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Research article

MOLECULAR DETECTION OF YELLOW MOSAIC VIRUS INFECTING BLACKGRAM (*VIGNAMUNGO* (L.) HEPPER) IN ANDHRA PRADESH

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ABSTRACT: The DNA of whitefly transmitted (WFT) geminivirus was amplified from a viral template present in infected leaves by Polymerase Chain Reaction (PCR) by using Coat Protein gene specific primers (RHA F and AC-abut), that amplify a viral DNA fragment of approximately 900bp CP gene product. Various dilutions were prepared and subject to PCR for detection of mungbean yellow mosaic virus. The virus could be detected upto 2.32 ng/µl concentration or 10^{-3} dilutions from infected plants but not in healthy plants. These results indicate that virus could be detected upto 2.32 ng/µl concentration levels by PCR test.

Key words: Blackgram, Polymerase Chain Reaction, CP gene primers.

INTRODUCTION

Mungbean yellow mosaic disease is the most destructive disease in blackgram (*Vignamungo*(L.) Hepper) which causes severe yield losses. It was first reported in 1960 and now occurs throughout the country. 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. Mungbean yellow mosaic virus belongs to the genus *begomovirus* and causes YMD in a number of economically important edible grain legumes including mungbean, urdbean and soybean. In India, it is grown in almost all States, comprising over an area of 3.18 m ha with an annual production of 1.44 m t. and an average yield of 453 kg ha^{-1} (Indian Farming, July 2010). In Andhra Pradesh, the crop is cultivated in an area of 0.421 m ha with an annual production of 0.25 m t. and an average yield of 593 kg ha^{-1} (Directorate of Economics and Statistics, Govt. of A.P., www.Indiastat.com, 2010-11).The virus reported from India is not mechanically transmitted but has been transmitted by the whitefly vector (*Bemisiatabaci*), not only to several species in the leguminosae [5] but also to *Brachiariaramosa* (Gramineae) and *Cosmos bipinnatus*, *Eclipta alba* and *Xanthium strumarium* (Compositae) [6, 7, 9].The MYMV causes 85-100 per cent yield loss in the plants that are infected at the seedling stage (Nene, 1973). DNA-DNA hybridization assays, molecular cloning and DNA sequencing of viral genomes have been used for detection and identification of whitefly transmitted geminiviruses. PCR and degenerate primers have been used for general detection of begomoviruses [12]. The total DNA obtained was used as a templateto detect the YMV by PCR using CP gene primers (RHA F and AC-abut) that amplify a 900bp CP gene product [10]. Various dilutions was prepared and subjected to PCR and YMV could be detected up to 2.32 ng/µl concentration or 10^{-3} dilutions from infected plants but not from healthy plants. Based on the present study we report here under accurate detection method for YMV infecting blackgram in Andhra Pradesh.

MATERIAL AND METHODS

DNA isolation

The DNA of YMV infected blackgram sample was extracted from leaves using the modified CTAB method [4]. Infected plant material (0.5g) was ground in a pre-sterilized pestle and mortar with liquid nitrogen 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), 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP, 0.1% Mercaptoethanal) and incubated for 1 hour in water bath at 65ºC.

Then tubes were centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was collected into eppendorf tubes. To this added equal volumes of chloroform and Isoamyl alcohol (24:1) and 1 µl RNase (100µg/µl) and incubated at room temperature for 10-20 min. Then centrifuged the tubes at 10,000 rpm for 10 min, separated the supernatant and added 0.1 volume of 3M sodium acetate (pH 4.8) and 0.6 volume of ice cold isopropanol then incubated at -20ºC for overnight. After incubation, the tubes were taken out and centrifuged at 13,000 rpm for 20 min at 4ºC. The supernatant was discarded and the pellet was washed with 70% alcohol and again centrifuged at 13,000 rpm at 4ºC for 10 min, discarded the supernatant, air dried the pellets and dissolved in 100µl of sterile distilled water. The DNA samples were quantified by nano drop spectrophotometer and stored at -20ºC for further use.

Polymerase Chain Reaction (PCR)

PCR was performed in 25 µl of reaction mixture using 1X PCR reaction buffer, 2.5 mM of $MgCl_2$, 10mM of dNTPs, 10 pmols of each primer (RHA F-TCAAGCTCCCGGTGCATGTTGCA, AC-abut-GTAAAGCTTTACGCATAATG), 2.5 U/ µl of Taq DNA polymerase (Fermentas, USA) and 100 ng of DNA template. The amplification was performed in a PCR machine (Eppendorf Pro S). The conditions for amplification of coat protein gene (RHA F, AC-abut primers) are; 1 cycle of 95°C for 4min , 30 cycles of 94°C for 1 min, 55°C for 1min ,72°C for 2min and 1 cycle of 72°C for 15min.

Analysis of PCR products by Agarose Gel Electrophoresis

Agarose gel electrophoresis of DNA was performed as described by Sambrook*et al*. [11]. The 1% agarose gel (W/V) was prepared by dissolving 0.5 g of agarose (Axygen, USA) in 50 ml of 1 X TBE buffer. The gel was allowed to cool for some time and then 2 µl of ethidium bromide (10 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 1xTBE buffer at 50V (Labemate Power Pack 300, USA) 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 (Alpha Innotech, USA) in an auto exposure mode.

RESULTS AND DISCUSSION

DNA-DNA hybridization, molecular cloning and DNA sequencing of viral genomes have been used for detection and identification of geminiviruses. Polymerase chain reaction and degenerate primers have been used for general detection of begomoviruses [12]. In the present study an attempt was made to detect the YMV by PCR using CP gene primers (RHA F and AC-abut) that amplify a 900bp CP gene product. Various dilutions (10^{-1} to 10^{-4})corresponding to respective concentration of total DNA (232.7 ng/µl, 23.27 ng/µl, 2.32 ng/µl and 0.232 ng/µl) was prepared and subjected to PCR as described earlier. The PCR results indicates that YMV could be detected up to 2.32 ng/µl concentration or 10^{-3} dilutions and virus was not detected in healthy plants (Fig 1). .

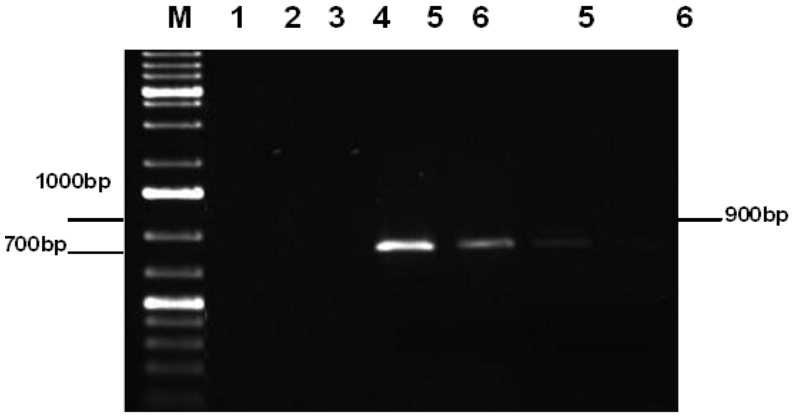

Fig 1. Detection of YMV in blackgram at different concentrations using coat protein gene primers (RHA F and AC-abut).

Lane M: 1 Kb DNA ladder; Lane 1: Healthy, Lane 2:Crude (2327 ng/µl); Lane3 : 10^{-1} dilution (232.7 ng/µl) ;Lane4 :10^{-2} dilution (23.27 ng/µl);Lane 5: 10^{-3} dilution (2.32 ng/µl), Lane 6: 10^{-4} dilution (0.232 ng/µl).

However, 23.27ng/µl could be taken as minimum DNA for detection of YMV infecting blackgram in Andhra Pradesh. From these above results, it is clear that the virus could be detected up to 2.32 ng/µl concentration levels by PCR test. The whitefly-transmitted geminiviruses 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 [1], and later establishment of B-biotype of *B.tabaci* in USA and elsewhere [2], begomoviruses have become recognized as emerging pathogens [3]. 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 blackgram in Andhra Pradesh. The PCR techniques described here allows rapid, sensitive and accurate detection of YMV even if present at low concentrations. [2] Reported detection of squash leaf curl virus even up to 10^{-4} fold dilutions. Ramaprasad and Umaharan [10] employed the standard PCR for detection of begomoviruses from tomato using clarified extract and they detected the virus amplification up to 2^{-8} dilutions.

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