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Distribution of Two Species of Begomoviruses Infecting Blackgram in Andhra Pradesh

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Authors' contributions

This work was carried out in collaboration among all authors. Author BHC collected diseased samples from different districts of AP, performed the research work as per guidance of author BVBR wrote the first draft of the manuscript. Author BVBR designed the study, analysis of results and corrected the manuscript. Authors LP and RSJD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Yellow mosaic diseases of blackgram is caused by two species of begomoviruses (MYMV, MYMIV). It is known fact that certain varities which are resistant to one species are susceptible to another species when screened by agroinoculations. Hence data on distribution of DNA-A &B components of MYMV / MYMIV under field conditions were not available in Andhra Pradesh.
Methods: In the present study total eighty yellow mosaic infected blackgram samples were colleted from seven districts of Andhra Pradesh during *rabi* and *kharif* 2016-17. The total DNA was isolated and detected for presence DNA-A and B components of MYMV/ MYMIV by PCR with specific primers. Samples which showed negative in normal PCR are further detected by RCA-PCR.
Results: PCR results showed that out of 80 YMD infected blackgram samples tested from seven districts, positve results were otained in 50% samples for MYMV-A, 98.75% for MYMIV-A,and 90% for MYMV-B and none for MYMIV-B. However RCA-PCR detected MYMIV-B presence in all 7

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samples (8.25%) collected from Guntur district, but none from other six districts. These results clearly shows that both species of begoviruses are present in Andhra Pradesh and MYMIV-A is predominant species in Andhra Pradesh as compared to MYMV-A. Hence germplasm and breedig material should be screened for both the species of begomovirses either under field or through agroinoculations to develop YMV resistant blackgram.

Keywords: Blackgram; mungbean yellow mosaic virus; mungbean yellow mosaic India virus; rolling circle amplification; begomoviruses.

1. INTRODUCTION

India is the largest producer and consumer of blackgram (*Vigna mungo* (L.) Hepper. The cultivated area is about 52.79 lakh ha^{-1}, production is 34.92 lakh $tonnes^{-1}$ and productivity is 662 Kg ha^{-1} during 2017-18. The major blackgram growing states in India are Madhya Pradesh, Rajasthan, Uttar Pradesh, Jharkhand, Tamil Nadu, Andhra Pradesh, Maharashtra and Karnataka. In Andhra Pradesh blackgram is cultivated in about 4.03 lakh ha^{-1} during 2017-18. The blackgram is affected several viral diseases, among them yellow mosaic disease (YMD) is major constraint in the productivity and it was first reported by Nariani [1] in mungbean. The YMD is caused by four species of begomoviruses *i.e., Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* (DoYMV) and *Horsegram yellow mosaic virus* (HgYMV) [2]. The genome of begomovirus is bipartite and composed of circular single standard DNA-A and DNA-B of 2.7Kb each in size. The DNA-A encodes proteins required for replication, transcription and encapsidation, whereas DNA-B encodes nuclear shuttle protein and movement protein required for inter and intracellular movement of the virus and both DNA-A&B are required to cause yellow mosaic disease in blackgram and other pulses [3].

Among four begomoviruses causing YMD in southern Asia, MYMIV and MYMV are most important as it infect large number of legumes in India. MYMIV is more predominant in northern, central and eastern regions of India [4] and MYMV in southern region [5,6], coexistence of MYMV and MYMIV were reported from Andhra Pradesh and Tamil Nadu [7,8,9]. In this study we attempted to identify predominant begomovirus species (MYMV/ MYMIV) infecting blackgram in six districts of Andhra Pradesh during *rabi* 2016-17 and in West Godavari district during *kharif* 2016 by PCR amplification of viral genomes with specific primers.

2. MATERIALS AND METHODS

2.1 Sample Collection and DNA Isolation

Yellow mosaic virus infected blackgram leaf samples were collected from seven districts (Kurnool, Kadapa,Chittoor, Nellore, Guntur, Krishna and West Godavari) of Andhra Pradesh during *rabi* and *kharif* 2016-17. Total genomic DNA was isolated by CTAB method [10] and quantified by Nanodrop spectrophotometer.

2.2 Polymerase Chain Reaction

PCR was performed in 25 µl of reaction mixture consist of 1X PCR buffer, 2.5 mM of $MgCl_2$, 10 mM of each dNTPs, 10 p mols of each primer, 2.5 U/µl of Taq DNA polymerase (Thermo Scientific) and 100 ng of DNA template. The amplifications were carried out in Eppendorf Pro thermocycler. The conditions for amplification of coat protein gene of MYMV, MYMIV and movement protein gene of MYMV were optimized and their annealing temperatures were given in Table 1. The amplified PCR products were separated on 1% agarose gel in 1x TBE buffer at 100 V. The banding pattern was documented in gel documentation system (BioRad) List of the four primers used, their annealing temperatures and product size were given in Table 1.

2.3 Rolling Circle Amplification (RCA)

Rolling circle amplification (RCA) was performed using Phi-29 DNA polymerase essentially as described by Packialakshmi et al. [11] in 25 µl reaction volume. About 50 ng of total DNA extracted from YMD infected blackgram samples were mixed with Phi-29 enzyme buffer (1X), 50 µM exo resistant random primer (Thermo Scientific, USA), 1mM dNTPs and denatured at 95°C for 3 min. After cooling the reaction mixture, 0.02 U of inorganic pyrophosphatase (Thermo Scientific, USA) and 10U of Phi 29 DNA polymerase (Thermo Scientific, USA) were added and incubated at 30°C for 20 hours. After

incubation the reaction was stopped by heat inactivation at 65°C for 10 min and amplified product were visualized on 1% agarose gel in 1XTBE buffer at 100V and photographed using gel doc system (Bio Rad).4

3. RESULTS AND DISCUSSION

The DNA isolated from 80 YMD infected blackgram samples that are collected from six districts during *rabi* 2016-17 and from West Godavari in *kharif* 2016 were amplified in PCR with specifc primers (Table.1) for detecting the DNA-A and B components of MYMV/ MYMIV. The results obtained by PCR amplification of 80 samples from seven districts with specific primers during *rabi* and *kharif* season were presented in Table.2 .The PCR amplification with 80 YMD infected blackgram samples revealed that 50% of tested samples were found positive with MYMV-CP primer, 98.75% with MYMIV-CP primer and 90% with MYMV-MP primer (Table 2 & Fig. 1). Efforts to amplify full length MYMIV-B in PCR was not sucessful due to absence or may be due to low concentration of MYMIV-B component in YMD infected leaves. To be infectious on blackgram, both DNA –A and DNA-B components of either MYMV or MYMIV are required [12]. All such PCR negative samples for MYMIV-B were subjected to rolling circle amplification and RCA products were again amplified in normal PCR with MYMIV-B primers (Table 1) and obtained full length 2.7Kb product in 6 YMD samples collected from Guntur district, but not from other districts (Table 2 & Fig. 2).

The distribution pattern of begomoviruses in 7 districts of Andhra Pradesh reveals that MYMIV-A (98.75%) and MYMV-B (90%) are predominant DNA-A & B component in Andhra Pradesh than MYMV-A (50%) and MYMIV-B (8.75%) during *rabi* season. PCR analysis of six samples collected from West Godavari district during *kharif* season revealed that MYMV-A, MYMIV-A and MYMV-B are present in all six samples tested (Table.2) . MYMV-A could not be detected in 12 YMD samples from Nellore and 3 YMD samples from Krishna district in normal PCR, however all 15 YMD samples from both districts were positive for MYMIV-A (Table.2). Further studies on RCA-PCR for Nellore and Krishna district samples were required to detect MYMV-A which could not be performed in this study.

Table 1. List of primers used for amplifying selected regions of DNA-A and DNA-B of MYMV and MYMIV

Primer Name	Nucleotide sequence (5'-3')	Target DNA	Product size	Annealing temp.
MYMV-CP	F-TGGGATCCATTGGTGAACGA R-TACGCACGACCTGATAACGA	DNA-A	700bp	59°C
MYMIV-CP	F-GGTCCCCTGATGTCCCTCGTG R-ATGCGTTCTCAGTATGGTTCT	DNA-A	500bp	55°C
MYMIV-B	F-CGGGATCCAATGATGCCTCTGGCAATTTGTG R-CGGATCCTGGAGATTCAATATCTCAG	DNA-B	2.7Kb	55°C
MYMV-MP	F-ATGGAGAATTATTCAGGCGCA R-TTACAACGCTTTGTTCACATT	DNA-B	900bp	55°C

Source: Reddy et al., 2015; Satya et al., 2015

Table 2. Detection of begomoviruses causing YMD in Andhra Pradesh by PCR with specific primers

Name of the District	No. of samples tested	Season	Amplified in PCR with specific primers			
			MYMV-A (700bp)	MYMIV-A (500bp)	MYMV-B (900bp)	MYMIV-B (2.7Kb)
Kurnool	9	Rabi,	9	8	9	0
Kadapa	11	Rabi,	6	11	10	0
Chittoor	32	Rabi	12	32	25	0
Nellore	12	Rabi,	0	12	12	0
Guntur	7	Rabi,	7	7	7	7
Krishna	3	Rabi,	0	3	3	0
West Godavari	6	Kharif	6	6	6	0
Total samples	80		40	79	72	7
Percentage			50.0	98.75	90.0	8.75

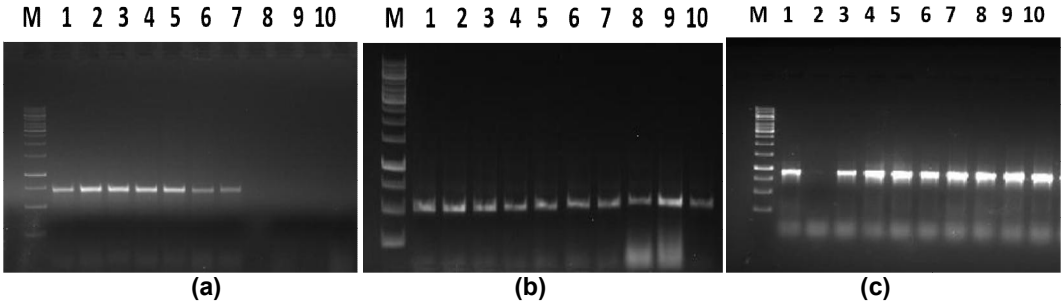

Fig. 1. PCR amplification of YMD infected blackgram samples with MYMV -CP primer-700bp (a), MYMIV-CP primer-500bp (b), and MYMV-MP primer-900bp (c). Lane M:1Kb ladder Lane 1-10:blackgram samples from different regions of Andhra Pradesh

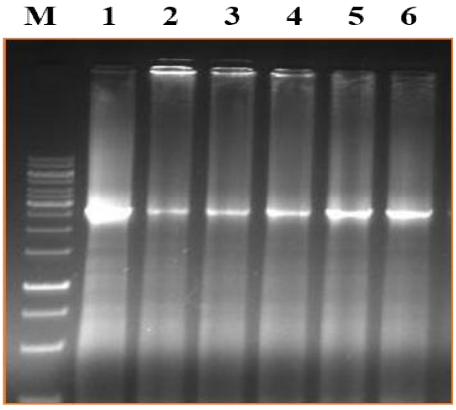

Fig. 2. PCR amplification of full length (2.7kb) MYMIV DNA-B with RCA product of Guntur district with MYMIV DNA-B primer. Lane M: 1Kb ladder, 1-6: RCA products of Guntur district

The negative results in PCR for MYMV-A presence in these two districts (Nellore and Krishna) may be due to low number of samples tested in the present study or absence of MYMV-A in those locations where samples were collected or it may require more sensitive diagnostic methods like qPCR or digital PCR. Hence more number of samples to be tested and RCA-PCR should be performed wherever negative results were obtained in normal PCR for further confirmation. RCA-PCR is a sensitive technique and which do not require PCR machine, can amplify any circular DNA in samples and sucessfully used by several workers in detection of geminiviruses and making full dimers for making infectious constructs in Agrobacterium [13,14,15,16].

Reddy et al. [7] developed duplex PCR technique for detection of begomovirus by designing primers to coat protein gene of MYMIV (500bp) and movement protein gene of MYMV (300bp) from Andhra Pradesh. Association of MYMIV with YMD of blackgram in Andhra Pradesh was reported by amplifying coat protein gene of YMV infecting blackgram [8]. Satya et al. [9] reported the association of MYMIV and MYMV with blackgram from Tamil Nadu. Similarly, Archana et al. [17] reported the association of MYMV with blackgram from Karnataka by amplification of MYMV-CP gene. From the study of three consecutive years, it was reported that MYMV-Urdbean strain was detected in green gram, black gram and soyabean from different states of North India, Similarly, MYMV Vigna strian predominant in South India whereas MYMIV strains were dominant in Eastern India and predominance of MYMIV was reported in Odisha [18] and he also reported the presence of MYMV infecting blackgram from Hyderabad during 2012-2014. Recently differential response of MYMIV / MYMV to certain blackgram genotypes in agroinoculation screening was observed (unpublished) . Hence this kind of studies on distribution DNA-A and B components of MYMV / MYMIV in various seasons across country will

helps the breeder to develop resistant genotypes to both species of begomoviruses .

4. CONCLUSION

Both the species of begomoviruses (MYMIV-A and MYMV-A) were prevalent in Andhra Pradesh during *rabi* and *kharif* seasons in samples collected from seven district during 2016-17. However , MYMIV-A is predominant species in *rabi* season (99.75%) as compared to MYMV-A (50%). Simillarly MYMV-B is predominant DNA-B component as compared to MYMIV-B (8.75%). Further studies on large scale sampling and testing is required to map the distribution of both the species of begomoviruses acroos state and country.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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