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Seed Transmission Behaviour of *Bean Common Mosaic Virus* in Green Gram

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ABSTRACT

Background: The virus-distribution and bimodal seed transmission of BCMV in green gram was studied. The *Bean common mosaic virus* (BCMV) infecting leguminous crops have been found to be the most devastating Potyvirus as they cause considerable yield loss. Emergence of symptom in the first trifoliate leaf stage of the plants under the natural condition confirms that the virus may be seed borne, which has been investigated in the present study.

Methods: The distribution of the virus in various parts of the seeds of mung bean (*Vigna radiata*) plants naturally infected in the field was determined by ELISA, polymerase chain reaction (PCR) and sequencing.

Result: Nucleotide sequencing of the PCR amplicons from the seed parts from groups of ten seeds revealed the presence of *Bean common mosaic virus* (BCMV) in the seed coat, cotyledon and embryonic axes. The grow out test performed with the same batch of seeds, there was symptom development after 3 weeks of sowing and the presence of virus was detected in all the seedlings through ELISA test. RT-PCR amplification of viral cDNA from whole seed, seed coat, cotyledons and embryo generated an amplicon of ~1300 with BCMV coat protein gene specific primer. The genomic sequence (GenBank accession No. ON944468) showed highest nucleotide identity of 90-91% sequence similarities with NL-1 strain (KF114860.1) and 94% sequence similarities with MY15-014 strain (MW079241.1) of previously available BCMV- CP virus gene sequence.

Key words: BCMV, Green gram, Legumes, Seed-borne viruses.

INTRODUCTION

First time the *Bean common mosaic virus* (BCMV) was described by Pierce (1934). BCMV belongs to the genus *Potyvirus*, which has been established as the largest among the eight genera currently assigned to the Family Potyviridae by the International Committee on Taxonomy of Viruses (ICTV, 2020). Potyvirus is plant-infecting positive-strand RNA viruses which includes 146 virus species (ICTV, 2013; Ivanov *et al*., 2014). The taxonomy of potyviruses is persistently changing due to frequent emergence of new viruses, inconclusive serological data between viruses and their isolates, variability in viral host differentials and different symptoms caused by strains of the same species (Ali *et al*., 2006). Gibbs *et al*. (2008) reported that the Potyviridae family have undergone a major speciation event approximately 6600 years ago, that coincides with the initiation of plant domestication.

A typical virus symptom, mosaic was observed in green gram, common bean,lime bean, rice bean and yard long bean, whereas, leaf rolling and leaf distortion was observed in black gram, polebean and snap bean (Manjunatha *et al*., 2017).

Seed transmission of BCMV has an important role to play in the epidemiological studies of the disease. Therefore, the detection of the location of virus in seed and its parts in view of seed quality control is much required (Hampton, 1967). Seed transmission ranges from 3 to 95% (Zaumeyer and Thomas, 1957) that actually depends on the tolerance of bean cultivar and their susceptible stage (Galvez *et al*., 1977).

BCMV traverse through the cell wall between the testa and the suspensor cell by an unknown mechanism that does not require plasmodesmata, or virus which can induce formation of new plasmodesmata as new avenue for the direct invasion of viruses (Ekpo and Saettler, 1974). Extensive spread of BCMV through the testa and endosperm has been reported only in the bean cultivars transmitting the virus via seed. In case of non-seed transmitting cultivars of been, BCMV leads to very limited invasion through testa and endosperm (Kaiser *et al*., 1968, Morales and Castano, 1987).

Previously, strain identification and differentiation of BCMV were done based on their virulent response on a set of host differential of *Phaseolus vulgaris* cultivars (Drifhout, 1978). Based on serological reactions, isolates/strains of BCMV were grouped into two serogroups, A and B (Vetten *et al*., 1992). Recent studies based on phylogenetic analysis established that viruses from these two different serotypes actually belong to two different species, where members of serotype A have been placed in the species, Bean common mosaic necrosis virus (BCMNV) and members of serotype B have been placed in the species, BCMV (Berger *et al*., 1997).

Manjunatha *et al*. (2017) have identified markers that may be used for genetic diagnosis of BCMV and isolation

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of the resistance gene against BCMV using map based cloning which can be further used in other crops for genetic transformation to induce the resistance for BCMV.

MATERIALS AND METHODS

he experiment was conducted in the year 2019-21 at the Department of Plant Pathology, Anand Agricultural University, Anand.

Sample collection

Green gram seeds were harvested from the BCMV infected plant showing symptoms like reduction and downward rolling of leaf lamina, necrosis of veins, leaf deformation and discolorations of interveinal area of leaf in the summer sown crop from plants grown in research fields at Anand Agricultural University, Anand. Seeds were harvested from infected green gram plants (variety GAM 5), labeled and stored. The seeds harvested from infected plants appeared darker in colour and shriveled (Fig 1).

Serological characterization

The detection of BCMV from different parts of infected seeds was done by performing the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using a Bean common mosaic virus antibody (provided by LOEWE® company) as per the manufacturer's protocol. Symptomatic samples (whole seed, seed coat, cotyledons and embryo) of mungbean variety GAM 5 and asymptomatic sample of *variety* GM 4 were assayed for the presence of BCMV by DAS-ELISA as described by Clark and Adams (1977) using polyclonal antibodies against BCMV. The absorbance of each well was measured at 405 nm (OD405) by ELISA-reader.

Molecular characterization

Isolation of RNA

Molecular detection was carried out for detection of the BCMV in GAM-5 variety of mungbean. The virus RNA was isolated through LiCl method from whole seed, seed coat, cotyledons as well as embryo of discoloured seeds of GAM-5 variety of mungbean.

RNA prurity check

Purity of isolated RNA was checked by electrophoresis. 3 µl of RNA was mixed with 2 µl of gel loading dye and loaded on 1 per cent agarose gel containing Ethidium Bromide (0.5 mg/ml) and electrophoresed in 0.5X TAE buffer at 80 volts for 2 hours. Then the gel was viewed under Gel Documentation System using UV transilluminator. High quality and purity RNA was then used for cDNA synthesis.

CDNA synthesis

First strand cDNA synthesis was initiated at the polyadenylated 3' terminus of the RNA with oligodT18 primer by reverse transcription performed in a 25 µl reaction mixture containing 1-2 µg of total RNA mixed with 5 µl of 5X reverse transcription buffer, 5 µl of 20 mMdNTP mix (Fermentas, USA), 10 µM of oligodT primer, 20 units of RNase inhibitor (0.5 µl) (Biogene), 200 units of reverse transcriptase (1 µl) and RNase free water added to make final volume. The reaction mixture was incubated at 37°C for 75 minutes and then heated for 5 minutes at 70°C to inactivate reverse transcriptase. RT-PCR was carried out using BCMV coat protein (CP) gene specific primers.

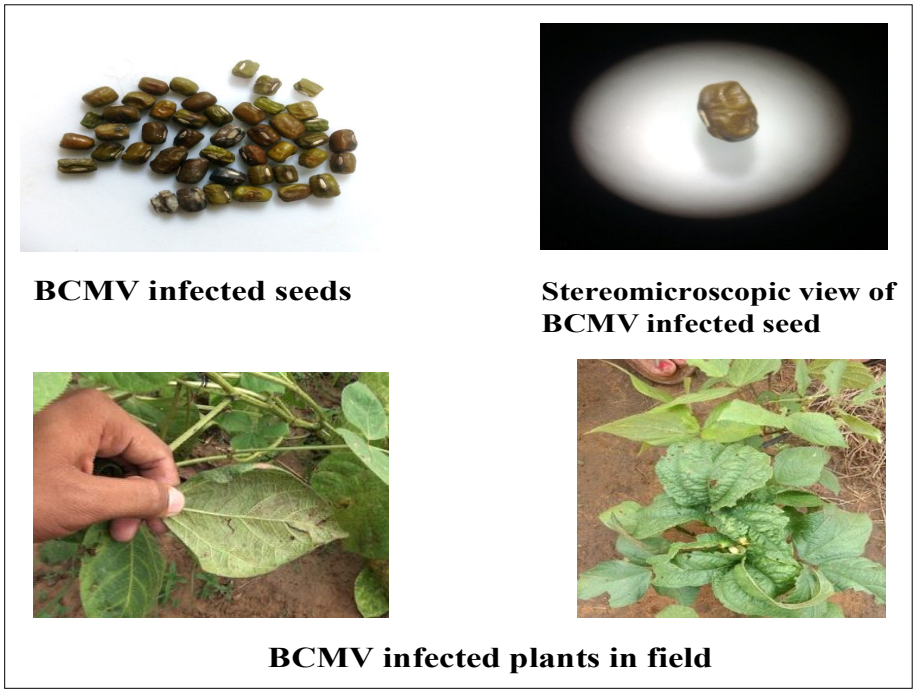

Fig 1: Symptomatic green gram seeds and plants infected with BCMV.

Detection of BCMV through polymerase chain reaction (PCR) using specific primers

The RNA extracted from different parts of a single seed and was reverse-transcribed and amplified using BCMV-CP virus specific primers *viz*., BCMV-CP-F (TGGCTGCTT GAGAGA GATGA) and BCMV-CP-R (ATCACTCTGCATGTCCTCAC). The amplified product was analyzed by gel electrophoresis. RT-PCR reaction was performed as one step of initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 50 seconds, annealing at 59°C for 1 minute, extension at 72°C for 1 minute. The last cycle of final extension was ended at 72°C for 30 minutes. The amplicons were analyzed on 1.8% agarose gel at 60 V in TAE (40.0 M Tris/acetate, 1.0 mM EDTA and pH 8.0) and stained with ethidium bromide.

Virus specific bands obtained in PCR amplification were eluted and sequenced by Eurofins Pvt. Ltd and results were analyzed in NCBI nucleotide BLAST to find out similarity of newly sequenced sample with previously available virus sequences.

Grow out test

Grow out test was conducted in insect proof cage under protected condition using BCMV infected seeds of mung bean (Var. GAM 5) and seeds of variety GM 4 were also sown under the protected condition in separate pot. The observations were taken periodically for symptom appearance. Symptoms of BCMV appeared under protected condition in GAM 5 seedlings. Further, the confirmation was done using the DAS-ELISA test.

Table 1: Detection of bean common mosaic virus in different parts of mungbean (Va. GAM-5).

Seed parts and control	O.D. value at 405 nm	Reaction
Positive control	0.613	+
Negative control	0.365	-
Seed	1.350	++
Seed coat	1.141	+++
Cotyledons	1.612	+++
Embryo	1.250	++

+++ = Strongly positive, ++ = mildly positive, + = weak reaction, - = Negative reaction.

Results and Discussion

Serological characterization

Detection of Bean Common Mosaic Virus (BCMV) was done in whole seed, seed coat, cotyledons and embryo of mungbean seeds of varieties GM-4 and GAM-5 through DAS-ELISA. The infected parts of mungbean variety of GAM-5 gave positive reaction and the O.D value of whole seed, seed coat, cotyledons and embryo recorded were 1.350, 1.141, 1.612 and 1.250 respectively as compared to negative control with O.D value of 0.365 (Table 1). Whereas, the whole seed, seed coat, cotyledons, as well as embryo of discoloured seeds of mungbean variety of GM-4 showed negative reaction and did not show the presence of the virus in these parts of the seeds.

Molecular characterization

RT-PCR amplification of viral cDNA from whole seed, seed coat, cotyledons and embryo generated an amplicon of ~1300 with BCMV coat protein gene specific primer (Fig 2). Virus specific bands obtained in PCR amplification were eluted and sequenced by Eurofins Pvt. Ltd and results were analyzed in NCBI nucleotide BLAST to find out similarity of newly sequenced sample with previously available virus sequences. From the results of PCR amplification by BCMV-CP virus specific primer it confirms survival of virus in different parts of the seed, followed by Sanger sequencing it is observed that BCMV- CP infected mungbean sample of GAM-5 variety has viral gene integration and the genomic

Description	Scientific name	Per. ident	Accession
BCMV isolate US1, complete genome	Bean common mosaic virus	91.16	KT175569.1
BCMV MY14-206 coat protein gene, partial cds	Bean common mosaic virus	94.52	MW079240.1
BCMV isolate MY15-014 coat protein gene, partial cds	Bean common mosaic virus	94.4	MW079241.1
BCMV strain NL-1n, complete genome	Bean common mosaic virus	90.39	KF114860.1

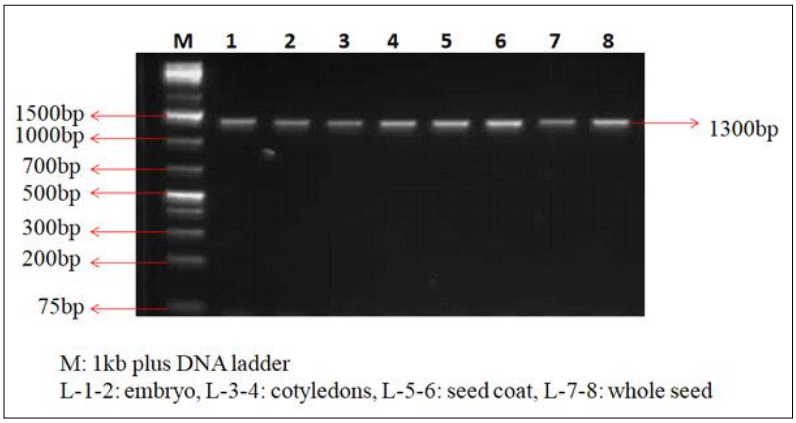

Fig 2: Gel image depicting presence of BCMV from different parts of mungbean seed.

Table 2: Serological detection of bean common mosaic virus from seedlings of mung bean (Var. GAM-5) grown for conducting grow out test.

Seed parts and control	O.D. value at 405 nm	Reaction
Positive control	0.613	+
Negative control	0.365	-
Seedling 1	1.271	+
Seedling 2	3.523	+++
Seedling 3	3.127	+++
Seedling 4	2.075	++

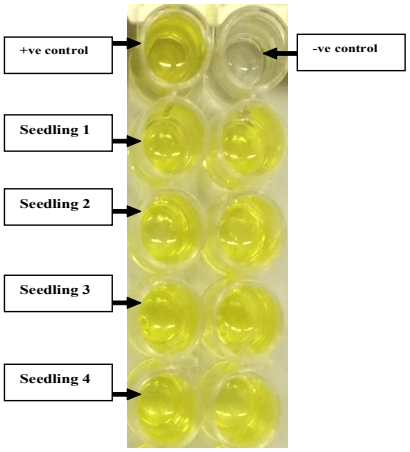



Fig 3: Symptom appearance after grow out test.

sequence (GenBank accession No. ON944468) showed highest nucleotide identity of 90-91% sequence similarities with NL-1 strain (KF114860.1) and 94% sequence similarities with MY15-014 strain (MW079241.1) of previously available BCMV- CP virus gene sequence.

Grow out test

The observations were taken periodically for symptom appearance. Symptoms of BCMV appeared at 6-7 leaf stage of the crop, under protected condition. It indicates the seed borne nature of BCMV. Seed transmission rates varied from 60-70 per cent. Symptom showed bronzing pattern on the lower side of the leaves along with some puckering (Fig 3).

Further, the confirmation was done using the ELISA test. The presence of BCMV was detected in infected leaf with BCMV antisera under ELISA test. Infected leaves from different seedlings showed positive reaction with O.D value of 1.271, 3.523, 3.127 and 2.075 respectively as compared to negative control with O.D value of 0.365 (Table 2).

Similarly, Schmidt, (1992) reported 93% seed transmission of BCMV. Morales and Bos, (1988) observed that the rate of BCMV transmission through seeds varies between genotypes of common bean and virus strains which may ranging from 0 to 83%. As per report of Sharma *et al*., (2009) BCMV was sap (90%), seed (75%) and aphid (70-80%) transmissible. The results are in conformity with the reports of Udayashankar *et al*. (2012); Puttaraju *et al*., (2004) and Pavitra (2013). As per new report transmission of BCMV and BCMNV may occur through seed or mechanical inoculation, or spread by aphids in a non-persistent manner, with the aphids retaining the virus on their stylets for a limited period of time (Worrall *et al*., 2015).

CONCLUSION

From the results of PCR amplification by BCMV- CP virus specific primer it confirms survival of virus in different parts of the seed, followed by Sanger sequencing it is observed that BCMV- CP infected mungbean sample of GAM-5 variety has viral gene integration and the genomic sequence (GenBank accession No. ON944468) showed highest nucleotide identity of 90-91% sequence similarities with NL-1 strain (KF114860.1) and 94% sequence similarities with MY15-014 strain (MW079241.1) of previously available BCMV- CP virus gene sequence. Grow out test was conducted in insect proof cage under protected condition using BCMV infected seeds of mungbean (Variety GAM-5). It was observed that mild symptoms of BCMV appeared under protected condition. At trifoliate stage serological test was conducted and positive result was obtained. This confirms the presence of BCMV in the seedlings.

Conflict of interest: None.

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