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Coat protein based characterization of Mungbean yellow mosaic virus in Tamil Nadu

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Abstract
Yellow mosaic disease caused by Begomovirus (Mungbean yellow mosaic virus (MYMV) or Mungbean yellow mosaic India virus (MYMIV)) has become an important constraint in mungbean production. Survey was conducted on five different mungbean growing localities in Coimbatore district of Tamil Nadu to explore the strain MYMV or MYMIV. PCR based characterization of strains (MYMV /MYMIV) were carried out using their gene specific primers respectively. Distinct viral gene specific PCR product corresponding to CP ~703 bp and MP ~893 bp was obtained for MYMV and no amplification was seen on MYMIV. The sample was further confirmed by CP gene sequencing. The obtained sequence was compared with the other selected begomovirus sequences from the NCBI blast database. Results were found to show high sequence identity to MYMV (100%). Phylogenetic analysis of CP gene sequence of our isolate with selected begomoviruses showed clustered with isolate of MYMV with more homology. While in contrast lesser homology was seen among MYMIV isolate, confirming that the begomovirus causing yellow mosaic disease of mungbean is explored to be as strain of MYMV and not as MYMIV among our geographical location isolates.

Keywords: mungbean, mosaic, survey, characterization, sequence, phylogeny

Introduction
Green gram (*Vigna radiata*) which is widely known as mung, moong, mungo, goldengram and chickasawpea is one of the most important annual pulse crops that are cultivated all around the South East Asian regions for their quality rich proteins. The proteins that are obtained from them nutritionally complement and balance the proteins in food when they are consumed together along with the cereals. In India, the crop is produced under an area of 43.05 lakh ha with the production of 20.70 lakh tones. Important mung bean growing states in India are found to be Rajasthan, Maharashtra, Karnataka, Andhra Pradesh, Odisha, Tamil Nadu and Uttar Pradesh. Inspite of its wide production, the crop undergo severe productivity losses that are majorly aggravated up due to environmental stresses which may be biotic or abiotic in origin. However, the productivity loss in mungbean is mainly due to biotic origin by several fungal and viral diseases which were reported to cause severe reduction in yield [9]. Fungal diseases like cercospora leaf spot, powdery mildew, root rot and viral diseases like cucumber mosaic virus, bean common mosaic virus, alfalfa mosaic virus, yellow mosaic disease were commonly observed in mungbean cultivated fields. Out of these viral diseases, Yellow mosaic disease was found to be the major one which involves Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV). Both MYMV and MYMIV are observed to be the members of the bipartite begomoviruses which composed of circular single-stranded DNA-A and DNA-B components of approx. 2.7 kb and are known to be 'Legumoviruses' [4] and are majorly transmitted by the vector whitefly, *Bemisia tabaci* [2]. The yellow mosaic symptoms due to MYMV /MYMIV appears as irregular alternate yellow and green patches on the leaves over the field. Early screening in breeding programme in India has been against a strain of MYMV, during their multi location trails. But the snag over the above fact in some areas is due to emergence of new strain/species of begomovirus with different virulence (i.e. MYMIV) that might have replaced the original strains [7]. The recurrent recombination among the geminivirus could be the cause, behind the diversity and emergence of new strains [3]. As above mentioned the YMV bipartite genome is composed of DNA A and DNA B genome. The DNA A encodes CP and the DNA B encodes MP. The NSP and MP encoded in DNA B are required for viral cell-to-cell and long-distance movement and play important roles in symptom development and host range [15]. The DNA A encoded coat protein (CP) contacts the cell and delivers the viral genome into plants and actively participates in the replication complex to suppress the innate immune response of the plant host [11].With the above background a field survey was conducted among the mungbean

cultivated areas to explore the differentiating YMV strains (MYMV or MYMIV) in Tamil Nadu with the aid of DNA A and DNA B specific primers.

Materials and Methodology

Collection of plant material

Young leaves from mungbean plants showing severe yellow mosaic symptoms along with respective healthy samples were collected from five different areas of Coimbatore district, Tamil Nadu namely Thondamuthur, Vaithegi falls, Naraseepuram, kuppepalayam, Booluvapatti were used in this research work.

The following experiment after sample collection was conducted at the Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore during 2018 - 2019.

Isolation of genomic DNA

Total DNA was extracted from collected young leaves using modified CTAB method of [14]. Briefly, the symptomatic leaves were ground with 600µl of CTAB buffer and are transferred into an eppendorf tube. After the addition of PVP and mercaptoethanol the tubes were incubated at 65°C (Water bath) for 30 min with occasional mixing. The incubated tubes were removed from the water bath and are allowed to cool at room temperature. Equal volume of chloroform: isoamyl alcohol mixture (24: 1) was added and inverted and incubated at room temperature for 15 min. It was centrifuged at 12000 rpm for 20 min and the clear aqueous phase was transferred to a new sterile tube. To the supernatant equal volume of phenol: chloroform (1:1) was added and centrifuged at 12000 rpm for 20 min and the clear aqueous phase was transferred to a new sterile tube. 1/3 volume of ice-cold isopropanol and 3M Sodium acetate was added to the collected supernatant and incubated in -20 overnight. On the next day, the tubes were centrifuged at 12000 rpm for 20 min to pelletize the DNA and the supernatant was discarded. The DNA pellet was washed with 70 % ethanol. After washing with 70% ethanol the DNA pellet was air dried completely and dissolved in sterile water.

Quality and quantity checks for isolated DNA

For checking purity and intactness, the crude genomic DNA was electrophoresed on 0.8 % agarose gel stained with ethidium bromide (EtBr) and was visualized in a gel documentation system (Alpha ImagerTM1200, Alpha Innotech Corp., CA, USA). DNA was quantified using Spectrophotometer. 1 µl of crude DNA was diluted to 1 ml deionized water. The absorbance for all accessions was measured at 260/280 nm. DNA has maximal absorbance at 260/230 nm. An optical density (OD) of 1.0 corresponds to 40 µg / ml for double stranded DNA.

Dilution of genomic DNA

Dilution of template DNA increased the chances of amplification of the yellow mosaic viral CP/MP specific PCR product. Various dilutions were prepared and subject to PCR for detection of mungbean yellow mosaic virus [8].

Preliminary detection of begomovirus

The diluted genomic DNA was amplified through Polymerase chain reaction (PCR) using univerasal primers for begomovirus namely PALIc1960 and PALIr772 [12]. The products were gel amplified for confirmation in 1.2 % agarose gel stained with ethidium bromide.

Amplification through gene specific primers

After confirmation as begomovirus the isolated DNA from infected plants was subjected to gene specific PCR amplification for DNA A and DNA B. Specific primers for DNA A and DNA B for MYMV and MYMIV (Table.1) were used. Reactions were performed in 20 µl mixture containing approximately 50 ng of genomic DNA, 50µM each dNTPs, 20 pmol of each forward primer and reverse primer and 0.5 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The reaction was carried out in an Eppendorf gradient S Master cycler (Eppendorf, Hamburg, Germany) programmed with initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min. Amplified products were analyzed by electrophoresis in 1.2% agarose gels in TBE buffer and visualized by staining with ethidium bromide and recorded with an Alpha Imager 2000 (Alpha Innotech, San Leandro, CA). The sizes of the PCR products were determined by comparison with standard 1 kb molecular marker (Thermoscientific, catalogue no-#SM0311).

Table 1: List of gene specific primers used in the present study for the characterization of MYMV

Primers	Sequences (5'-3')
MYMIV DNA A F	GTATTTGCAKCAWGTTCAAGA
MYMIV DNA A R	AGGDGTCATTAGCTTAGC
MYMIV DNA B F	ATGGAAAATTATTCAGGTGCA
MYMIV DNA B R	CTACAACGCTTTGTTCACATT
MYMV DNA B F	TTACAACGCTTTGTTCACATT
MYMV DNA B R	ATGGAGAATATTCAGGCGCA
MYMV DNA A F	GATGCATGAGTACATGCC
MYMV DNA B R	GCGGAATTACGATACCGCC

Sequencing and comparison of coat protein gene of MYMV with other selected Begomovirus

Gel amplified PCR product from one isolate were sequenced from out sourcing (Eurofins, Karnataka) for further confirmation using coat protein forward and reverse primers. The phylogenic neighbor-joining trees and evolutionary analysis were conducted using MEGA X software package [5] based on coat protein gene sequences of our isolate with other selected begomovirus sequences downloaded from NCBI Genbank. Robustness of trees was determined by bootstrap sampling of multiple sequence alignment with 1000 replications.

Results and Discussion

Field survey was conducted over the mungbean cultivated areas (Fig.1) and the leaves from both healthy and symptomatic leaves were collected subjected to DNA isolation. A single, unsheared, good quality genomic DNA of ~20 kb in size was observed on running 0.8 per cent agarose gel. The quantity of genomic DNA was determined by taking absorption at 260nm in Nanodrop spectrophotomer and the concentration was found to be ~1.9 µg/µl.

(A)

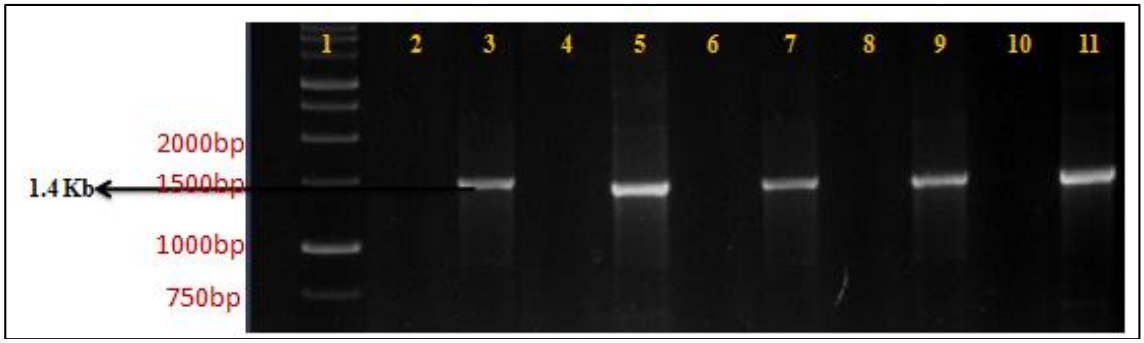
(B)

(C)

a) Mungbean field with Yellow Mosaic disease infected and healthy plants, b) Typical yellow mosaic symptom, c) whiteflies (*Bemisia tabaci*)

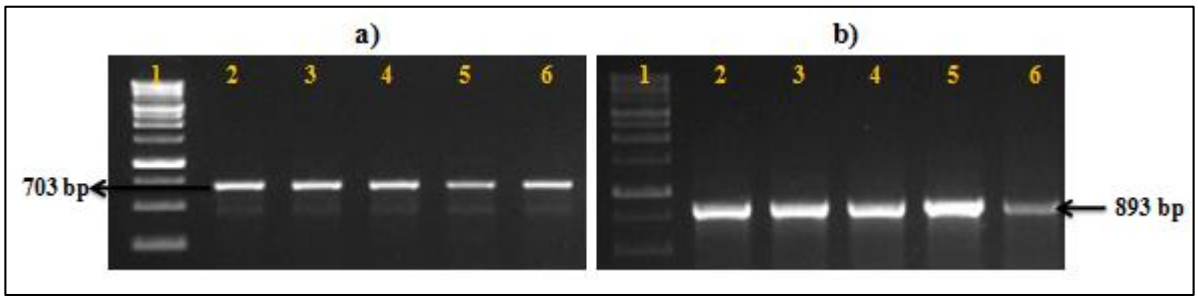
Fig 1: Overview of mungbean field with characteristic YMV symptom

The extracted DNA samples were subjected to universal Roja's primer amplification. The expected band size of about 1.4 kb was seen in symptomatic plants while healthy plants do not showed any amplification (Fig.2). After the above confirmation the symptomatic DNA samples were further characterized for the presence of MYMV or MYMIV. The results were as follows using gene specific MYMV primers (coat protein and movement protein primers) for DNA A and DNA B the expected band size of about 700 bp and 893 bp respectively were amplified in all symptomatic plants (Fig.3). The Yellow mosaic virus was found to be consistently PCR detected in infected samples which was diluted in the ratio 1: 20. Similarly presence of YMV in lima bean was characterized using MP and CP Primers [2]. Coat protein genes were found to be traditionally proven as useful for identification and classification plant virus because of its high degree of conservation. Since, International Committee on Taxonomy of Viruses approves the coat protein sequences for determining the identity of begomo virus [6]. The CP gene is the exclusive structural protein of Gemini viruses that has been observed to play a determinative function in the transmission of the viruses [10].



1. 1Kb ladder, 2.Healthy (Thondamuthur), 3.Infected (Thondamuthur), 4.Healthy (Vaithegi falls), 5.Infected (Vaithegi falls), 6.Healthy (Naraseepuram), 7.Infected (Naraseepuram), 8. Healthy (Kuppepalayam), 9.Infected (Kuppepalayam), 10.Healthy (Booluvapatti), 11.Infected (Booluvapatti).

Fig 2: PCR confirmation of begomovirus using universal primer (Roja's primer)



a) Coat protein primer, b) Movement protein primer. from left to right 1.1Kb ladder, 2. Infected (Thondamuthur), 3. Infected (Vaithegifalls), 4.Infected (Naraseepuram), 5.Infected (Kuppepalayam), 6.Infected (Booluvapatti).

Fig 2: PCR confirmation of begomovirus using universal primer (Roja's primer)

On contrast on observing with MYMIV primers the samples showed no amplification for both DNA A and DNA B suggesting that our isolate belongs to MYMV. For further confirmation the PCR product was sequenced and the obtained nucleotide sequence was blasted using NCBI blast programme. The results showed it was similar to that of MYMV isolate (DQ865201.1).The cluster phylogenetic dendrogram were constructed based on nucleotide sequence of the CP gene of our isolate with other begomovirus and the results showed 100 % homology with MYMV and 98% homology with HgMV and while on comparing with MYMIV our isolate showed lesser homology (Fig. 4). The International Committee on Taxonomy of Viruses (ICTV) accepts the classification of begomoviruses based on CP gene sequences, when full length sequences are not available [13]. Thus the results of the phylogenetic analysis of the present study revealed that coat protein gene obtained from the sequenced sample is a Mungbean yellow mosaic virus (MYMV) but not Mungbean yellow mosaic India (MYMIV) virus.

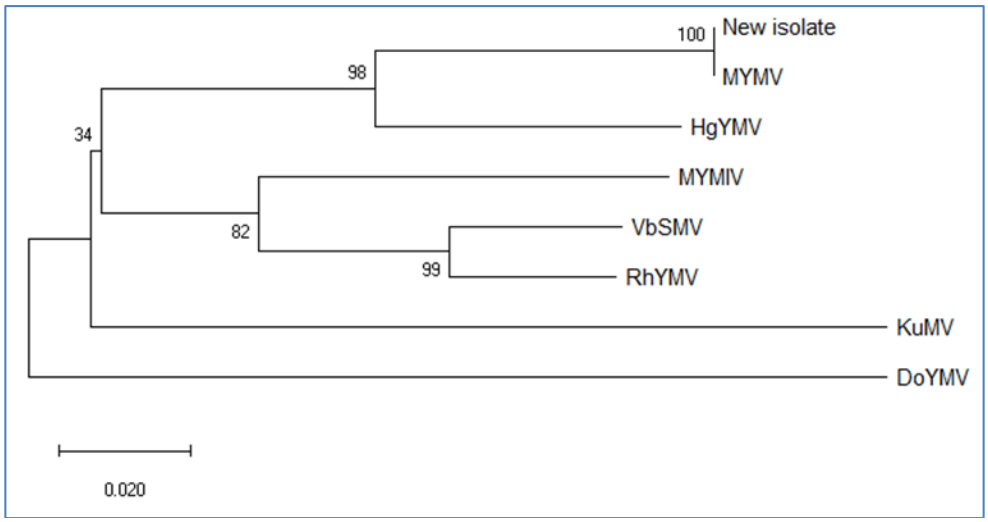


Fig 4: Phylogenetic tree obtained from comparison of complete nucleotide sequence of coat protein gene of MYMV with other begomovirus from database. MYMV - mungbean yellow mosaic virus, HgYMV - horsegram yellow mosaic virus, MYMIV - mungbean yellow mosaic india virus, RhYMV - rhynchosia yellow mosaic virus, KuMV - Kudzu yellow mosaic virus, DoYMV - dolichos yellow mosaic virus.

Conclusion

The above obtained results clearly indicates that the isolate which we have collected in our study is confirmed as MYMV and in future prospects the obtained results can be further utilized for infectious clone construction by which the breeding society can get supported in the disease resistance breeding programme through agroinoculation screening technique.

References

1. Bhagyashree M, Basavaraj S, Prameela HA, Jyoti G, Hurakadli MS, Rangaswamy KT. Coat protein and movement protein based characterization of Lima bean yellow mosaic virus disease in Karnataka. Legume Research: An International Journal, 2017, 40(2).
2. Chhabra SK, Kooner BS. Field resistance in Blackgram (*Vigna mungo* L.) against insect-pest complex and yellow mosaic virus. Indian Journal of Entomology. 1981; 43:288-293.
3. Fauquet CM, Bisaro DM, Briddon RW, Brown JK, Harrison BD, Rybicki EP *et al*. Revision of taxonomic criteria for species demarcation in the family Geminiviridae and an updated list of begomovirus species. Achieves of Virology. 2003; 148:405-421.
4. Ilyas M, Qazi J, Mansoor S, Briddon RW. Molecular characterisation and infectivity of a "legumovirus" (genus Begomovirus: Family Geminiviridae) infecting the leguminous weed *Rhynchosia minima* in Pakistan. Virus Research. 2009; 145(2):279–284. doi:10.1016/j.viruses. 2009.07.018
5. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 2018; 35(6):1547-1549.
6. Mayo MA, Pringle CR. Virus taxonomy-1997. J. Gen. Virol. 1998; 79:649-657.
7. Nair RM, Gotz M, Winter S, Giri RR, Boddepalli VN, Sirari A, Boopathi M. Identification of mungbean lines

with tolerance or resistance to yellow mosaic in fields in India where different begomovirus species and different *Bemisia tabaci* cryptic species predominate. European journal of plant pathology. 2017; 149(2):349-365.
8. Obaiah S, Bhaskara Reddy BV, Eswara Reddy NP, Siva Prasad Y. Molecular detection of Yellow Mosaic Virus infecting Blackgram [*Vigna mungo* (L.) hepper] in Andhra Pradesh. Int. J. Pl. Ani. Envi. Sci., 2013, 4.
9. Paul PC, Biswass MK, Mandal D, Paul P. Studies on host resistance of mungbean against Mungbean Yellow Mosaic Virus in the agro-ecological condition of lateritic zone of west Bengal. The Bio scan. 2013; 8(2):583-587.
10. Pradeep Sharma, Narayan Rishi, Malathi VG. Molecular cloning of coat protein gene of an Indian Cotton Leaf Curl Virus (CLCuV-HS2) isolate and its phylogenetic relationship with other members of geminiviridae. Virus Genes. 2005; 30:85-91.
11. Reichert VL, Choi M, Petrillo JE, Gehrke L. Alfalfa mosaic virus coat protein bridges RNA and RNA dependent RNA polymerase in vitro. Virology. 2007; 364:214 226.
12. Rojas MR, Gilbertson RL, Russell DR, Maxwell DP. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted Gemini viruses. Plant Disease. 1993; 77:340-347.
13. Rybicki EP. A proposal for naming Gemini viruses: A reply by the Gemini viridae study group chair. Achieves of Virology. 1998; 143:421-424.
14. Sudha Manickam. DNA isolation protocol for *Vigna radiata* with free of phenolics. Nature, Protocol Exchange, 2009.
15. Zhou YC, Garrido-Ramirez ER, Sudarshana MR, Yendluri S, Gilbertson RL. The N-terminus of the Begomovirus Nuclear Shuttle Protein (BV1) Determines Virulence or A virulence in Phaseolus vulgaris. MPMI 2007; 20:1523-1534.