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Symptomatology, Electron Microscopy and RT- PCR in Detection of Dolichos Mosaic Virus Infecting Field Bean (*Lablab purpureus* L.)

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Field bean (*Lablab purpureus* (L.) Sweet) is one of the important pulse crops in tropics. Dolichos mosaic virus (DMV) infected field bean plants produced symptoms like vein clearing, uneven leaf lamina, twisting of leaves, mosaic mottling, puckering and blistering on newly formed trifoliate leaves. The purified preparation of the virus under electron microscopy revealed the presence of long flexuous rod shaped particles approximately measuring ~750 nm in size. RT-PCR product ~ 340 bp amplified by using BCMV specific partial CP gene primers indicated the infection of BCMV in dolichos.

Keywords: *Lablab purpureus* (*L.*), Puckering, DMV, BCMV and Electron microscopy.

Field bean, *Lablab purpureus* L. also called as *Dolichos* bean or hyacinth bean belongs to the family Fabaceae and its native of India and mainly cultivated as an inter crop with cereals. It is presently grown throughout the tropical parts of Asia, Africa, West Indies, China and India. Within India, Field bean is cultivated to a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra.

In Southern India, this crop is grown for fresh green pods used as vegetable, dry seeds for preparations of various dishes and the other plant parts as fodder for livestock. The green pods contain a small amount of vitamin A, C, iron and rich in calcium. It contains protein 20- 28 per cent.

Field bean is susceptible to a variety of diseases caused by fungi, bacteria, viruses and nematodes. Among the virus diseases, Dolichos enation mosaic, leaf roll and Dolichos yellow mosaic disease have been reported to occur in India under field condition (Capoor and Verma, 1950). Further, the crop has been reported to be susceptible to a strain of *Bean common mosaic virus*. First Report of *Bean common mosaic virus* infecting Field bean in India was reported by Udayashankar *et al.* (2011). The virus belongs to the genus Potyvirus (family Potyviridae). It causes yield loss upto 40 per cent.

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MATERIAL AND METHODS

To study the symptoms of virus causing mosaic, sap inoculation technique was used. Young leaves of 15-20 days old showing characteristic mosaic symptoms were collected from infected field bean plants washed in tap water to remove the dust particles adhering to them and dried between the folds of blotting paper. The leaves were then macerated in chilled mortar and pestle using potassium phosphate buffer (pestle and mortar pH 7.0, 0.05M) at the rate of 1ml/gm of leaf tissue. The resultant pulp was squeezed through absorbent cotton and the extract thus obtained was used as standard inoculum. Then 8-10 days old healthy seedlings of Field bean were inoculated with sap extracted from diseased plants. Field bean cv. HA - 4 was used for mechanical sap inoculation. The inoculated plants were kept in the glasshouse and symptom expressions recorded

periodically.

Electron Microscopy

The final viral suspension that was obtained by purification was taken for electron microscopic studies. The formvar coated grids were floated on purified viral suspension for 10 minutes. Then, stained the grids with 2 per cent phosphotungstic acid (PTA) for five minutes and allowed to dry. After drying the grids were observed under JEOL 100S transmission electron microscope and taken the picture of viral particles. The length of the virus particle was measured by the following formula.

$$\text{Actual size of the particle (nm)} = \frac{\text{Measured size in mm}}{\text{Magnification}} \times 10,00,000$$

Reverse transcriptase polymerase chain reaction (RT-PCR)

The total RNA isolation from the sample was done using kit method (Shrimpex biotech services PVT.Ltd) according to the prescribed protocol.

The RNA isolated from the plant samples was used for RT-PCR. First c-DNA was synthesized from viral RNA in a 20 µl reaction using RT enzyme. Reverse transcription was carried out in eppendorf thermo cycler. 8µl of template RNA + 2 µl of reverse primer were added into PCR tube. The mixture was incubated at 70°C for 5 min in PCR machine. Mixture was immediately chilled on ice for 2 min. All the reaction component including 1X BIOSCRIPT reaction buffer (BIOLINE), 1µl 10mM dNTPs, 2 µl dTT and 3 µl of DEPC water were added into the tube and the tube was incubated at 37°C for 1 min. 2 µl of RT enzyme was added to the tube and incubated at 42°C for 60 min. The reaction was stopped by heating the mixture at 70°C for 10 min and stored at -20°c. The c-DNA thus obtained was used for performing PCR.

The c-DNA synthesized by reverse transcription was amplified by PCR. PCR reaction mixture of 25µl was prepared as follows: Eppendorf tubes of 0.2 ml were selected, labelled and kept on ice crystals. Samples were taken for PCR along with positive control (virus c-DNA) and negative control (Distilled water). 25µl of reaction mixture was prepared by adding the following ingredients into the eppendorf tube.

Reagents	**Volume**
c-DNA	2.0µl
Forward primer (20pmol/µl)	2.0µl
PCR Master mixture	12.5 µl
Deionised nuclease free water	8.5µl
Total	25.0µl

Primers

BCMV Primer 1 (Reverse)	Rv3-52 - GGTTCTTCCGGCTT ACTCATAAACAT-32
BCMV Primer 2 (Forward)	FW3-52 -GCAGTAGCACAGA TGAAGGCA-32 :

The PCR amplification was carried out in a thermal cycler (Eppendorf) with the following conditions; Initial denaturation at 94°C for 5 min followed by 40 cycles having the following parameters 30 sec of denaturation at 94°C, 30 seconds of annealing at 61°C and extension for 1 min at 72^0C followed by a final extension for 10 min at 72^0C. Amplified DNA fragments were electrophoresed in 0.8 per cent Agarose gel.

The electrophoresis apparatus connected to the power supply and electrophoresis was carried out at 50 V for 3 hr or up to deep blue dye migrated to the end of the gel. It was then visualized and documented by Alpha digidoc 1000 system (Alpha Innotech Corporation, USA).

RESULTS AND DISCUSSION

The symptoms produced by the virus on field bean plants cv. HA- 4 Mechanical/sap inoculated under glass house conditions appeared 8 - 10 days after sap inoculation in the form of vein clearing, vein banding, uneven leaf lamina, and twisting of newly emerged trifoliate leaf followed by mosaic mottling, puckering and blistering (Plate No. 1).

The symptoms produced by the present causal virus on field bean plants were similar to those described by Udayashankar *et al.* (2011). Basavaraja and Keshava Murthy (1992) reported that a virus disease of Field bean characterized by uneven leaf lamina, twisting of leaves, vein clearing, mosaic mottling and puckering was investigated. Capoor and Sawant (1986) with Lima bean mosaic virus, Nariani and Pingaley (1960), with *Soybean mosaic virus*; Pudashini *et al.,* 2013 with Sweet bean plants infected by *Bean common mosaic necrosis virus*.

Electron microscopic studies revealed the presence of flexuous rod shaped particles measuring approximately ~750 nm in length in diseased samples of field bean which were not found in healthy samples. These particles have morphology similar to those of Potyviruses (Plate No. 2). The above result confirmed with the reports of several workers, who reported rod shaped flexuous virus particles in their studies {(Udayashankar *et al.* (2011), Bharadwaj *et al.* (1990), and Sharma Deepti and Chalam (2009)}

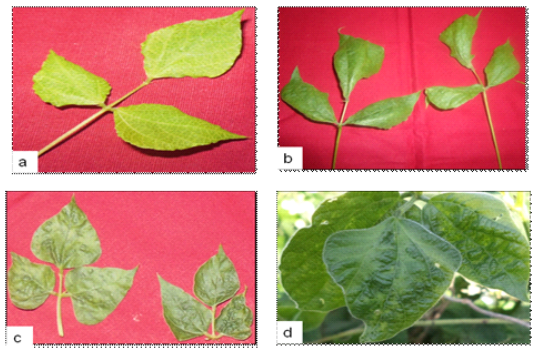


Plate 1. Field bean plants showing different symptoms under green house condition a) Vein clearing b) curling and uneven leaf lamina c) Blistering d) Mosaic mottling symptoms

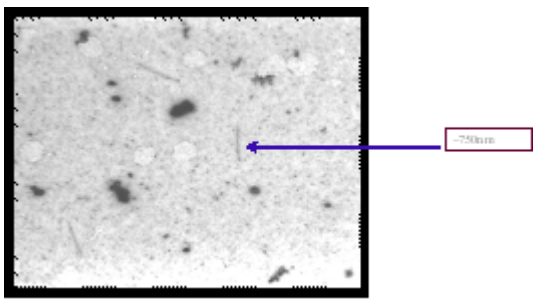
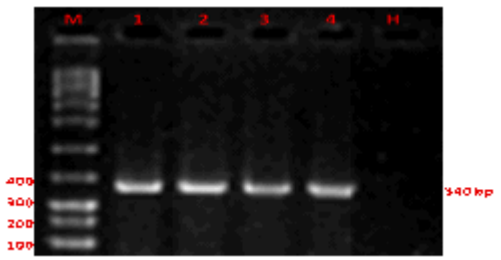


Plate 2. Flexuous virus particles of DMV infects field bean as seen under Electron Microscope (Magnification of 10,000X)



Lane M- 100 bp Marker
Lane 1, 2, 3 and 4 BCMV infected samles
Lane H - Field bean healthy leaf sample

Plate 3. Gel picture showing amplification of partial CP gene in BCMV infected Leaf samples

The PCR successfully amplified the partial CP gene of ~340bp from infected field bean leaf samples. The amplicon of partial CP genes were confirmed by electrophoresis and ~ 340 bp bands were found confirming the presence of partial CP gene (Plate No.3) and no amplification obtained in healthy leaf samples.

The results are in conformity with the reports of Udayashankar *et al.* (2011) reported that an immune capture reverse transcription (IC-RT)-PCR assay employing degenerate primers for amplifying partial coat protein (CP). RT- PCR was performed with a virus-specific primer pair designed to amplify 340 bp, the partial coat protein gene of DMV.

Based on the above studies such as symptomatology, electron microscopy and molecular detection through PCR, it was concluded that the mosaic disease in dolichos is caused by a strain of *Bean common mosaic virus* belongs to potyvirus group.

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