Eco. Env. & Cons. 28 (May Suppl. Issue) : 2022; pp. (S300-S305)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i03s.045

Molecular characterization of virus species causing yellow mosaic disease of redgram in Andhra Pradesh

B.H. Chaithanya[1]*, B. V. Bhaskara Reddy[2], L. Prasanthi[2] and R. Sarada Jayalakshmi Devi[3]

[1]Regional Agricultural Research Station, Nandyal, ANGRAU, India
[2]Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, ANGRAU, India
[3]S.V. Agricultural College, Tirupati, ANGRAU, India

(Received 30 September, 2021; Accepted 30 October, 2021)

ABSTRACT

Redgram is animportant drought tolerant leguminous crop in the semi-arid subtropics. Many fungal, bacterial and viral diseases are major constraints for redgram production, apart from all these diseases, yellow mosaic disease (YMD) is observed in redgram crop in few localities of Chittoor district, Andhra Pradesh during 2017. The present study was aimed to find out associated begomo virus species causing YMD in redgram.The total genomic DNA was isolated from YMV infected redgram leaves by modified CTAB method. The rolling circle amplification based full length clones were constructed in pUC18 vector. The two constructed clones (DNA-A and DNA-B) were confirmed by using gene specific primers and were sequenced.The coat protein gene andcomplete nucleotide sequence analysis was carried out with the known begomoviruses sequences obtained from Genbank database.The phylogenetic analysis of coat protein gene of YMV-AP-Red gram isolate shared 94.3-98.1% nucleotide identity with HgYMV isolates, followed by MYMV (81.2-85.2%), MYMIV (78.1-80.2%) and DoYMV(72.2-74%). Hence it was confirmed that viral species causing YMD in redgram from Tirupati, AndhraPradesh is a Horsegram yellow mosaic virus and it is a variant of HgYMV as it shared>94% similarity with other HgYMV isolates at nucleotide level.

Keywords: *Redgram, yellow mosaic disease, Horsegram yellow mosaic virus and rolling circle amplification*

Introduction:

Redgram is an important rainfed and drought tolerant crop which is suitable for both deep and shallow soils. The cultivated area of redgram crop in India is around 53.87 lakh ha with a production of 904 lakh tonnes during 2017-18 as per reports from Directorate of pulse development in India. The yellow mosaic disease of redgram (YMD) was first reportedby Williams *et al.* (1968). Further, the causal agent of YMD in redgram was identified as mungbean yellow mosaic virus (MYMV) on the basis of vector (*Bemisiatabaci*) transmission and symptomatology (Nene *et al.*, 1971) and considered it as one of the important hosts of MYMV in other cultivated legumes (Nene *et al.*, 1973). Yellow mosaic disease of redgram was first reported in southern and northern states of India *i.e.* Delhi, Uttar Pradesh and Andhra Pradesh. The yellow mosaic virus of redgram was transmitted by *B. tabaci* to *Glycine max* (soyabean), *Macrotyloma uniflorum* (horse gram), *Phaseolus lunatus* (lima bean) and *Vigna radiata* (green gram) (Muniyappa and Veeresh, 1984). The symptomology of yellow mosaic disease in red gram was characterized by small yellow diffused spots on the leaves which gradually enlarged to form broad yellow patches (Maramorosch and Muniyappa, 1981).

Recently, yellow mosaic disease was observed on few redgram cultivars in Tirupati region of Andhra Pradesh during kharif 2017. In the present study, we attempted to detect and characterize the causal agent associated with YMD in redgram.

Materials and Methods

Sample collection and nucleic acid extraction

Red gram plants showing yellow mosaic symptoms were collected from Regional Agricultural Research Station (RARS), Tirupati, Andhra Pradesh during 2016-2017. The genomic DNA was extracted from infected leaf samples using modified CTAB method. The Quantity of DNA was calculated at 260 nm and 280 nm using Nanodrop and quality of extracted DNA was checked by agarose gel electrophoresis.

Polymerase Chain Reaction

PCR was performed in 25 µl of reaction mixture using 1X PCR reaction buffer, 2.5 mM of $MgCl_2$, 10 mM of dNTPs, 10 pmols of each primer, 2.5 U/ µl of Taq DNA polymerase and 10-20ng of DNA template. The amplification was performed in a PCR machine. The conditions for amplification of coat protein and movement protein specific primers for HgYMV are; 1 cycle of 95 °C for 4 min, 30 cycles of 94 °C for 1 min, 55 °C for 1 min , 72 °C for 2 min and 1 cycle of 72 °C for 15min. PCR products were analyzed on 1% agarose gel electrophoresis in 1X TBE buffer. The primers used for amplification was given in Table 1.

Cloning and sequencing of full length clones

Rolling circle amplification (RCA) was performed using extracted DNA samples from YMV infected redgram samples as per protocol developed by Packialakshmi *et al*, 2010. The amplified product was digested with BamH1 (1U/µl) and HindIII (1U/µl), the resultant 2.7kb gene fragment was cloned in pUC18 vector and recombinant clones were confirmed by gene specific primers and restriction digestion analysis. The confirmed clones were sequenced at automated DNA sequencing facility by PCR walking. The Sequence data were assembled and analysed using the software programme Bio Edit v 7.2.5. The phylogenetic tree was constructed using the neighbour-joining method with bootstrapping (1000 replicates) in Mega software version 7.0.

Results and Discussion

PCR detection, Cloning and Sequencing of DNA-A and DNA-B

The total genomic DNA was isolated from the yellow mosaic virus infected red gram leaves using modified CTAB method. The isolated DNA samples from infected leaves were used for detection of begomoviruses by Polymerase Chain Reaction using specific primers (Table 1). An expected band size of approximately 1000 bp, 2.7kb and 500bp was amplified with HgYMV-CP-F and vR, HgYMV-A-F and R and HgYMV-MP-F and R primersrespectively (Fig. 2). Further, RCA was performed with isolated DNA from infected samples. The RCA product was digested with Hind III and BamH1 enzymes which results approx. 2700bp length and cloned in pUC 18 vector and transformed into DH5⟨ cells of *Escherichia coli.* Both DNA-A and DNA-B full length clones of red gram were identified by using gene specific primers and confirmed by restriction digestion analysis.

Sequence Analysis

Table 1. List of oligonucleotide primers used for amplification of HgYMV

Primer Name	Nucleotide sequence (5'!3')	Target molecule	Product size	Annealing temperature	Reference
HgYMV-CP-F	ATGCTTGCAATTAAGTAC TTGCA	Coat Protein	1000bp	55 ^{0}C	Naimuddin and Akram (2010)
HgYMV-CP-R	TAGGCGTCATTAGCATAGGCA				
HgYMV-A-F	ATCATACTGAGAACGCTTTG	Full length DNA-A	2.7kb	55 ^{0}C	Anburaj *et al.* (2010)
HgYMV-A-R	TGTCATACTTCGCAGCTTC				
HgYMV-MP-F	AAGAACGACGAAGAGA	Movement protein	500bp	55 ^{0}C	Anburaj *et al.* (2010)
HgYMV-MP-R	GTGTCGTCCTTGAGGGAA				

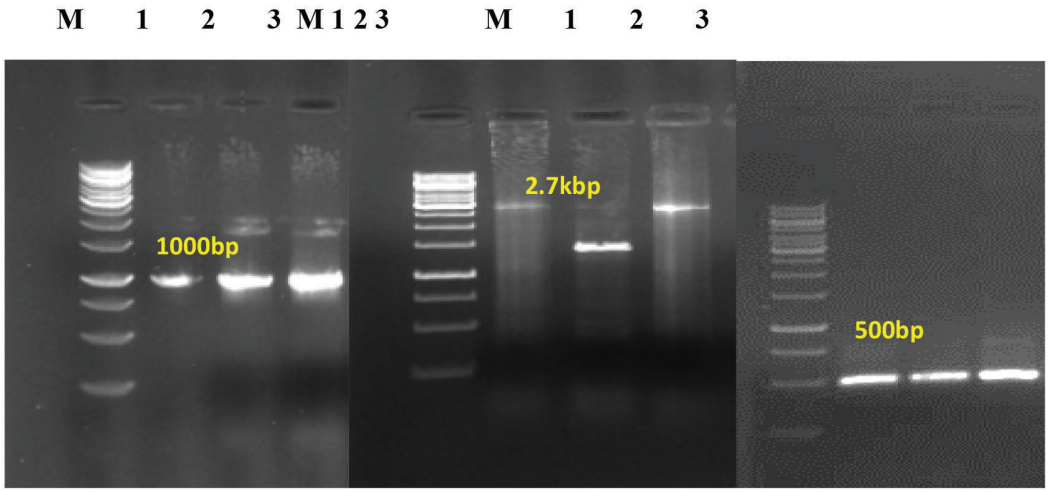

Fig. 1. Detection of HgYMV in infected redgram leaf samples by PCR amplification of coat protein and full-length gene of HgYMV (A&B) and movement protein gene of HgYMV (C).
Lane M: 1Kb ladder and Lane 1-3: DNA samples from YMV infected redgram leaves.

The complete nucleotide sequence of the DNA-A (2736bp) and DNA-B (2674bp) components ofYMV-AP-Red gram isolate were determined in both orientations and sequence data were assembled and analyzed using the Bioedit software (version 7.2.5) and submittedin NCBI (Accession numbers DNA-A: MW284411, DNA-B:MW284412).

The DNA-A of YMV-AP-Red gram encodes seven predicted open reading frames (ORF), two in the virion sense *i.e* AV1 encodes coat protein and AV2 encodes a protein of unknown function, five ORFs in complementary sense *i.e.* AC1 encodes replication protein, AC2 encodes transcriptional activator protein, AC3 encodes replication enhancer protein, AC4 and AC5 of unknown function. Similarly, The DNA-B of YMV-AP-Red gram encodes two predicted ORFs, one in the virion sense (BV1) encodes nuclear shuttle protein (NSP) and another in complementary sense (BC1) encodes movement protein.

The phylogenetic analysis based on full length coat protein gene and full lengthcomplete nucleotide sequence of yellow mosaic virus infecting redgram was carried out with other known begomoviruses sequences obtained from GenBank database. Gen Bank accession numbers of different begomoviruses used for sequence comparison and phylogenetic analysis of DNA-A and DNA-B component of YMV-AP-Red gram isolate were presented separately in Table 2 and 3.

The complete nucleotide sequence of the DNA-A component of YMV isolate infecting Red gram in Andhra Pradesh with other sequences of begomoviruses from database showed that it has close association (96.5-99.4%) with HgYMV isolates. The constructed phylogenetic tree forms four unique clusters representing HgYMV-Horsegram yellow mosaic virus, MYMV-Mungbean yellow mosaic virus,MYMIV-Mungbean yellow mosaic India virus and DoYMV-Dolichus yellow mosaic virus isolates (Fig. 2). Similar phylogeny results were observed in comparison of coat protein gene of YMV-AP-Red gram with other begomoviruses. The AV1 (coat protein) gene sequence analysis of YMV-AP-Red gram isolate with other begomoviruses shared 94.3-98.1% identity with HgYMV isolates, followed by MYMV (81.2-85.4%), MYMIV (78.1-79.7%) and DoYMV (72.4-74%) isolates (Table 2). The CP gene is the most highly conserved gene in the family Geminiviridae. Coat protein genes have traditionally proven useful for plant virus identication and classication (Mayo and Pringle 1997).

The nucleotide identity at coat protein or complete DNAA sequence is 94–100% will be considered as variant and 85–93% will be considered as strain of same virus and and<85% will be considered as distinct species in Begomovirus classication (Fauquet *et al*. 2008). Asper these guidelines, the present YMV-AP-Redgram isolate shared >96.5%sequence homology with HgYMV isolates at complete

nucleotide level and >94.3% identity at coat protein gene level. From above results it is clear that virus species causing yellow mosaic disease in red gram from Tirupati region of Andhra Pradesh are closely related to HgYMV than MYMV, MYMIV and DoYMV isolates and it was considered as a variant of previously identified HgYMV. Raj *et al.* (2005) confirmed the association of Tomato leaf curl New Delhi virus (ToLCNDV) with the yellow mosaic disease of pigeon pea in Uttar Pradesh state based on sequence identities of coat protein gene. But in our study, we found that virus species causing YMD in

Table 2. List of begomoviruses nucleotide sequences (NCBI, Gen Bank) used for comparison with nucleotide sequences of yellow mosaic virus DNA-A from Redgram host in Andhra Pradesh and construction of phylogenetic tree

S. No.	Virus Acronym	Accession number	Geographic origin	Host	% nucleotide similarity	
					AV1	Full length
1	HgYMV	KC019306	India	Frenchbean	97.4	98
2	HgYMV	AM932427	India	Cowpea	97.6	98
3	HgYMV	GU323321	Srilanka	Frenchbean	94.3	96.5
4	HgYMV	AJ627904	India	Horsegram	97.6	97.9
5	HgYMV	AM932425	India	Frenchbean	98.1	99.4
6	DoYMV	AM157413	India	Dolichus	73.6	62.9
7	DoYMV	AJ968370	India	Dolichus	74	63.3
8	DoYMV	JX315325	India	Dolichus	72.2	62.7
9	MYMV	AY269991	Pakisthan	Soyabean	84.1	83.9
10	MYMV	JQ398669	India	Urdbean	81.2	83
11	MYMV	JX244176	India	Mungbean	85.2	84
12	MYMV	KC911721	India	Urdbean	84.8	83.9
13	MYMV	DQ400848	India	Urdbean	84.8	83.9
14	MYMV	DQ865201	India	Mothbean	85.4	83.8
15	MYMIV	FM208836	Pakisthan	Mungbean	79.7	82
16	MYMIV	KC019304	India	Frenchbean	79.3	81.8
17	MYMIV	DQ400847	India	Urdbean	79.2	81.7
18	MYMIV	AF126406	India	Mungbean	78.1	75.5

Table 3. List of begomoviruses nucleotide sequences (NCBI, GenBank) used for comparison with nucleotide sequences of yellow mosaic virus DNA-B from Redgram host in Andhra Pradesh and construction of phylogenetic tree

S. No.	Virus Acronym	Accession number	Geographic origin	Host	% nucleotide similarity
1	HgYMV	KC019307	India	Frenchbean	99.1
2	HgYMV	AM932428	India	Cowpea	96.6
3	HgYMV	AM932430	India	Limabean	96.6
4	HgYMV	KP752089	India	Frenchbean	98.3
5	DoYMV	KJ481206	India	Horsegram	48.5
6	DoYMV	KJ481205	India	Horsegram	48.5
7	MYMV	KC911729	India	Urdbean	67.5
8	MYMV	DQ865202	India	Mothbean	69.1
9	MYMV	KC911731	India	Urdbean	68.7
10	MYMV	DQ400849	India	Urdbean	68.9
11	MYMV	KP319016	India	Mungbean	68.9
12	MYMIV	FM955604	Pakisthan	Mungbean	69.2
13	MYMIV	AY271894	India	Mungbean	68.9
14	MYMIV	FM202442	Pakisthan	Mungbean	68.9
15	MYMIV	KC019305	India	Frenchbean	68.4
16	MYMIV	JN543396	Nepal	Frenchbean	68.3
17	MYMIV	AY939925	India	Urdbean	68.4
18	MYMIV	KP828155	India	Soyabean	68.6

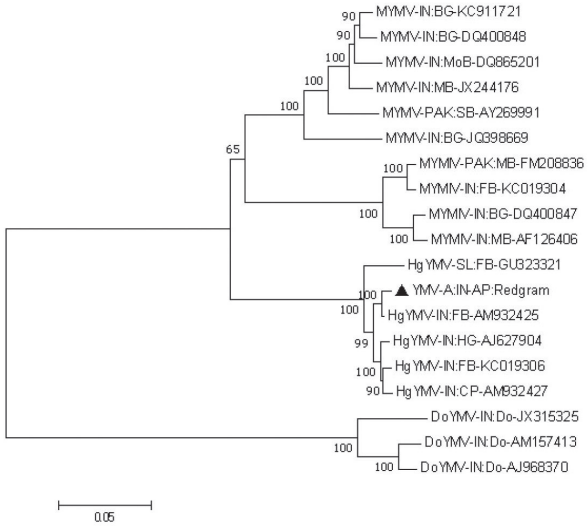

Fig. 2. The phylogenetic tree constructed from comparison of complete nucleotide sequence of DNA-A component of yellow mosaic virus from red gram host in Andhra Pradesh with other begomoviruses. Mega 7.0 version was used for construction of phylogenetic tree.

redgram is closely related to HgYMV. Similarly, manjunatha *et al.* (2015) reported that the virus infecting redgram in Karnataka was identified as HgYMV based on nucleotide sequence analysis of coat protein gene, it shared maximum nucleotide identity (98%) with previously identified strains of HgYMV from southern India followed by MYMV (87%).

The complete nucleotide sequence analysis of DNA-B component of YMV-AP-Redgram was done using other begomoviruses from NCBI database (Table 3) and phylogeny tree was constructed (Fig. 3). It shared >96.4% homology with HyYMV isolates followed by MYMV (67.8-69.2%), MYMIV (68.3-68.9%) and DoYMV (48.9%) isolates.

Conclusion

In this study, we attempted to find the associated begomovirus species infecting red gram in Andhra Pradesh. International Committee on Taxonomy of Viruses (ICTV) accepts the classification of begomoviruses based on coat protein gene sequences. The nucleotide sequence analysis of coat protein gene revealed that yellow mosaic virus infecting Red gram (YMV-Andhra Pradesh: Red gram) is a Horse gram yellow mosaic virus

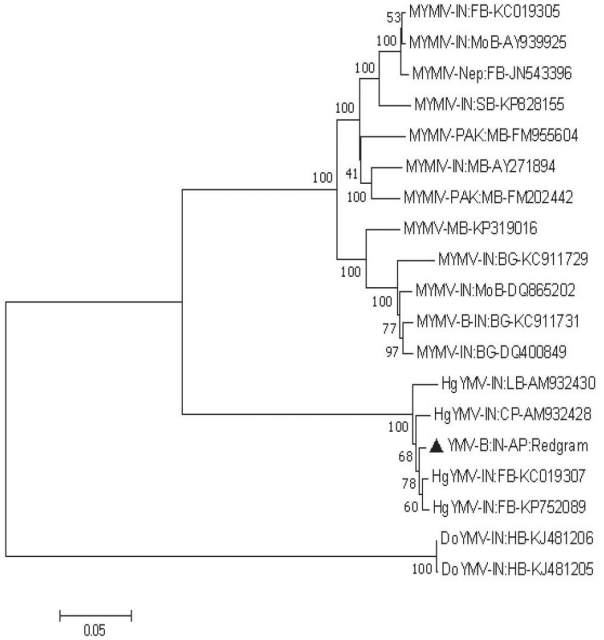

Fig. 3. The phylogenetic tree constructed from comparison of complete nucleotide sequence of DNA-B component of yellow mosaic virus from red gram host in Andhra Pradesh with other begomoviruses. Mega 7.0 version was used for construction of phylogenetic tree.

(HgYMV) since it showed >94.3per cent identity at nucleotide level with other HgYMV isolates.

References

Fauquet, C.M., Briddon, R.W., Brown,J.K., Moriones, E., StanleyJ., Zerbini, M., Zhou, X. 2008. Geminivirus strain demarcation and nomenclature. *Archieves of Virology*. 153 : 783–821.

Manjunatha, N., Noorulla, H., Anjaneya, R., Archana, S and Manjunath, S.H. 2015. Molecular detection and characterization of virus causing yellow mosaic disease of redgram (*Cajanus cajan* L. Millsp) in Karnataka. *International Journal of pure and Applied Bioscience*. 3(4) : 258-264.

Maramorosch, K and Muniyappa, V. 1981. White fly transmitted plant disease agents in Karnataka, India. In *International workshop on pathogens transmitted by whiteflies.* July 31,(Oxford: Association of Applied Biologists, Wellesboume)

Mayo, M.A. and Pringle, C.R. 1997. Virus taxonomy. *Journal of General Virology*. 79 : 649–657.

Muniyappa, V. and Veeresh, G.K. 1984. Plant virus diseases transmitted by whiteflies in Karnataka, Proceedings of the Indian Academy of Sciences (Animal

Science). 93: 397-406.
Nene, Y. L. 1973. Viral disease of some warm weather pulse crops in India. *Plant Disease Reports*.57: 463-467.
Nene, Y. L., Naresh, J. S. and Nair, N. G.1971. Additional hosts of mungbean yellow mosaic virus. *Indian Phytopathology*. 24 : 415-417.
Packialakshmi, R.M., Srivastava, N., Girish, K.R. and Usha R. 2010. Molecular characterization of a distinct *begomovirus* species from *Vernonia cinerea* and its associated DNA-beta using the bacteriophage Phi 29 DNA polymerase. *Virus Genes.* 41 : 135-143.
Raj, S.K., Khan, M.S. and Singh, R. 2005. Natural occurrence of begomovirus on pigeonpea in India. *Plant Pathology*. 54(6) : 809.
Williams, F.J., Grewal, J.S. and Amin, K.S. 1968. Serious and new diseases of pulse crops in India in 1966. *Plant Disease Reports*. 52 : 300-304.