RESEARCH ARTICLE Legume Research- An International Journal, Volume 45 Issue 7: 907-913 (July 2022)

Genetic Variability Studies of Yellow Mosaic Virus Infecting Blackgram [*Vigna mungo* (L.) Hepper] from Andhra Pradesh, India

D. Vijay Kumar Naik, B.V. Bhaskara Reddy, R. Sarada Jayalakshmi Devi, L. Prasanthi, R. Lakshminarayana Vemireddy, Akkari Srividhya, B.H. Chaithanya, T.M. Hemalatha, K. Sailaja

10.18805/LR-4800

ABSTRACT

Background: The productivity of blackgram is affected by many biotic and abiotic stresses. Among the biotic stresses, yellow mosaic disease (YMD) cause severe yield loss and it is caused by four distinct viruses (belongs to genus begomovirus) collectively known as yellow mosaic virus (YMV). Hence there is need to characterize various YMV isolates associated with blackgram in Andhra Pradesh. **Methods:** YMV infected blackgram samples were collected from East Godavari, Kurnool and Prakasam districts of Andhra Pradesh. The Rolling Circle Amplification (RCA) based full length MYMIV DNA-A and MYMV DNA-B of three isolates were cloned and sequenced. **Result:** Nucleotide sequence of full length DNA-A of MYMIV-East Godavari isolate showed >96% similarity at nucleotide and >90% at amino acid level with other MYMIV isolates in NCBI database. The complete DNA-A nucleotide sequence of MYMIV-Kurnool and MYMIV-Prakasam isolates shared >99% similarity at nucleotide and >98% at amino acid level with other MYMIV isolates. The complete nucleotide sequence of MYMV DNA-B of three isolates (East Godavari, Kurnool and Prakasam) had >97% homology with other MYMV DNA-B isolates from database. The predicted amino acid sequence of MYMV DNA-B of three isolates shared >96% homology with other MYMV-B isolates. The common region (CR) sequence similarity between MYMIV-As and MYMV-Bs of East Godavari and Kurnool isolates was 76.4% and 78.3% with Prakasam isolate. The divergence between the MYMIV-A and MYMV-B of present three isolates (East Godavari, Kurnool and Prakasam) under study were ranged from 22.2 to 22.6%.

Key words: Common region, Complete genome sequence, Genetic variability, MYMIV DNA-A, MYMV DNA-B, YMV.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] or urdbean is an important short duration self pollinated pulse crop cultivated in almost all parts of India. In term of area and production, blackgram is the fourth most important cultivated grain legume crop in India after chickpea, pigeonpea and mungbean. In India, the cultivated area is about 46 lakh ha, production is 24.5 lakh tonnes and productivity is 533 Kg/ha during 2020-21 (agricoop.nic.in). The productivity of blackgram is affected by many biotic and abiotic stresses. Many plant pathogens affect blackgram crop production of which, yellow mosaic disease (YMD) cause severe yield loss and it is caused by four distinct viruses (belongs to genus begomovirus) collectively known as yellow mosaic virus (YMV) *i.e. Mungbean yellow mosaic India virus* (MYMIV), *Mungbean yellow mosaic virus* (MYMV), *Horsegram yellow mosaic virus* (HgYMV) and *Dolichos yellow mosaic virus* (DoYMV). Begomoviruses are being transmitted by whitefly (*Bemisia tabaci*) from infected to healthy plants in persistent circulative manner (John *et al.*, 2008). The begomoviruses are icosahedral particles (20×30 nm) having circular single stranded DNA. The majority of members of the geminiviruses have bipartite genome *i.e.*, DNA-A and DNA-B. The DNA-A and DNA-B components of YMV has highly conserved common region (CR) that possesses the origin of replication (ori), promoter, stem loop structure and iterons. The stem loop or hairpin structure will have invariant nonanucleotide (TAATATTACA) which is nicked at T/A position by the Rep protein to initiate the rolling circle amplification (Kumar *et al.*, 2017).

Acharya N.G. Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India.

Corresponding Author: D. Vijay Kumar Naik, Department of Plant Pathology, Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India. Email: dvnaik24@gmail.com

How to cite this article: Naik, D.V.K., Reddy, B.V.B., Devi, R.S.J., Prasanthi, L., Vemireddy, R.L., Srividhya, A., Chaithanya, B.H., Hemalatha, T.M. and Sailaja, K. (2022). Genetic Variability Studies of Yellow Mosaic Virus Infecting Blackgram [*Vigna mungo* (L.) Hepper] from Andhra Pradesh, India. Legume Research. 45(7): 907-913. DOI: 10.18805/LR-4800.

Submitted: 27-09-2021 **Accepted:** 18-01-2022 **Online:** 28-03-2022

In India, MYMV was first reported in infected mungbean fields at Indian Agricultural Research Station (IARI), New Delhi (Nariani, 1960) and YMD on blackgram was first reported by Williams *et al.* (1968). The MYMIV is more common in northern, central and eastern regions of India whereas MYMV in southern and western India (Mishra *et al.*, 2020) but both MYMIV and MYMV species were recorded in Andhra Pradesh (Reddy *et al.*, 2015). In the present article, we have discussed about genetic variability in YMV species associated with different blackgram isolates from three districts of Andhra Pradesh.

MATERIALS AND METHODS

DNA isolation and PCR amplification

During 2019-20, YMV infected (Fig 1) and healthy blackgram

young leaves were collected from geographically different and major blackgram growing areas *i.e.* Kurnool (15.4391101, 78.5101757), Prakasam (15.8238069, 80.3848281) and East Godavari (16.7969001, 81.8620407) districts of Andhra Pradesh (Single isolate from each district was collected). The leaf tissue (100mg) was used for isolation of DNA and DNA from YMV infected and healthy leaf samples were extracted using modified CTAB method (Murray and Thomson, 1980). The total extracted DNA was subjected to PCR by using MYMIV coat protein gene specific primer (F/R: 5′GGTCCCCTGATGTCCCTCGTG3′/ 5′ ATGCGTTCTCAG TATGGTTCT3′) and MYMV movement protein gene specific primer (F/R: 5′ATGGAGAATTATTCAGGCGCA3′/5′ TTACA ACGCTTTGTTCACATT3′). PCR reaction was performed in a 10 µl final volume of mix containing the components of 10x PCR reaction buffer, 2.5 mm of $MgCl_2$, 10 mm of dNTPs, 10 pM of each primer, 2.5 units of Taq DNA polymerase and 100 ng of DNA template. The amplification was performed in a PCR machine (Eppendorf Pro S). The conditions for amplification of target DNA are; 1 cycle of 94°C for 4 min for initial denaturation, 94°C for 30s, 55°C for 45s, 72°C for 1 min extension (35 cycles) and 1 cycle of 72°C for 10 min final extension. The PCR amplified products were analyzed on 1% agarose gel (W/V) electrophoresis.

Rolling circle amplification and gel elution

After confirmation of YMV by PCR, circular genomic DNA of YMV was amplified by rolling circle amplification (RCA) technique (Lizardi *et al.*, 1998). The RCA mixture was performed in 20 µl volume mix containing Target DNA (30 ng/µl), Exo-randem primer (50 µM), Phi29 DNA buffer (1X), dNTPs (10 mM) and Sterile distilled water. The RCA amplified products was analysed in 1% agarose gel and DNA fragments of 2.7 kb from three isolates were extracted from the excised gel slices using QIA quick columns (Qiagen, Germany) kit as per the manufacturer's protocol.

Cloning and sequencing

The eluted 2.7 kb RCA products from three isolates were cloned in *pUC*18 vector and clones containing YMV insert were identified by colony PCR using MYMIV-coat protein (CP), MYMV-movement protein (MP) specific primers and further confirmed by restriction double digestion of plasmid DNA with *BamHI* (5u/µl) and *EcoRI* (5u/µl). The full length 2.7 kb clones of MYMIV DNA-A and MYMV DNA-B were sequenced at automated DNA sequencing facility (Eurofin Genomics India Pvt.Ltd., Bangalore) and sequences were deposited in NCBI GenBank. Both nucleotide and amino acid sequences were compared with other begomoviruses collected from NCBI GenBank database (http://www.ncbi.org) and a phylograms were constructed from aligned sequences using neighbor-joining method and boot strap option using Mega 7.0 software.

RESULTS AND DISCUSSION

The extracted DNA was amplified by PCR with specific primers, yielded 500 bp for MYMIV-A and 900 bp for MYMV-B in all infected samples collected from three districts but not from healthy samples. Both MYMIV and MYMV were reported in blackgram from Andhra Pradesh, Tamil Nadu and Karnataka (Reddy *et al.*, 2015).

Sequence homology and phylogenetic analysis of MYMIV DNA-A

The complete genome sequence of MYMIV DNA-A (2.7 Kb) clones from East Godavari, Kurnool and Prakasam isolates were submitted to NCBI GenBank and obtained accession numbers (East Godavari-MT312254, Kurnool-MT350281 and Prakasam-MT300190). Complete multiple nucleotide sequence alignment of MYMIV-East Godavari (E.G) isolate with other begomoviruses in database showed >96 and >90% similarity with MYMIV-Meghalaya (KU950430) followed by Satna (MF683072) and West Bengal (HF922628) isolates at nucleotide and amino acid level respectively. At nucleotide level, it shared 90.5-96.9% similarity with MYMIV DNA-A, 81.4-85.9% with MYMV DNA-A and 81.6-88.5% with HgYMV. The predicted amino acid sequence of MYMIV-East Godavari isolate shared 81.9-91.4% similarity with MYMIV DNA-A, 58.1-64.1% with MYMV DNA-A and 55.3-69.2% with HgYMV DNA-A. The comparison of complete DNA-A nucleotide sequence of MYMIV-Kurnool (KNL) and MYMIV-Prakasam (PRA) isolates with other sequences collected from NCBI GenBank showed that, both isolates shared maximum similarity (>99%) with MYMIV-Raichur (MN698280) followed by 86% with MYMV-

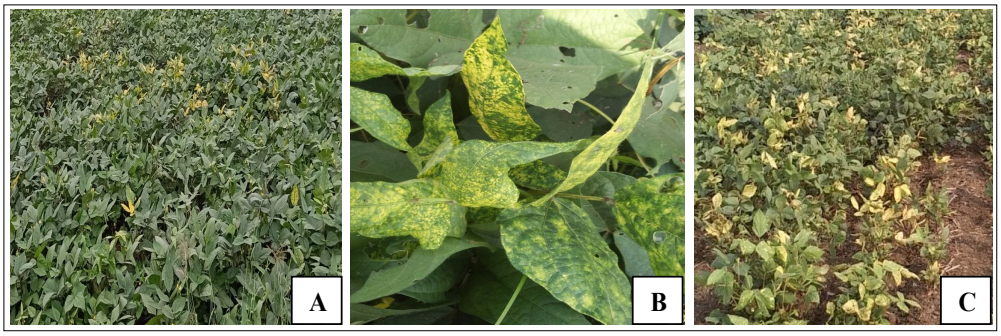

Fig 1: Blackgram showing typical yellow mosaic disease symptoms at different districts in Andhra Pradesh. (A). East Godavari; (B). Kurnool; (C). Prakasam.

New Delhi (JQ398669) and 90.4% with HgYMV-Raichur (MN698282) isolates. The analysis of predicted amino acid sequence of MYMIV-Kurnool and MYMIV-Prakasam isolates showed maximum (>98%) similarity with MYMIV-Raichur (MN698280) isolate followed by 64.4% with MYMV-New Delhi (JQ398669) and 74.2% with HgYMV-Raichur (MN698282) isolates (Table 1).

Among the three isolates under study when compared with each other at nucleotide level, MYMIV-East Godavari isolate shared 95.9% and 95.6% similarity with MYMIV-Kurnool and MYMIV-Prakasam respectively. At amino acid level, MYMIV-East Godavari isolate shared 88.9% and 88.4% identity with MYMIV-Kurnool and MYMIV-Prakasam isolates respectively. The sequence identity between the MYMIV-Kurnool and MYMIV-Prakasam was 99.3% and 98.3% at nucleotide and amino acid level respectively (Table 1). The above results revealed that Kurnool and Prakasam isolates belongs to same variant and East Godavari isolate belongs to different variant. According to ICTV, if coat protein (AV1) of DNA-A or complete nucleotide sequence similarity is 85-93% then they will be considered as strain of same virus and less than 85% homology will be considered as separate species and identity between 94-100% will be considered as isolate or variant (Fauquet *et al*., 2008). The MYMIV-A isolate of three districts shared >94% homology with full length and coat protein gene of MYMIV-A isolates from NCBI database, hence they are treated as same variants of MYMIV. A phylogenetic tree for complete nucleotide sequence of MYMIV-A of East Godavari, Kurnool and Prakasam isolates was constructed. The MYMIV-EG isolate formed cluster with MYMIV-Central and North Indian isolates *i.e.,* Meghalaya (KU950430), Satna (MF683072), West Bengal (HF922628) and Coimbatore (KP313758) isolates. The MYMIV-KNL and MYMIV-PRA isolates form separate cluster with MYMIV-Raichur from Karnataka (MN698280) which is adjoining state to Andhra Pradesh (Fig 2). The members of begomovirus genus are known to form clusters according to geographical origin with distinct branches (Prema and Rangaswamy, 2018).

Sequence homology and phylogenetic analysis of MYMV DNA-B

The complete genome sequences of MYMV DNA-B of three isolates (East Godavari, Kurnool and Prakasam) were submitted to NCBI GenBank (East Godavari- MT312255, Kurnool-MT318837 and Prakasam- MT312256). The nucleotide sequence analysis revealed that, MYMV-East Godavari and MYMV-Prakasam isolates had maximum

Table 1: Complete genome sequence similarity matrix of MYMIV DNA-A of three isolates with other begomoviruses at nucleotide level and amino acid level.

Isolate name	Nucleotide level			Amino acid level		
	E. G	KNL	PRA	E. G	KNL	PRA
MYMIV-IN:E.G-MT312254	ID	0.959	0.956	ID	0.889	0.884
MYMIV-IN:KNL-MT350281	0.959	ID	0.993	0.889	ID	0.983
MYMIV-IN:PRA-MT270290	0.956	0.993	ID	0.884	0.983	ID
MYMIV-IN:Bg-JX110618	0.914	0.907	0.906	0.826	0.816	0.813
MYMIV-IN:Bg-AF126406	0.905	0.904	0.902	0.819	0.816	0.815
MYMIV-IN:Cp-AY937195	0.955	0.942	0.94	0.87	0.836	0.832
MYMIV-IN:Sb-KC852204	0.948	0.949	0.948	0.849	0.855	0.854
MYMIV-IN:Sb-MH324445	0.949	0.948	0.948	0.859	0.861	0.862
MYMIV-IN:Cb-MN698280	0.958	0.994	0.991	0.887	0.986	0.977
MYMIV-IN:Sb-HF922628	0.966	0.965	0.962	0.909	0.9	0.891
MYMIV-IN:Gg-KP313758	0.965	0.961	0.957	0.904	0.889	0.879
MYMIV-IN:Gg-KU950430	0.969	0.958	0.956	0.914	0.886	0.884
MYMIV-IN:Tm-MF683072	0.968	0.957	0.954	0.911	0.882	0.877
MYMIV-IN:Pp-MF693401	0.948	0.949	0.949	0.851	0.86	0.861
MYMIV-IN:Sb-EU523045	0.951	0.953	0.952	0.86	0.869	0.867
MYMIV-IN:Bg-LC271790	0.926	0.925	0.925	0.836	0.842	0.843
MYMIV-NEP:Gg-AY271895	0.955	0.943	0.94	0.879	0.849	0.845
MYMIV-BAN:Gg-AF314145	0.923	0.907	0.904	0.859	0.818	0.814
MYMIV-PAK:Gg-AM950268	0.955	0.953	0.953	0.873	0.875	0.875
MYMIV-OMN:Cs-MF818047	0.95	0.938	0.935	0.86	0.826	0.822
MYMV-IN:Gg-MN698275	0.817	0.821	0.819	0.584	0.594	0.593
MYMV-IN:Bg-JQ398669	0.859	0.86	0.859	0.641	0.644	0.645
MYMV-IN:Gg-MN698275	0.817	0.821	0.819	0.584	0.594	0.593
MYMV-VN:Gg-JX244172	0.814	0.82	0.82	0.581	0.594	0.595
HgYMV-IN:Cb-MN698282	0.885	0.904	0.902	0.692	0.742	0.739
HgYMV-SRI:Cb-GU323321	0.816	0.819	0.816	0.553	0.57	0.566

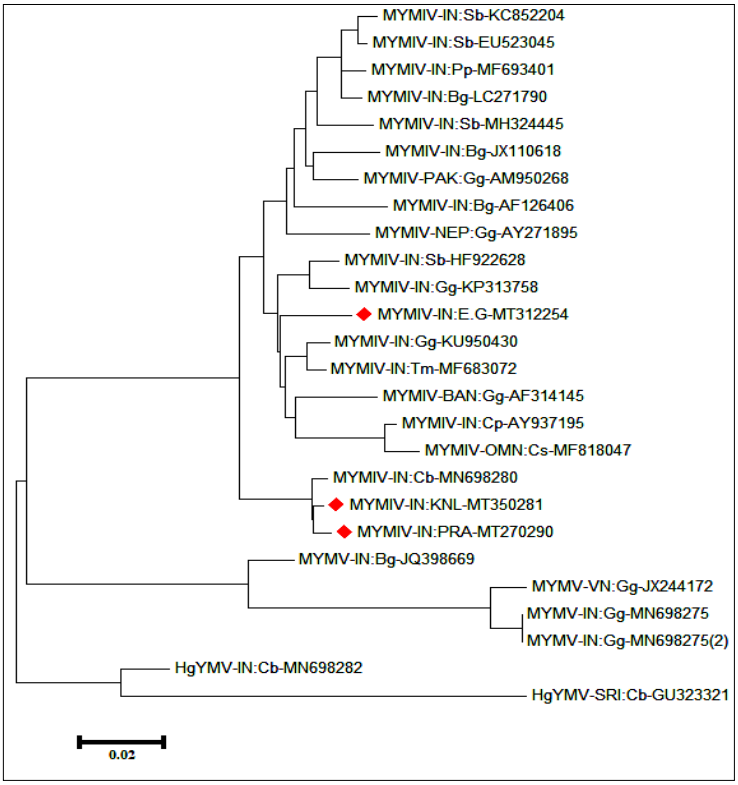

Fig 2: Phylogenetic tree (1000 boot strap replications) of the full length DNA-A of MYMIV isolates (marked) from Andhra Pradesh with other begomoviruses from database.

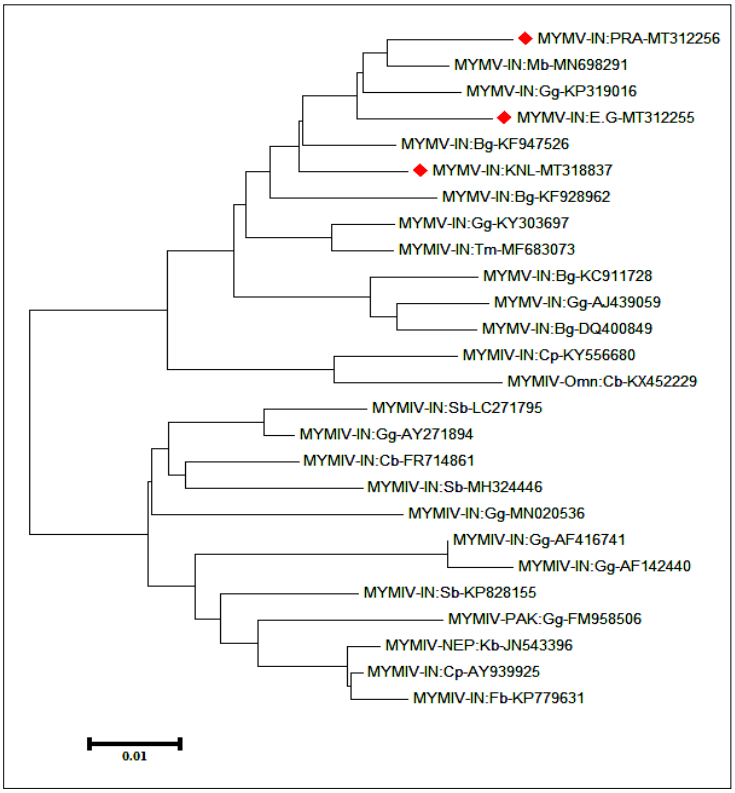

Fig 3: Phylogenetic tree (1000 boot strap replications) of the full length DNA-B of MYMV isolates (marked) from Andhra Pradesh with other begomoviruses from database.

Table 2: Complete genome sequence similarity matrix of MYMV DNA-B of three isolates with other begomoviruses at nucleotide level and amino acid level.

Isolate name	Nucleotide level			Amino acid level		
	E.G.	KNL	PRA	E.G.	KNL	PRA
MYMV-IN:E.G-MT312255	ID	0.968	0.966	ID	0.965	0.956
MYMV-IN:KNL-MT318837	0.968	ID	0.964	0.965	ID	0.956
MYMV-IN:PRA-MT312256	0.966	0.964	ID	0.956	0.956	ID
MYMV-IN:Bg-KF928962	0.953	0.963	0.949	0.924	0.92	0.915
MYMV-IN:Bg-KF947526	0.967	0.974	0.963	0.96	0.958	0.949
MYMV-IN:Mb-MN698291	0.973	0.971	0.98	0.963	0.967	0.97
MYMV-IN:Gg-KP319016	0.971	0.968	0.976	0.951	0.951	0.963
MYMV-IN:Gg-AJ439059	0.941	0.947	0.948	0.91	0.908	0.913
MYMV-IN:Bg-KC911728	0.941	0.948	0.945	0.91	0.915	0.913
MYMV-IN:Bg-DQ400849	0.945	0.949	0.948	0.917	0.913	0.917
MYMV-IN:Gg-KY303697	0.953	0.965	0.961	0.938	0.935	0.94
MYMIV-IN:Tm-MF683073	0.954	0.964	0.963	0.933	0.933	0.944
MYMIV-IN:Cp-KY556680	0.846	0.847	0.843	0.828	0.83	0.834
MYMIV-IN:Sb-LC271795	0.914	0.921	0.923	0.878	0.881	0.885
MYMIV-IN:Gg-AF416741	0.911	0.916	0.914	0.874	0.883	0.883
MYMIV-IN:Gg-AY271894	0.919	0.926	0.929	0.885	0.888	0.894
MYMIV-IN:Cb-FR714861	0.917	0.926	0.928	0.881	0.883	0.89
MYMIV-IN:Gg-AF142440	0.898	0.903	0.9	0.851	0.86	0.858
MYMIV-IN:Cp-AY939925	0.914	0.918	0.913	0.863	0.872	0.87
MYMIV-IN:Gg-MN020536	0.903	0.909	0.91	0.856	0.863	0.863
MYMIV-IN:Fb-KP779631	0.906	0.91	0.905	0.853	0.863	0.86
MYMIV-IN:Sb-KP828155	0.91	0.917	0.915	0.876	0.878	0.878
MYMIV-IN:Sb-MH324446	0.912	0.917	0.918	0.861	0.868	0.866
MYMIV-NEP:Kb-JN543396	0.914	0.918	0.913	0.865	0.874	0.872
MYMIV-PAK:Gg-FM958506	0.903	0.906	0.905	0.858	0.865	0.867
MYMIV-Omn:Cb-KX452229	0.935	0.934	0.933	0.892	0.887	0.885

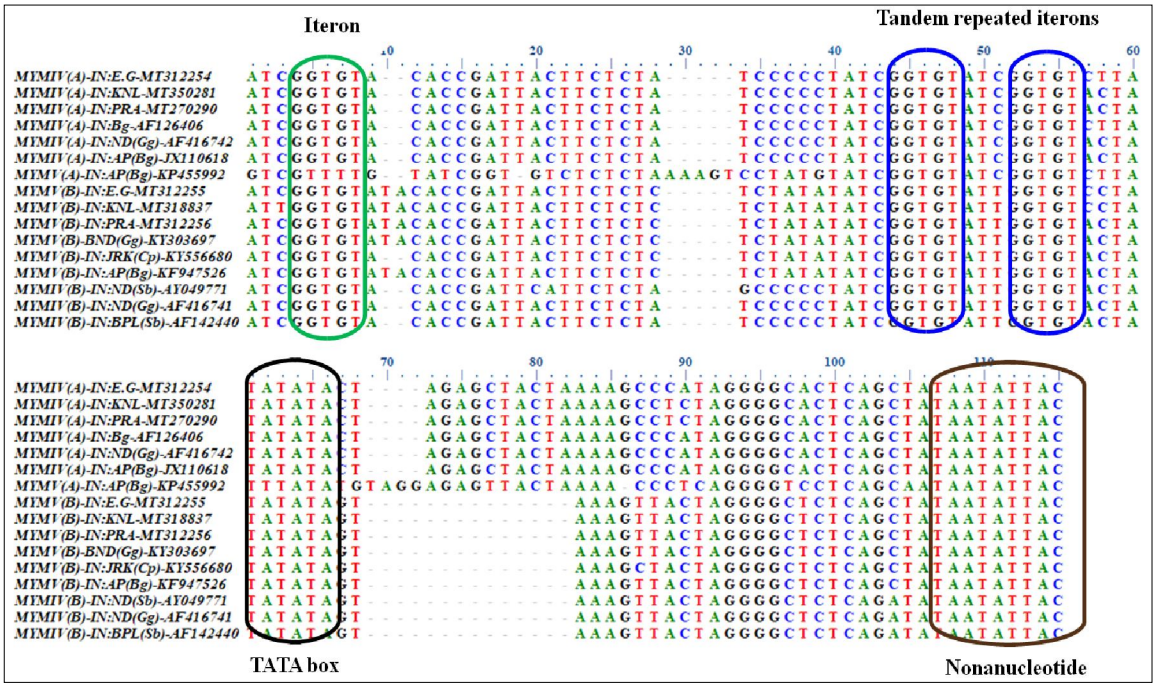

Fig 4: Alignment of common region of MYMIV-A and MYMV-B isolates under study with other begomoviruses.

homology (>97%) with DNA-B of MYMV-Belgaum (MN698291) followed by MYMV-Coimbatore (KP319016) and MYMV-Tirupati (KF947526) isolates. The MYMV-Kurnool isolate showed 97.4% similarity with DNA-B of MYMV-Tirupati (KF947526) isolate. The MYMV DNA-B of three isolates shared 94.1 to 97.6% similarity with MYMV DNA-B (KC911728 and KP319016) and 84.3 to 96.4% with MYMIV DNA-B (KY556680 and MF683073) isolates. The predicted amino acid sequence of MYMV DNA-B of three isolates shared >95% homology with MYMV-Belgaum (MN698291), Coimbatore (KP319016) and Tirupati (KF947526) isolates. The predicted amino acid sequence similarity of three isolates had 91 to 97% homology with MYMV DNA-B (KC911728 and KP319016) and 82.8 to 94.4% with MYMIV DNA-B (KY556680 and MF683073) when compared with NCBI database (Table 2). In phylogenetic tree (Fig 3), all three isolates formed individual clusters *i.e.,* MYMV-East Godavari with MYMV- Coimbatore (KP319016), MYMV-Kurnool with MYMV-Tirupati (KF947526) and MYMV-Prakasam isolate with MYMV-Belgaum (MN698291) isolates. All three isolates under study formed unique cluster with MYMV-B of south Indian isolates. Karthikeyan *et al.* (2004) cloned and sequenced five highly variable DNA-Bs from single YMV infected blackgram plants collected from Vamban, Tamil Nadu. Based on nucleotide sequence and phylogenetic analysis, five highly variable DNA-Bs were divided into two groups *i.e.,* first group (KA27) showed 95% similarity with MYMV DNA-B and second group comprising (KA21, KA22, KA28 and KA34) shared 89-90% similarity with MYMIV DNA-B. Haq *et al.* (2011) characterized the association of YMV infecting blackgram from Vamban, Tamil Nadu. The comparative study of complete nucleotide sequence of VamBg5 isolate of DNA-A and DNA-B with other begomoviruses sequence showed maximum similarity with MYMV DNA-A and MYMIV DNA-B respectively.

Table 3: The common region (CR) nucleotide sequence similarity matrix of MYMIV-A and MYMV-B of three isolates with other begomoviruses.

Isolate name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MYMIV(A)-IN:E.G-MT312254	ID	0.961	0.961	1	0.98	0.98	0.69	0.764	0.754	0.754	0.754	0.778	0.754	0.778	0.807	0.807
MYMIV(A)-IN:KNL-MT350281	0.961	ID	1	0.961	0.98	0.98	0.663	0.773	0.764	0.783	0.783	0.807	0.783	0.807	0.836	0.836
MYMIV(A)-IN:PRA-MT270290	0.961	1	ID	0.961	0.98	0.98	0.663	0.773	0.764	0.783	0.783	0.807	0.783	0.807	0.836	0.836
MYMIV(A)-IN:Bg-AF126406	1	0.961	0.961	ID	0.98	0.98	0.69	0.764	0.754	0.754	0.754	0.778	0.754	0.778	0.807	0.807
MYMIV(A)-IN:ND(Gg)-AF416742	0.98	0.98	0.98	0.98	ID	1	0.672	0.764	0.754	0.773	0.773	0.798	0.773	0.798	0.826	0.826
MYMIV(A)-IN:AP(Bg)-JX110618	0.98	0.98	0.98	0.98	1	ID	0.672	0.764	0.754	0.773	0.773	0.798	0.773	0.798	0.826	0.826
MYMV(A)-IN:AP(Bg)-KP455992	0.69	0.663	0.663	0.69	0.672	0.672	ID	0.573	0.565	0.565	0.565	0.584	0.565	0.548	0.548	0.548
MYMV(B)-IN:E.G-MT312255	0.764	0.773	0.773	0.764	0.764	0.764	0.573	ID	0.989	0.989	0.989	0.958	0.989	0.875	0.906	0.906
MYMV(B)-IN:KNL-MT318837	0.754	0.764	0.764	0.754	0.754	0.754	0.565	0.989	ID	0.979	0.979	0.947	0.979	0.864	0.895	0.895
MYMV(B)-IN:PRA-MT312256	0.754	0.783	0.783	0.754	0.773	0.773	0.565	0.989	0.979	ID	1	0.968	1	0.885	0.916	0.916
MYMV(B)-BND(Gg)-KY303697	0.754	0.783	0.783	0.754	0.773	0.773	0.565	0.989	0.979	1	ID	0.968	1	0.885	0.916	0.916
MYMV(B)-IN:JRK(Cp)-KY556680	0.778	0.807	0.807	0.778	0.798	0.798	0.584	0.958	0.947	0.968	0.968	ID	0.968	0.893	0.925	0.925
MYMV(B)-IN:AP(Bg)-KF947526	0.754	0.783	0.783	0.754	0.773	0.773	0.565	0.989	0.979	1	1	0.968	ID	0.885	0.916	0.916
MYMIV(B)-IN:ND(Sb)-AY049771	0.778	0.807	0.807	0.778	0.798	0.798	0.548	0.875	0.864	0.885	0.885	0.893	0.885	ID	0.968	0.968
MYMIV(B)-IN:ND(Gg)-AF416741	0.807	0.836	0.836	0.807	0.826	0.826	0.548	0.906	0.895	0.916	0.916	0.925	0.916	0.968	ID	1
MYMIV(B)-IN:BPL(Sb)-AF142440	0.807	0.836	0.836	0.807	0.826	0.826	0.548	0.906	0.895	0.916	0.916	0.925	0.916	0.968	1	ID

Common region of MYMIV DNA-A and MYMV DNA-B

The common region (CR) of MYMIV DNA-A and MYMV DNA-B present between non coding regions of ORFs AC1/AV2 in DNA-A and BC1/BV1 in DNA-B. The non-coding regions of six isolates under study (3 MYMIV DNA-A and 3 MYMV DNA-B) were aligned with other begomoviruses in database (Fig 4). The DNA-A and DNA-B of begomoviruses have a common region (CR) which contains stem loop structure with a loop containing conserved nonanucleotide motif (5′-TAATA TTAC-3′), TATA box and several repeated *iteron* sequences (GGTGT). Three direct repeats are found before TATA box and two repeats out of three are tandem repeat sequences. The repeated iterons of present six isolates (MYMIV-A and MYMV-B) have similar *iteron* sequences *i.e.,* GGTGT. The function of iteron and TATA box was described by Arguelo-Astorga *et al.* (1994) as initiator for rolling circle amplification (RCA). During RCA, replication was initiates by hydrolysis of phosphodiester bond between the 7th and 8th residues of the nonanucleotide *i.e.,* 5′-TAATATT/AC-3′ (Pant *et al.*, 2001).

The CR region of MYMIV DNA-A three isolates shared maximum identity (96.1 to 100%) with MYMIV DNA-A isolates (AF126406, AF416742 and JX110618) and minimum homology (66.3 to 69%) with MYMV-A of Andhra Pradesh (KP455992) isolate. The MYMV DNA-B common region of three isolates shared maximum identity (100%) with MYMV-B of Bangladesh (KY303697), Tirupati (KF947526) isolates. The CR sequence similarity between MYMIV DNA-As and MYMV DNA-Bs of East Godavari and Kurnool isolates was 76.4% and 78.3% with Prakasam isolate (Table 3). The divergence between the MYMIV DNA-A and MYMV DNA-B of present three isolates (East Godavari, Kurnool and Prakasam) under study were from 22.2 to 22.6%. Haq *et al.*, (2011) reported 14% diversity between the DNA-A and DNA-B of MYMV isolate in blackgram from Vamban, Tamil Nadu. The sequence diversity in CR region between DNA-A and DNA-B might reflect the genetic diversity within the viral genomes and intracellular copy number between DNA-A and DNA-B of MYMV (Pant *et al.*, 2001). The length of common region in DNA-A is longer than DNA-B due to deletions of DNA-B genome. The above results are contrasting with results obtained by Pant *et al.* (2001) who reported that CR of DNA-B is longer than the DNA-A of MYMV. Further work on making infectious constructs for agroinoculation screening of blackgram genotypes for 3 isolates were in progress in our lab to study any impact on symptomatology due to variations in viral genomes.

Based on our results we concluded that, more number of YMV infecting blackgram isolates from entire state is need to characterize at molecular level for identification of different species associated with YMD of blackgram because, YMD in blackgram is caused by two species of begomovirus *i.e.* MYMV and MYMIV. Genetic variability studies are essential to understand YMV epidemiology in different locations of Andhra Pradesh for effective management of YMD and to develop resistant varieties to YMV.

Conflict of interest: None.

REFERENCES

Archana, S., Venkatesh., Padmaja, A.S., Manjunatha, N and Nagaraju, N. (2017). Molecular detection and characterization of yellow mosaic virus (YMV) infecting blackgram (*Vigna mungo* L.) in Karnataka with the aid of coat protein (cp) gene. Journal of Food Legumes. 30(4): 277-280.

Arguelo-Astorga, G.R., Guevara-Gonzalez, R.G., Herrera-Estrella, L.R., Rivera-Bustamante, R.F. (1994). Geminivirus replication origins have a group specific organization of iterative elements: A model for replication. Virology. 203: 90-100.

Fauquet, C.M., Briddon, R.W., Brown, J.K., Moriones, E., Stanley, J., Zerbini, M and Zhou, X. (2008). Geminivirus strain demarcation and nomenclature. Archives of Virology. 153: 783-821.

Haq, Q.M.I., Rouhibakhsh, A., Ali, A. and Malathi, V.G. (2011). Infectivity analysis of a blackgram isolate of Mungbean yellow mosaic virus and genetic assortment with MYMIV in selective hosts. Virus Genes. 42: 429-439.

John, P., Sivalingam, P.N., Haq, Q.M.I., Kumar, N., Mishra, A., Briddon, R.W. and Malathi, V.G. (2008). Cowpea golden mosaic disease in Gujarat is caused by a Mungbean yellow mosaic India virus isolate with a DNA B variant. Archives of Virology. 153: 1359-1365.

Karthikeyan, A.S., Vanitharani, R., Balaji, V., Anuradha, S., Thillaichidambaram, P., Shivaprasad, P.V., Parameswari, C., Balamani, V., Saminathan, M. and Veluthambi, K. (2004). Analysis of an isolate of Mungbean yellow mosaic virus (MYMV) with a highly variable DNA-B component. Archives of Virology.149: 1643-1652.

Kumar, S., Bhaben, T., Sunil, K.M., Lingaraj, S. (2017). Molecular characterization and infectivity of Mungbean yellow mosaic India virus associated with yellow mosaic disease of cowpea and mungbean. Biocatalysis and Agricultural Biotechnology. 11: 183-191.

Lizardi, P.M., Hauang, X., Zhu, Z., Bray-ward, P., Thomas, D.C. and Ward, D.C. (1998). Mutation detection and single-molecule counting using isothermal rolling-circle amplification. Nature Genetics. 19(3): 225-432.

Mishra, G.P., Dikshit, H.K., Ramesh, S.V., Tripathi, K., Kumar, R.R., Aski, M., Singh, A., Roy, A., Priti., Kumari, N., Dasgupta, U., Kumar, A., Praveen, S. and Nair, R.M. (2020). Yellow Mosaic Disease (YMD) of mungbean [*Vigna radiata* (L.) Wilczek]: Current status and management opportunities. Frontiers in Plant Science. 11: 1-24.

Murray, M.G. and Thompson W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 8: 4321-4326.

Nariani, T.K. (1960). Yellow mosaic of mung (*Phaseolus aureus* L.). Indian Phytopathology. 13: 24 29.

Pant, V., Gupta, D., Roy Choudhury, N., Malathi, V.G., Varma, A. and Mukherjee, S.K. (2001). Molecular characterization of the rep protein of the blackgram isolate of Indian Mungbean yellow mosaic virus. Journal of General Virology. 82: 2559-2567.

Prema, G.U. and Rangaswamy, K.T. (2018). Molecular characterization of coat protein gene of blackgram yellow mosaic virus (BGYMV) from Karnataka, India. International Journal of Current Microbiology and Applied Sciences. 7(7): 2225-2235.

Reddy, B.V.B., Obaiah, S., Prasanthi, L., Sivaprasad, Y., Sujitha, A. and Krishna, T.G. (2015). Mungbean yellow mosaic India virus is associated with yellow mosaic disease of blackgram (*Vigna mungo* L.) in Andhra Pradesh, India. Archives of Phytopathology and Plant Protection. 48(4): 345-353.

Satya, V.K., Alice, D., Malathi, V.G and Velazhahan, R. (2015). Molecular identification and genetic diversity of yellow mosaic virus in blackgram. Biochemical and Cellular Archives. 15(2): 475-479.

Tamura, K., Dudley, J., Nei, M and Kumar, S. (2007). MEGA 7: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 24: 1596-1599.

Williams, F.J., Grewal, J.S. and Amin, K.S. (1968). Serious and new diseases of pulse crops in India in 1966. Plant Disease Reporter. 52: 300-304.