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Research Article

Molecular Characterization of Begomovirus Infecting Black Gram (*Vigna mungo* L.) from East Godavari, Andhra Pradesh

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ABSTRACT

For molecular characterization of Yellow Mosaic Virus (YMV) associated with Yellow Mosaic Disease (YMD) of blackgram, infected blackgram samples were collected from farmer field of East Godavari district, Andhra Pradesh. The total DNA was isolated by CTAB method, amplified in PCR with coat protein and movement protein gene specific primers. The resulting PCR products were sequenced and submitted to NCBI GenBank. Comparison of coat protein and movement protein nucleotide sequence with other begomoviruses collected from NCBI GenBank revealed that DNA-A of MYMIV and MYMV of present isolate shared 99.3% and 97.2% similarity with MYMIV-Raichur (MN698280) and MYMV-Vamban (KC911722) isolates respectively. MYMV DNA-B of present isolate shared 96% identity with MYMV-Tirupati (KF947526) isolate. Phylogenetic tree based on coat protein gene nucleotide sequence of MYMIV, MYMV and movement protein gene nucleotide sequence of MYMV infecting East Godavari isolate with other isolates collected from NCBI GenBank formed unique clusters with DNA-A of MYMIV, MYMV and DNA-B of MYMV respectively. The results revealed that both MYMIV and MYMV are associated with YMD of blackgram in East Godavari district of Andhra Pradesh.

Keywords: *Begomoviruses, Coat protein, Movement protein, Characterization, Nucleotide sequence similarity, East Godavari isolate, Phylogenetic tree.*

INTRODUCTION

Blackgram is most important grain legume crop in South Asia. In term of area and production, blackgram is the fourth most important cultivated grain legume crop in India after chickpea, pigeonpea and mungbean. India is the largest producer of blackgram, the cultivated area is about 52.79 lakh ha, production is 34.92 lakh tonnes and productivity is 662 Kg/ha during 2017-18.

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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, production is 3.70 lakh tonnes and productivity is about 920 Kg/ha during 2017-18 (www.indiastat.com)

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 yellow mosaic virus (YMV) belongs to family Geminiviridae, genus begomovirus. The begomovirus is the largest genera and it comprises approximately 322 species and more than 500 isolates. The begomoviruses are twinned icosahedral particles measures upto 30 nm in length and 20 nm in wide having circular single stranded DNA. The size of the DNA-A and DNA-B components of YMV is approximately 2.7 Kb. Begomoviruses are transmitted by whitefly (*Bemisia tabaci*) from infected to healthy plants in persistent circulative manner (Mishra et al., 2020).

The YMD is caused by four distinct viruses (belongs to genus begomovirus) *i.e*. *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* (DoYMV) and *Horsegram yellow mosaic virus* (HgYMV) (John et al., 2008). Of these MYMIV and MYMV are most important species, MYMV is more common in southern and western India, whereas MYMIV in northern, central and eastern regions of India (Mishra et al., 2020). However, both are present in Andhra Pradesh (Reddy et al., 2015)

Most of the genera under Geminiviridae have monopartite genomes except begomoviruses which have either monopartite or bipartite genomes. All bipartite begomoviruses had two genomic components *i.e.* DNA-A and DNA-B. The DNA-A has 7 Open Reading Frames (ORFs) which encodes for pre-coat protein (AV2), coat protein (AV1) on virus sense stand, replication initiation protein (AC1), transcription activator protein (AC2), replication enhancer protein (AC3), symptoms determination protein (AC4) and AC5 on complementary stand. The DNA-B has two ORFs *i.e.* movement protein (BC1) involved in virus movement between the cells and nuclear shuttle protein (BV1) involved in movement of the virus within the cell (Kumar et al., 2017). If complete genome sequence is not available, the full length coat protein gene (AV1) sequences are accepted by International Committee on Taxonomy of Viruses (ICTV) for classification of begomoviruses (Fauquet et al., 2008).

In India, the yellow mosaic disease (YMD) on blackgram was first reported by Williams et al. (1968). Association of *Mungbean yellow mosaic India virus* (MYMIV) with YMD of blackgram in Andhra Pradesh was reported by Reddy et al. (2015) for the first time from South India. The present article described the molecular characterization of coat protein and movement protein gene of YMV infecting blackgram from East Godavari district of Andhra Pradesh.

MATERIAL AND METHODS

YMV infected blackgram leaf samples were collected from East Godavari district of Andhra Pradesh during 2019 (Fig 1). The total DNA from YMV infected and healthy leaf samples were extracted using CTAB method (Murray and Thomson, 1980). The purity and concentration of the DNA was quantified using Nanodrop spectrophotometer.

The extracted DNA was subjected to PCR by using YMV coat protein and movement protein gene specific primers (Table.1). PCR reaction was performed in a 25µl 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 conditions for amplification of target DNA are; 1 cycle of 94°C for 4min 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 products

were analyzed on 1 % agarose gel (W/V) electrophoresis.

The PCR amplified products were sequenced at automated DNA sequencing facility (Eurofin Genomics India Pvt. Ltd., Bangalore). Sequence assembling, nucleotide alignment and percent identity matrix were done with BioEdit version 7.0 software (Hall, 1999). Both nucleotide and amino acid sequences are 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 (Tamura et al., 2007).

RESULTS AND DISCUSSION

The blackgram leaves showing yellow mosaic symptoms were collected from farmer field, total DNA was extracted from infected and healthy leaves of blackgram plants by using the CTAB method. The total extracted DNA was subjected to PCR amplification using YMV coat protein (CP) and movement protein (MP) gene specific primers. The PCR amplified products were analyzed on 1% agarose gel and yielded an amplicon product size 920 bp, 900 bp and 1000 bp from infected plants and but not from healthy plants (Fig. 2). The PCR products were purified, coat protein gene of MYMIV and MYMV, movement protein gene of MYMV were sequenced at automated DNA sequencing facility (Eurofins Genomics India Pvt.Ltd., Bangalore) and the sequences were submitted to NCBI GenBank (MT066228-MYMIV DNA-A: MT119671-MYMV DNA-A: MT071114-MYMV DNA-B).

The comparison of coat protein (CP) nucleotide sequence of MYMIV infecting blackgram of East Godavari (MT066228) isolate with other begomoviruses collected from NCBI GenBank indicated that, DNA-A of MYMIV-East Godavari isolate shared 99.3% and 99.6% similarity with DNA-A of MYMIV-Raichur (MN698280) isolate at nucleotide and amino acid level respectively followed by DNA-A of MYMIV-Kurnool (JN181004) and Kadapa (KC243785) isolates (99% at nucleotide level and 99.2% at amino acid level by both isolates). The similarity percentage of coat protein gene sequence of present isolate with other begomoviruses ranged from 99.3%-93.5% at nucleotide level and 99.6%-96.8% at amino acid level (Table. 2). The phylogenetic tree was constructed based on coat protein gene nucleotide sequence of MYMIV-East Godavari isolate with other begomoviruses. The MYMIV-East Godavari isolate formed unique cluster with MYMIV from Raichur (MN698280), Kurnool (JN181004), Chittoor (KJ747961) and Kadapa (KC243785) isolates. Based on phylogenetic results it is clear that MYMIV infecting East Godavari isolate closely related to south Indian MYMIV isolates (Fig. 3).

The present results were in agreement with earlier results reported by Reddy et al. (2015). The coat protein sequence analysis of six isolates collected from six districts of Andhra Pradesh revealed that the isolates from Telangana and Rayalaseema regions are the same variant with > 99.5 % sequence identity and isolate from coastal Andhra is different variant with >95.4 % identity when compared to Rayalaseema and Telangana regions isolates in the database. Similar results were reported by Naimuddin et al. (2011) from IIPR, Kanpur. The sequence analysis of coat protein gene of YMV infecting wild species of blackgram showed 97% and 99% similarity with *Mungbean yellow mosaic India virus* (MYMIV) at nucleotide and amino acid level respectively.

Based on pairwise and multiple nucleotide sequence alignment of coat protein gene of MYMV-East Godavari isolate (MT119671) with other begomoviruses collected from NCBI GenBank revealed that the present isolate shared 97.2% similarity with MYMV-Vamban (KC911722) and minimum similarity (78.1%) with MYMV-Punjab (MT345791) isolate. The predicted amino acid sequence of coat protein gene of MYMV-East Godavari isolate showed 95.6% similarity with MYMV isolates from database. The coat protein nucleotide sequence

similarity with MYMV ranged from 97.2 - 78.1% at nucleotide level and 95.6-82.5% at amino acid level (Table. 3). A phylogenetic tree for MYMV-A of East Godavari isolate was constructed by neighbour-joining method with other sequences available in NCBI database. The present isolate form unique cluster with MYMV-Vamban (KC911722) and MYMV-Tirupati (KP455992) isolates (Fig. 4).

Prema and Rangaswamy (2018) reported that yellow mosaic virus infecting blackgram from Hebbal, Bangalore was a variant of *Mungbean yellow mosaic virus* (MYMV). Coat protein nucleotide sequencing analysis indicated that MYMV-Hebbal shared maximum similarity (98.7%) with MYMV-Namakkal, followed by MYMV-Madurai (98.4%) and MYMV-Tamil Nadu (98.3%). Association of MYMV-A with blackgram was reported by Archana et al. (2017) from Karnataka state. Hence the present results were in agreement with earlier reports.

The analysis of movement protein gene sequence of MYMV-East Godavari (MT071114) isolate with other begomoviruses collected from NCBI database showed 96% and 91.6% similarity with MYMV-Tirupati (KF947526) isolate at nucleotide and amino acid level respectively. It shared 95.5% and 90.2% similarity with MYMV-Belgaum isolate (MN698291) at nucleotide and amino acid level respectively. The percentage of similarity with MYMV ranged from 96%-81% at nucleotide level and 91.6%-55.6% at amino acid level (Table.4). By using neighbour-joining method, phylogenetic tree was constructed from movement protein gene sequence of MYMV-East Godavari isolate with other isolates from NCBI GenBank. The present isolate form unique cluster with MYMV-Tirupati (KF9487526) isolate (Fig.5).

John et al. (2008) cloned several MYMV DNA-Bs from YMV infected cowpea samples collected from Gujarat. The sequence analysis indicated that DNA-B component of the isolate shared >92% similarity with MYMV DNA-B and <92% with MYMIV-B. 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. The above data analysis revealed that there is a variability in DNA-B component of MYMV under field conditions. Hence there is need to collect more number of samples for sequencing and analysis of MYMV-B variability.

Fig. 1: Black gram plants showing typical symptoms of yellow mosaic disease

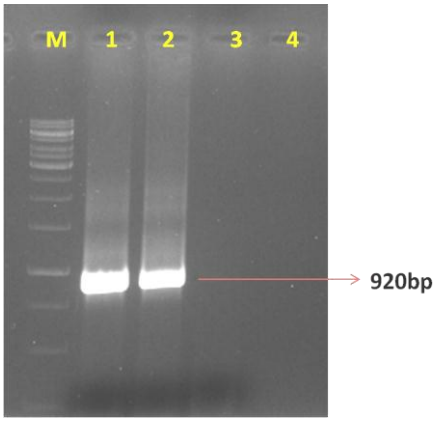

Fig. 2 (a): PCR amplification of MYMIV coat protein gene with specific primer (MYMIV CP-F/R) from YMV infected blackgram plants collected from East Godavari district of Andhra Pradesh.
Lane: M-1 Kb known marker
Lane: 1&2: Infected blackgram plants
Lane: 3&4: Healthy blackgram plants

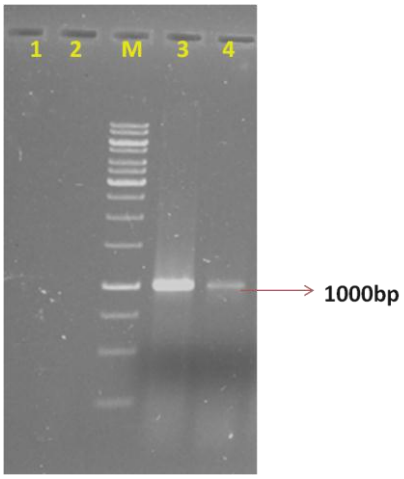

Fig. 2 (b): PCR amplification of MYMV coat protein gene with specific primer (MYMV CP-F/R) from YMV infected blackgram plants collected from East Godavari district of Andhra Pradesh.
Lane: M-1 Kb known marker
Lane: 1&2: Healthy blackgram plants
Lane: 3&4: Infected blackgram plants

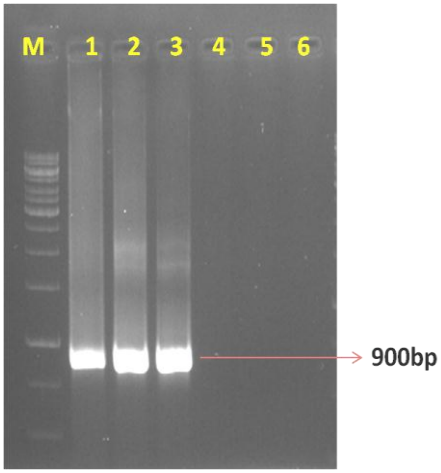

Fig. 2 (c): PCR amplification of MYMV movement protein gene with specific primer (MYMV MP-F/R) from YMV infected blackgram plants collected from East Godavari district of Andhra Pradesh.
Lane: M-1 Kb known marker
Lane: 1-3: Infected blackgram plants
Lane: 4-6: Healthy blackgram plants

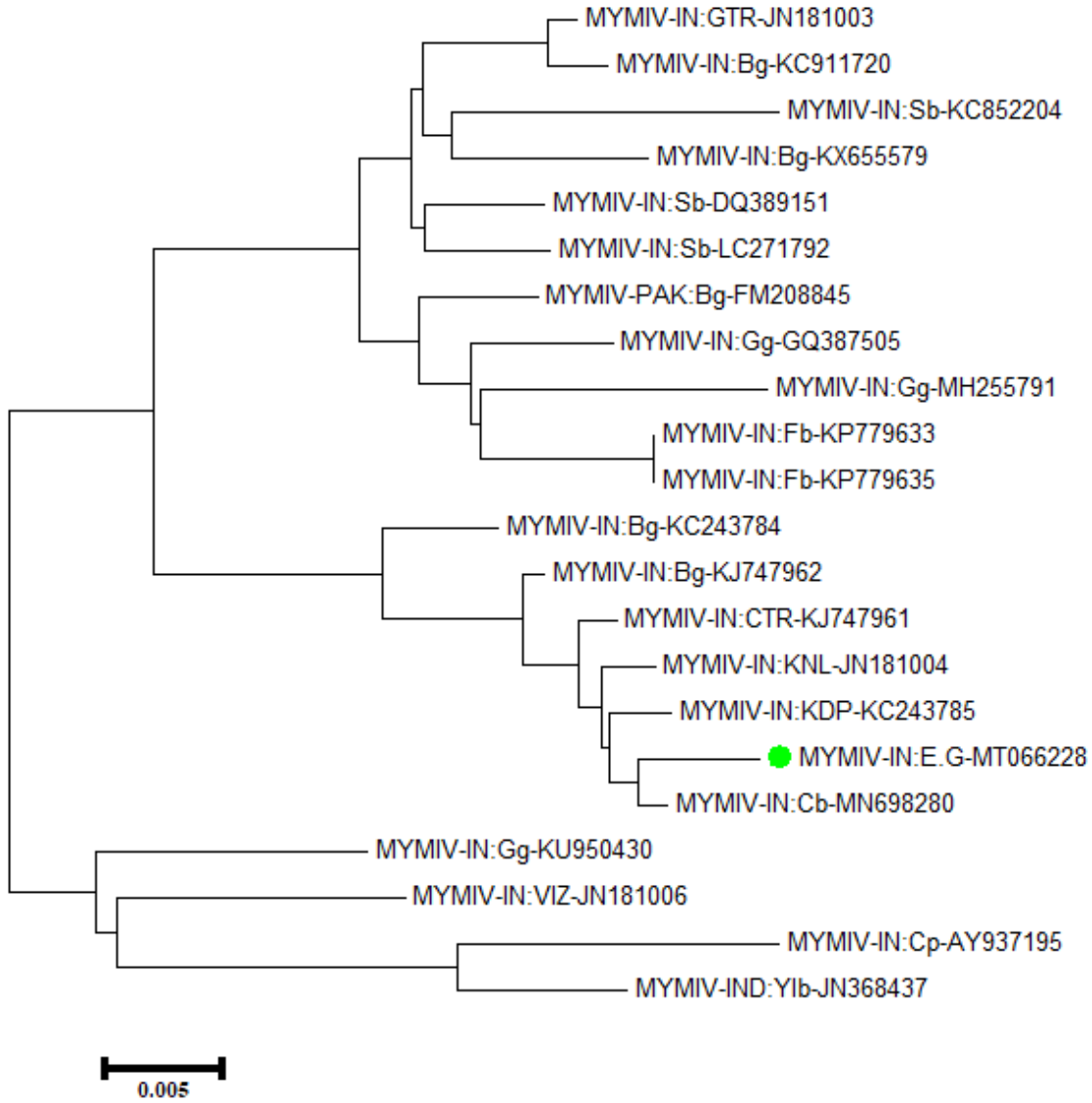

Figure.3: Phylogenetic tree (1000 boot strap replications) derived from coat protein gene sequence of MYMIV (marked) from East Godavari district of Andhra Pradesh with other begomoviruses. Abbreviations and accession numbers of begomoviruses sequences used were given in table 2.

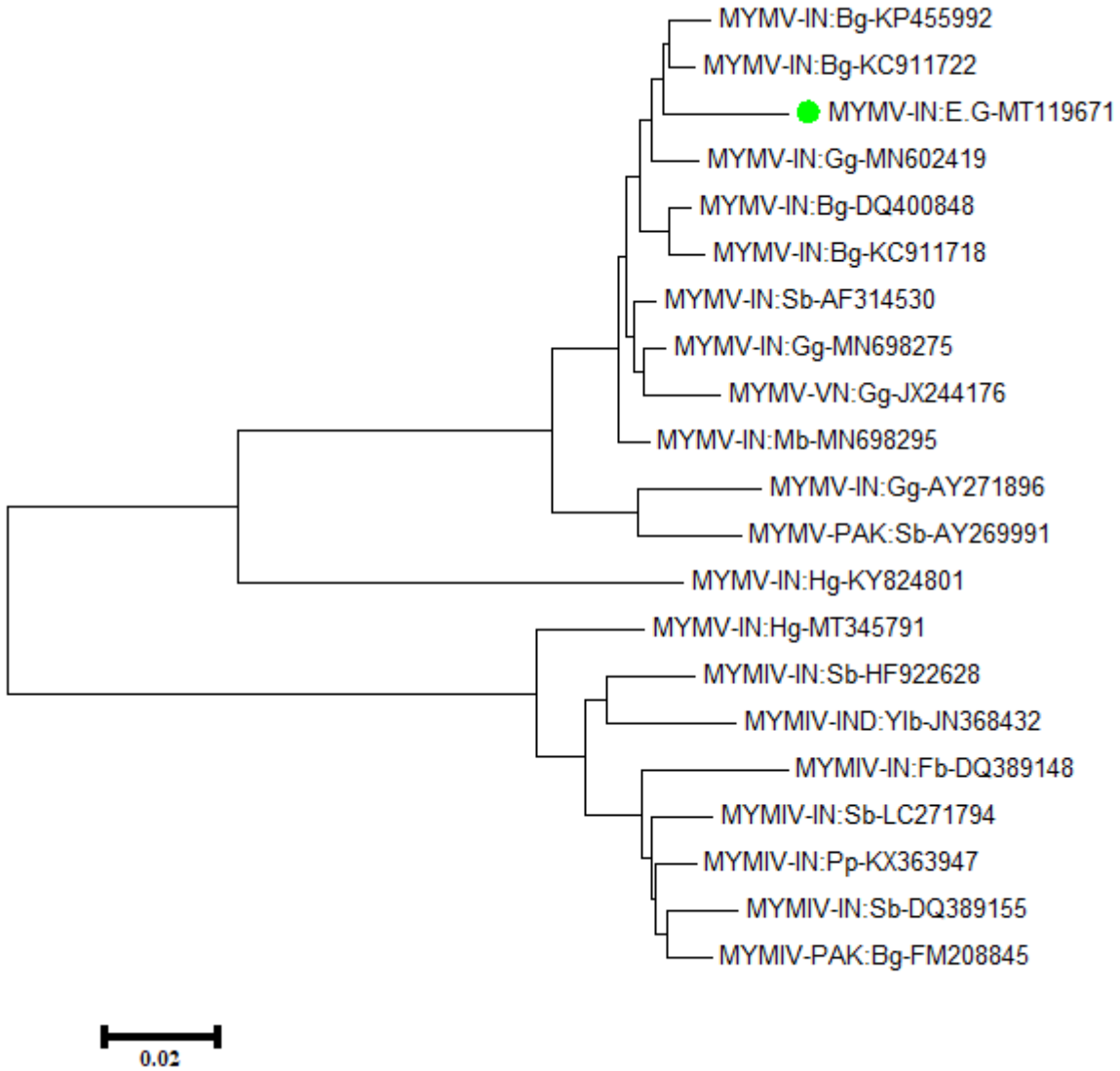

Fig. 4: Phylogenetic tree (1000 boot strap replications) derived from coat protein gene sequence of MYMV (marked) from East Godavari district of Andhra Pradesh with other begomoviruses. Abbreviations and accession numbers of begomoviruses sequences used were given in table 3.

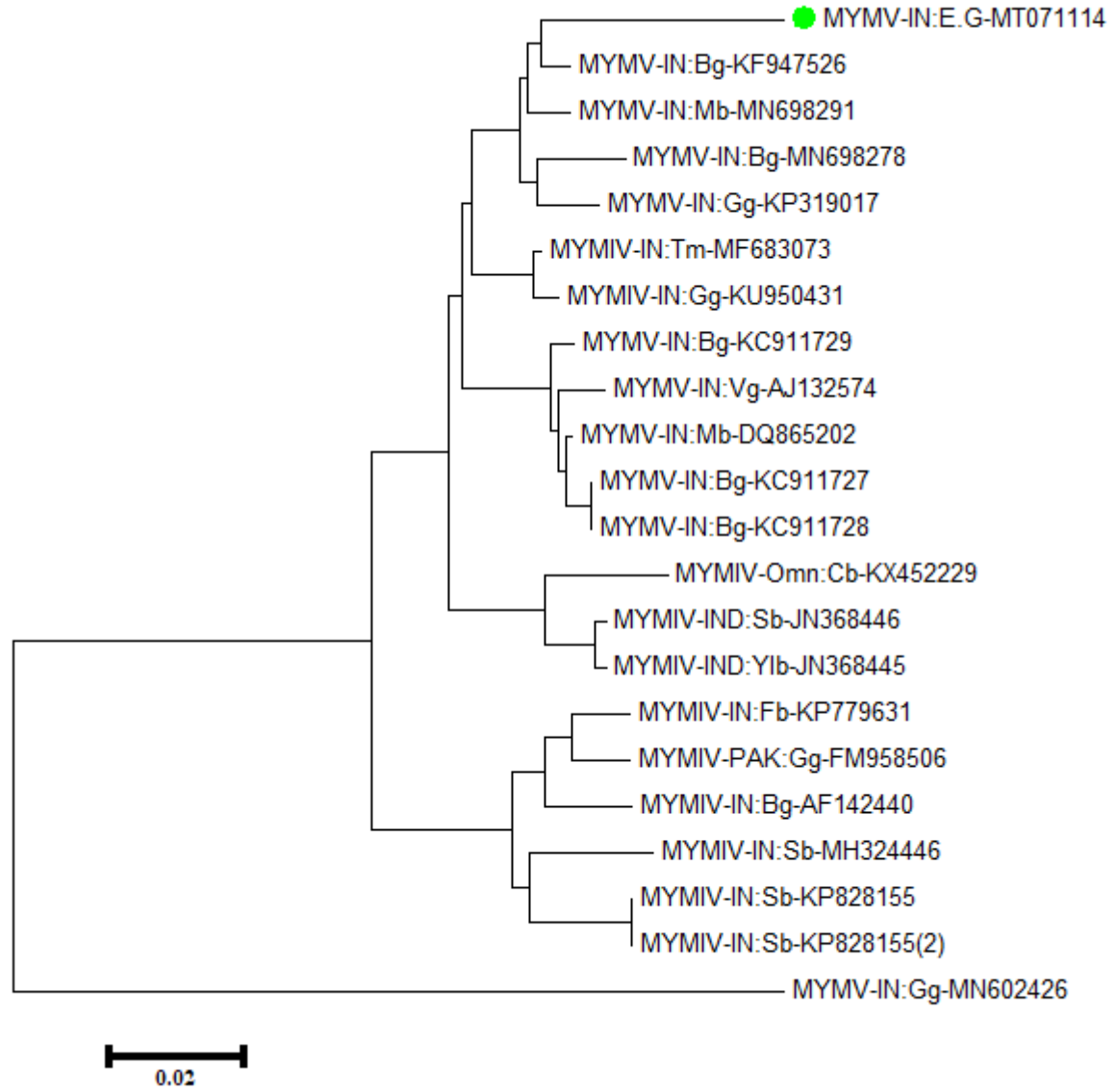

Fig. 5: Phylogenetic tree (1000 boot strap replications) derived from movement protein gene sequence of MYMV (marked) from East Godavari district of Andhra Pradesh with other begomoviruses. Abbreviations and accession numbers of begomoviruses sequences used were given in table 4.

Table 1: List of oligonucleotide primers used for amplification of yellow mosaic virus infecting blackgram

S. No.	Nucleotide Sequence (5'-3')	Specific to	Product size	Annealing Temp	Reference
MYMIV CP-F	TCAAGCTCCCGGTGCATGTTGCA	MYMIV coat protein	920 bp	55°C	Rouhibakhsh et al. (2008)
MYMIV CP-R	GTAAAGCTTTACGCATAATG				
MYMV CP-F	ATGGGTCCGTTGTATGCTTG	MYMV coat protein	1000 bp	55°C	Prema and Rangaswamy. (2018)
MYMV CP-R	GGCGTCATTAGCATAGGCAAT				
MYMV MP-F	ATGGAGAATTATTCAGGCGCA	MYMV movement protein	900 bp	55°C	Naimuddin et al. (2011)
MYMV MP-R	TTACAACGCTTTGTTCACATT				

Table 2: Nucleotide and amino acid sequence similarity matrix of coat protein gene of MYMIV infecting blackgram East Godavari isolate with other begomoviruses collected from NCBI GenBank

S. No.	Virus species	Geographical origin	Abbreviation	Nucleotide level	Amino acid level
1	*Mungbean yellow mosaic India virus*	India (East Godavari)	MYMIV-IN:E.G-MT066228	ID	ID
2	*Mungbean yellow mosaic India virus*	India (Guntur)	MYMIV-IN:GTR-JN181003	0.954	0.968
3	*Mungbean yellow mosaic India virus*	India (Kurnool)	MYMIV-IN:KNL-JN181004	0.99	0.992
4	*Mungbean yellow mosaic India virus*	India (Vizianagaram)	MYMIV-IN:VIZ-JN181006	0.968	0.984
5	*Mungbean yellow mosaic India virus*	India (Kadapa)	MYMIV-IN:KDP-KC243785	0.99	0.992
6	*Mungbean yellow mosaic India virus*	India (Hyderabad)	MYMIV-IN:Bg-KC243784	0.984	0.988
7	*Mungbean yellow mosaic India virus*	India (Gujarat)	MYMIV-IN:Cp-AY937195	0.935	0.976
8	*Mungbean yellow mosaic India virus*	India (Madhya Pradesh)	MYMIV-IN:Sb-KC852204	0.948	0.949
9	*Mungbean yellow mosaic India virus*	India (Raichur)	MYMIV-IN:Cb-MN698280	0.993	0.996
10	*Mungbean yellow mosaic India virus*	India (Meghalaya)	MYMIV-IN:Gg-KU950430	0.954	0.988
11	*Mungbean yellow mosaic India virus*	India (Chittoor)	MYMIV-IN:CTR-KJ747961	0.989	0.992
12	*Mungbean yellow mosaic India virus*	India (Tamil Nadu)	MYMIV-IN:Bg-KJ747962	0.988	0.988
13	*Mungbean yellow mosaic India virus*	India (Haryana)	MYMIV-IN:Sb-DQ389151	0.957	0.968
14	*Mungbean yellow mosaic India virus*	India (Coimbatore)	MYMIV-IN:Bg-KC911720	0.953	0.968
15	*Mungbean yellow mosaic India virus*	India (Madhya Pradesh)	MYMIV-IN:Sb-LC271792	0.957	0.972
16	*Mungbean yellow mosaic India virus*	India (Haldwani)	MYMIV-IN:Bg-KX655579	0.953	0.972
17	*Mungbean yellow mosaic India virus*	India (Kanpur)	MYMIV-IN:Gg-GQ387505	0.953	0.976
18	*Mungbean yellow mosaic India virus*	India (New Delhi)	MYMIV-IN:Gg-MH255791	0.95	0.976
19	*Mungbean yellow mosaic India virus*	India (Satna)	MYMIV-IN:Fb-KP779633	0.947	0.972
20	*Mungbean yellow mosaic India virus*	India (Akola)	MYMIV-IN:Fb-KP779635	0.952	0.976
21	*Mungbean yellow mosaic India virus*	Indonesia	MYMIV-IND:Ylb-JN368437	0.94	0.98
22	*Mungbean yellow mosaic India virus*	Pakistan	MYMIV-PAK:Bg-FM208845	0.959	0.972

Table 3: Nucleotide and amino acid sequence similarity matrix of coat protein gene of MYMV infecting blackgram East Godavari isolate with other begomoviruses collected from NCBI GenBank

S. No.	Virus species	Geographical origin	Abbreviation	Nucleotide level	Amino acid level
1	*Mungbean yellow mosaic virus*	India (East Godavari)	MYMV-IN:E.G-MT119671	ID	ID
2	*Mungbean yellow mosaic virus*	India (Tirupati)	MYMV-IN:Bg-KP455992	0.97	0.951
3	*Mungbean yellow mosaic virus*	India (Vamban)	MYMV-IN:Bg-DQ400848	0.967	0.956
4	*Mungbean yellow mosaic virus*	India (Coimbatore)	MYMV-IN:Bg-KC911718	0.966	0.956
5	*Mungbean yellow mosaic virus*	India (Hyderabad)	MYMV-IN:Gg-MN698275	0.969	0.956
6	*Mungbean yellow mosaic virus*	India (Maharashtra)	MYMV-IN:Sb-AF314530	0.963	0.956
7	*Mungbean yellow mosaic virus*	India (Dharward)	MYMV-IN:Gg-MN602419	0.966	0.956
8	*Mungbean yellow mosaic virus*	India (Haryana)	MYMV-IN:Gg-AY271896	0.928	0.917
9	*Mungbean yellow mosaic virus*	India (Vamban)	MYMV-IN:Bg-KC911722	0.972	0.956
10	*Mungbean yellow mosaic virus*	India (Jhansi)	MYMV-IN:Hg-KY824801	0.842	0.873
11	*Mungbean yellow mosaic virus*	India (Punjab)	MYMV-IN:Hg-MT345791	0.781	0.825
12	*Mungbean yellow mosaic virus*	India (Belgaum)	MYMV-IN:Mb-MN698295	0.963	0.956
13	*Mungbean yellow mosaic virus*	Vietnam	MYMV-VN:Gg-JX244176	0.954	0.943
14	*Mungbean yellow mosaic virus*	Pakistan	MYMV-PAK:Sb-AY269991	0.931	0.93
15	*Mungbean yellow India mosaic virus*	India (New Delhi)	MYMIV-IN:Pp-KX363947	0.775	0.816
16	*Mungbean yellow India mosaic virus*	India (West Bengal)	MYMIV-IN:Sb-HF922628	0.78	0.812
17	*Mungbean yellow India mosaic virus*	India (Haryana)	MYMIV-IN:Sb-DQ389155	0.771	0.812
18	*Mungbean yellow India mosaic virus*	India (Uttara Pradesh)	MYMIV-IN:Fb-DQ389148	0.764	0.807
19	*Mungbean yellow India mosaic virus*	India (Madhya Pradesh)	MYMIV-IN:Sb-LC271794	0.772	0.812
20	*Mungbean yellow India mosaic virus*	Indonesia	MYMIV-IND:Ylb-JN368432	0.775	0.803
21	*Mungbean yellow India mosaic virus*	Pakistan	MYMIV-PAK:Bg-FM208845	0.774	0.812

Table 4: Nucleotide and amino acid sequence similarity matrix of movement protein gene of MYMV infecting blackgram East Godavari isolate with other begomoviruses collected from NCBI GenBank

S. No.	Virus species	Geographical origin	Abbreviation	Nucleotide level	Amino acid level
1	*Mungbean yellow mosaic virus*	India (East Godavari)	MYMV-IN:E.G-MT071114	ID	ID
2	*Mungbean yellow mosaic virus*	India (Tirupati)	MYMV-IN:Bg-KF947526	0.96	0.916
3	*Mungbean yellow mosaic virus*	India (Belgaum)	MYMV-IN:Mb-MN698291	0.955	0.902
4	*Mungbean yellow mosaic virus*	India (Raichur)	MYMV-IN:Bg-MN698278	0.947	0.872
5	*Mungbean yellow mosaic virus*	India (Namakkal)	MYMV-IN:Mb-DQ865202	0.933	0.828
6	*Mungbean yellow mosaic virus*	India (Madurai)	MYMV-IN:Vg-AJ132574	0.928	0.818
7	*Mungbean yellow mosaic virus*	India (Vamban)	MYMV-IN:Bg-KC911727	0.933	0.823
8	*Mungbean yellow mosaic virus*	India (Coimbatore)	MYMV-IN:Bg-KC911729	0.935	0.833
9	*Mungbean yellow mosaic virus*	India (Dharward)	MYMV-IN:Gg-MN602426	0.81	0.556
10	*Mungbean yellow mosaic virus*	India(Coimbatore)	MYMV-IN:Gg-KP319017	0.954	0.892
11	*Mungbean yellow mosaic virus*	India (Vamban)	MYMV-IN:Bg-KC911728	0.933	0.823
12	*Mungbean yellow mosaic India virus*	India (Satna)	MYMIV-IN:Tm-MF683073	0.944	0.872
13	*Mungbean yellow mosaic India virus*	India (Meghalaya)	MYMIV-IN:Gg-KU950431	0.943	0.867
14	*Mungbean yellow mosaic India virus*	India (New Delhi)	MYMIV-IN:Bg-AF142440	0.909	0.754
15	*Mungbean yellow mosaic India virus*	India (Indore)	MYMIV-IN:Sb-KP828155	0.908	0.751
16	*Mungbean yellow mosaic India virus*	India (Madhya Pradesh)	MYMIV-IN:Sb-MH324446	0.908	0.754
17	*Mungbean yellow mosaic India virus*	India (Varanasi)	MYMIV-IN:Fb-KP779631	0.909	0.765
18	*Mungbean yellow mosaic India virus*	India (Indore)	MYMIV-IND:Sb-JN368446	0.924	0.805
19	*Mungbean yellow mosaic India virus*	Indonesia	MYMIV-IN:Sb-KP828155	0.908	0.751
20	*Mungbean yellow mosaic India virus*	Indonesia	MYMIV-IND:Ylb-JN368445	0.924	0.805
21	*Mungbean yellow mosaic India virus*	Oman	MYMIV-Omn:Cb-KX452229	0.916	0.791
22	*Mungbean yellow mosaic India virus*	Pakistan	MYMIV-PAK:Gg-FM958506	0.909	0.762

CONCLUSION

The present results from the coat protein gene sequence (MYMIV and MYMV), movement protein gene sequence (MYMV) alignment and construction of phylogenetic tree with other begomoviruses have clearly established that both MYMIV and MYMV are associated with YMD of blackgram in East Godavari district of Andhra Pradesh.

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