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Molecular Detection of Yellow Mosaic Disease of Greengram Through Polymerase Chain Reaction

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Yellow mosaic disease of greengram is very important viral disease of greengram and it is widely occurred throughout the country and it takes heavy toll in Indian subcontinent and adjacent areas of South-East Asia, causing upto 100 per cent losses in yield if it infects at seedling stage. Initially total DNA of each sample was extracted using modified CTAB method. Further begomovirus coat protein specific forward primer (AV494 (F): 5'GCC(CT)AT(GA)TA(TC)AG(AG)AAGCC(AC)AG 3' (21nt) and reverse primer AC1048 (R): 5' GG(AG)TT(AGT)GA(GA)GCATG(TAC)GTACATG 3'(22 nt)) were used to detect for the presence of virus in naturally infected and artificially inoculated plants through polymerase chain reaction. The PCR products were visualized through gel document unit. A band of approximately 520 bp was consistently amplified from total DNA extracted from infected greengram and inoculated greengram samples, while it was absent in other samples like healthy and water blank.
Key words: *PCR, MYMV, Primers*

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INTRODUCTION

Yellow mosaic disease (YMD), which affects five important pulse crops greengram, blackgram, french bean, pigeon pea and soybean, causes an annual yield loss of about $ 300 million (Varma *et al.,* 1992). Greengram is the third major pulse crop cultivated in the Indian sub-continent. It is highly prone to YMD (Nene 1973) which is a widespread problem because all cultivated varieties are susceptible to the disease. YMD in *V. radiata* (mungbean) was first reported by Nariani (1960). It causes 85–100% yield loss when the plants are infected at the seedling stage (Nene 1973).

Mungbean Yellow Mosaic Virus belongs to the family Geminiviridae consists of viruses with circular, single-stranded (ss) DNA genomes. These are transmitted from plant-to-plant by arthropod vectors with twin particles. Taxonomically the family is divided into four genera based on host range, genome arrangement and insect vector. Those are *Mastrevirus, Curtovirus*, *Topocuvirus* and *Begomovirus*. The genus *Begomovirus* contains viruses that are transmitted by the whitefly (*Bemisia tabaci*) to dicotyledonous plants with *Bean golden yellow mosaic virus* as the type species (Honda and Ikegami 1986, Mandal *et al* 1997). Various isolates of the begomoviruses causing YMD have been placed in two virus species, *Mungbean yellow mosaic India virus* (MYMIV) and *Mungbean yellow mosaic virus* (MYMV) on the basis of nucleotide sequence identity (Fauquet *et al* 2003).

This paper reports the molecular studies on detection of MYMV using polymerase chain reaction to detect the virus in infected plants and artificially inoculated samples of greengram.

MATERIAL AND METHODS

Maintenance of Yellow mosaic virus culture: Greengram plants showing characteristic mosaic symptoms of irregular green and yellow patches in older leaves and complete yellowing of younger leaves, infected plants produce fewer flowers and pods, pods often remain small contain few seeds that are malformed and discolour seed was brought to the laboratory from greengram fields of Main Agricultural Research Station, University of Agricultural Sciences, Raichur, and virus culture was

maintained by inoculating to two leaf stage old healthy greengram seedlings using whiteflies (*B. tabaci*). All process was carried out under nylon net (40 mesh) protected greenhouse.

Maintenance of whitefly culture: Initially, whiteflies (*B. tabaci*) were collected from greengram plants at Main Agricultural Research Station (M.A.R.S), Raichur and the colony was established on freshly grown cotton (*Gossypium hirsutum*) and Brinjal plants kept in an insect proof net house. Four weeks after release, freshly emerged whiteflies were collected using an aspirator and were transferred to healthy cotton plants kept in an insect proof cages. The colony so developed was referred to be virus free (aviruliferous) colony and further same colony was periodically maintained by frequently introducing healthy cotton plants grown in pots (6 ×10 cm) into the insect proof cages maintained in an insect proof polyhouse of controlled temperature of 28 to 30°C.

DNA extraction from host plants

Total genomic DNA of the virus was extracted from the infected field samples as well as artificially whitefly transmitted greengram seedlings from glass house by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Lodhi *et al*. (1994) modified by Maruthi *et al*. (2002). Extracted DNA samples were qualitatively monitored in 1 per cent agarose gel electrophoresis. The concentration was spectrophotometrically determined at OD of 260 nm and 280 nm.

Detection of MYMV Virus Host Plants by Polymerase Chain Reaction:

PCR reactions was performed in a PCR reaction were carried out in 25µl reaction mixture containing 6.0 mM Tris HCl, 2.5mM dNTPs, 20µM of each primer (Coat protein specific forward primer (AV494 (F): 5'GCC(CT)AT(GA)TA(TC)AG(AG)AAGCC(AC)AG 3' (21nt) and reverse primer AC1048 (R): 5' GG(AG)TT(AGT)GA(GA)GCATG(TAC)GTACATG 3'(22 nt), 2.5 units of Taq DNA polymerase (Bangalore Genie Pvt Ltd, Bangalore) were used to detect for the presence of virus in artificially inoculated plants and field collected samples and 10-15 µg of DNA with PCR protocol consisted of 2 minutes at 94°C for initial denaturation followed by 30 cycles of 1 min at 94°C, 1 min at 61°C for annealing, 2 min at 72°C for extension. Finally PCR products were visualized through 1.5% gel electrophoresis.

RESULT

The greengram leaves infected by Yellow Mosaic Disease were brought from field as well as whitefly transmitted seedlings from glass house and subjected to PCR amplification using begomovirus coat protein specific primer. The PCR products were visualized through gel document unit. A band of approximately 520 bp was amplified from total DNA extracted from infected greengram and inoculated greengram samples, while amplification was absent in other samples like healthy and water blank (Figure 1).

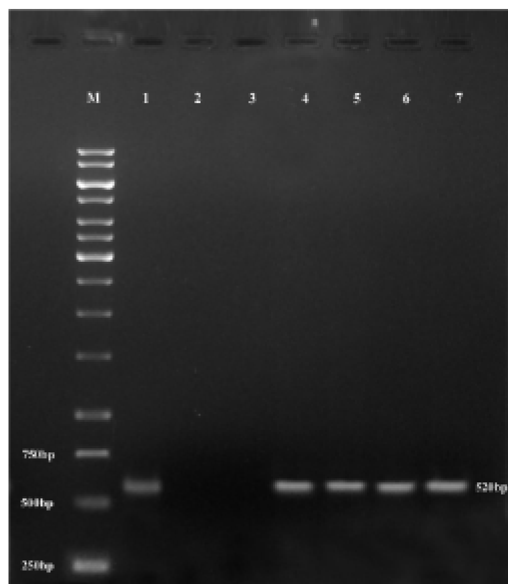

Figure 1. Agarose gel showing PCR products of MYMV obtained with CP primers in greengram in field collected and virus inoculated greengram samples

M- 1kb ladder, Lane 1- Positive control Sunflower leaf curl virus, Lane 2- negative control, Lane 3 – Water blank, Lane 4 and 5- MYMV infected greengram field samples, Lane 6and 7- MYMV inoculated greenngram samples

DISCUSSION

Polymerase Chain Reaction test was employed and amplified PCR product by using begomovirus coat protein specific primers. A band of approximately 520 bp was amplified from CP primers from total DNA extracted from infected greengram and inoculated greengram samples, while it was absent in other samples like healthy and water blank. These primers designed to amplify the conserved region of the CP gene of begomoviruses employed by many workers (Brown *et al*., 2001 and Aswathanarayana *et al*., 2005) to identify and confirm the diseases caused by begomoviruses in wide range of crop plants.

Further, these primers used elsewhere for detection of begomovirus such as Obaiah *et al*. (2014) amplified the DNA of blackgram infected by MYMV through PCR by using coat protein gene specific primer that amplify a viral DNA fragment of approximately 730 bp CP gene product and Govindan *et al*. (2014) revealed virus concentration was proven to be persistently discrete fragments of 703 bp when amplified through PCR in MYMV acquired whiteflies.

CONCLUSION

Molecular detection of MYMV virus on greengram helps us to know the presence of virus in field samples as well as in whitefly inoculated samples this reports that whitefly could transmit the disease from infected plant to healthy plant successfully with characteristic symptoms. Detection of virus is very important because it gives genetic and molecular information and also we can frame best management practices to reduce disease incidence.

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