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Molecular Detection of Yellow Mosaic Virus Infecting Legumes

D. Ashwini

Department of Plant Pathology, JNKVV, Jabalpur, India.

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The DNA of whitefly transmitted (WFT) geminivirus was amplified from a viral template present in infected leaves by Polymerase Chain Reaction (PCR) by using degernative primers (PBL1v2040 and PCRc154)that amplify viral DNA-B fragment of approximately500-650bpfor detection of mungbean yellow mosaic virus.

Key words: Legumes, Polymerase Chain Reaction, degenerate primers.

Yellow mosaic diseases (YMD) are major constraints in improving the productivity of grain legumes in India. Yield loss per annum due to YMD was estimated to be $ 300 million taking blackgram, mungbean and soybean together[1]. YMD of soybean was first observed in North India[2,3] as early as 1970s and since then it had spread at alarming proportions. Whitefly transmission, enzyme linked immunosorbent assay (ELISA) and immuno-specific electron microscopic (ISEM) studies suggested that the aetiological virus causing YMD in soybean is a begomovirus of the family Geminiviridae. Begomoviruses have characteristic icosahedral geminate particles that encapsidate genome of circular single-stranded DNA. They infect dicots and are transmitted by the whitefly *Bemisiatabaci*, Gennadius. They have monopartite or bipartite genome. In bipartite begomoviruses, DNA A encodes proteins required for replication, transcription and encapsidation whereas DNA B encodes proteins required for movement functions[4].

The diseased plants show alternating green and yellow patches. Leaf size is generally not affected, but sometimes the green areas are slightly raised and the leaves show a slight puckering and reduction in size. The leaves become paper white and thin in case of black gram. PCR and degenerate primers have been used for general detection of begomo viruses[5]. The total DNA obtained was used as a templateto detect the YMV by PCR using degernative primers (PBL1v2040 and PCRc154) that amplify viral DNA-B fragment of approximately 500-650bp for detection of mungbean yellow mosaic virus.

* To whom all correspondence should be addressed.

MATERIALS AND METHODS

DNA isolation

The DNA of YMV infected legumes (Soybean, Urd bean, French bean, Dolichos bean)samples was extracted from leaves using the modified CTAB method[6]. Infected plant material (100mg) was ground in a pre-sterilized pestle and mortar in the presence of ice flakes until a fine powder was obtained and transferred to sterile eppendorf tube. To this added 1ml of pre-heated (65ºC) extraction buffer (100 mMTris (pH 8.0), 5 MNaCl, 20 mM EDTA, 10% CTAB, 2% Mercaptoethanal) and incubated for 30min in water bath at 65ºC. Then tubes were centrifuged at 10,000 rpm for 10 min at 4°C temperature and the supernatant was collected into eppendorftubes. To this added 0.7 volume of chloroform and Isoamyl alcohol (24:1) and mixing was done by inverting the tubes 10-20 min to form emulsion. Then centrifuged the tubes at 10,000 rpm for 10 min,

separated the supernatant and added 0.8 volume of cold isopropanol to precipitate nucleic acid. Then Centrifugation was carried out @ 10,000 for 10 min (temperature 4°C). Pellet was washed with 70% cold ethanol and centrifugation was carried @ 10,000 for 10 min (4°C) and the ethanol was completely removed and DNA pellet was dried at 37°C. The pellet was dissolved in 50-70 µl sterile distilled water

Purification of DNA

Purification of DNA includes removal of RNA, proteins, lipids, tannins, polyphenols. The genomic DNA was treated with 1-2 µl RNAse A per 50 µl DNA solution and incubated at 37°C min followed by addition of equal amount of phenol: chloroform: isoamyl alcohol (25:24:1) and mixed gently by inverting tube. Centrifugation is done @ 10,000 rpm for 10 min at room temperature and the top aqueous was transferred to 1.5 ml centrifuge tube, 0.6 volume of cold isopropanol was added after adding 0.1 volume of 3M solution of sodium acetate (pH 5) and mixed by inversion to precipitate nucleic acid. Incubated at -20°C for 2 hours. Centrifugation is carried out @ 10,000 rpm for 10 min at 4°C and supernatant was poured out followed by wash with 70% ethanol and spinning is done at 10,000 rpm for 10 min at 4°C. Ethanol was completely removed and DNA pellet was dried by leaving the tubes uncovered at 37°C for 5 min. The pellet was dissolved in 40µl sterile double distilled water and stored at -20 or -70°C.

Polymerase Chain Reaction (PCR)

A degenerate primer is a mixture of molecules in which the nucleotides at one or more defined positions vary by design. A degenerate primer for DNA – B, PBL1v2040 was designed to anneal to the complementary sense strand of the replicative form of the BL1 ORF sequence encoding the amino acid sequence CysMetLysI1eAspHisCys, located in the amino terminus region of the predicted protein of the BL1 ORF. PBL1v2040 was paired with the common region complementary sense primer (PCRc154) to amplify a DNA – B fragment.

PCR was performed in 50µl of reaction mixture using 1 µl l template DNA (< 100ng), 5 µl 10X Taq buffer, 4 µl $MgCl_2$ (25 mM), 1 µldNTP mix (10mM), 2 µl primers (PBL1v2040- 5' GCCTCTGCAGCARTGRTCKATCTTCATACA 3' and PCRc154- 5' GGTAATATTATAHCGGATGG 3'), 1µl of Taq DNA polymerase (Fermentas, USA) and 14 µl sterile distilled water. The amplification was performed in a PCR machine (Eppendorf Pro S). The conditions for amplification viral DNA-B fragment (PBL1v2040 and PCRc154- about primers) are; 1 cycle of 94°C for 4min , 30 cycles of 94°C for 1 min, 50°C for 1min,72°C for 3min and 1 cycle of 72°C for 10min.

Analysis of PCR products by Agarose Gel Electrophoresis

Agarose gel electrophoresis of DNA was performed as described by Sambrook *et al*.[7]. The 1% agarose gel (W/V) was prepared by dissolving 1 g of agarose in 100 ml of 1 X TAE buffer. The gel was allowed to cool for some time and then 5 µl of ethidium bromide (0.5 mg / ml) was added and poured into gel casting tray of mini horizontal electrophoresis unit (Hoefer, USA). The DNA samples were mixed with loading dye (Fermentas, USA) and the electrophoresis was carried in 1xTAE buffer at 60V till the dye front reached the lower part of the agarose gel. The migration pattern of the DNA fragments in the gel was recorded using gel documentation system in an auto exposure mode.

RESULTS AND DISCUSSION

Polymerase chain reaction and degenerate primers have been used for general detection of begomoviruses[5]. The Yellow mosaic viral DNA extracted from the samples brought from the fields was amplified in thermocycler using degenerate primers specific for begomovirus (PCRv154 and PBL1v2040), a thick band was observed at 500-650 base pairs. Thus Begomovirus

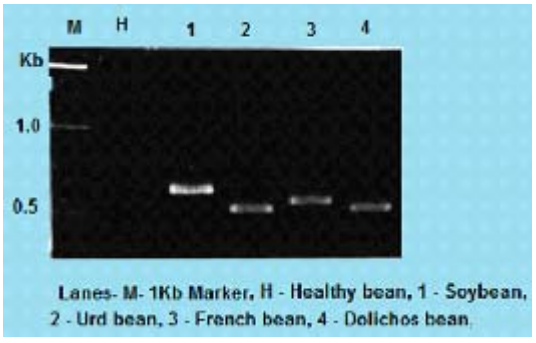

Fig. 1. Detection of yellow mosaic virus in infected hosts using specific (deng) primer in PCR

was detected in the host plants including Soybean, Urd bean, French bean and Dolichos bean (Fig. 1).

The whitefly-transmitted gemini viruses of the genus Begomoviruses are important pathogens of vegetables and fibre crops in subtropical and tropical agro-ecosystems. Since the dramatic increase in population densities of the *B. tabaci* vector in 1970s[8], and later establishment of B-biotype of *B.tabaci*in USA and elsewhere[9], begomoviruses have become recognized as emerging pathogens[10]. The plethora of new and uncharacterized begomoviruses isolated from diverse locations worldwide necessitates the development of an accurate and simple methodology for their rapid and accurate identification. Serology is not suitable for begomovirus characterization because high titre antisera are difficult to prepare and lack sufficient specificity. Consequently the DNA based approaches, including PCR has supplemented serology for detection, identification and classification of begomoviruses

The objective of this study was to develop and optimize a PCR method that permits sensitive and accurate detection method for YMV infecting legumes. The PCR techniques described here allows rapid, sensitive and accurate detection of YMV even if present at low concentrations[9].

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