Enzyme-linked immunosorbent assay (DAC-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) diagnostics were used to detect and identify the BCMV resistant accessions. The study revealed that a total of 29 accessions were found to be immune to BCMV infections. However, these accessions need to be further evaluated in BCMV disease hotspots and under multilocation trials. Therefore, in the present study, accessions that were immune to BCMV may serve as valuable donors for the BCMV resistant mungbean breeding programme.

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