The Pharma Innovation Journal 2023; 12(12): 2078-2083

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 2078-2083

www.thepharmajournal.com
Received: 30-09-2023
Accepted: 06-11-2023

K Sailaja
Ph.D., Scholar, Department of Plant Pathology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

BV Bhaskara Reddy
Principal Scientist, Department of Plant Pathology, Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, Andhra Pradesh, India

R Sarada Jayalakshmi Devi
Vice Chancellor, Administrative Office, ANGRAU, LAM, Guntur, Andhra Pradesh, India

L Prasanthi
Director of Research, Administrative Office, ANGRAU, LAM, Guntur, Andhra Pradesh, India

G Mohan Naidu
Professor and Head, Department of Statistics and Computer Applications, Agricultural College, Naira, Andhra Pradesh, India

Corresponding Author:
K Sailaja
Ph.D., Scholar, Department of Plant Pathology, S.V. Agricultural College, Tirupati, Andhra Pradesh, India

Molecular characterization of begomovirus infecting green gram from Kadapa, Andhra Pradesh

K Sailaja, BV Bhaskara Reddy, R Sarada Jayalakshmi Devi, L Prasanthi and G Mohan Naidu

Abstract
Green gram is the most important pulse crop grown in India. Yellow mosaic infected and healthy green gram leaves were collected from Kadapa district of Andhra Pradesh during *Rabi* season of 2021-22. Total DNA was isolated from both diseased and healthy leaf tissue by CTAB method. The viral presence was confirmed by PCR amplification with viral specific primers. The PCR Samples were sent for sequencing and results were submitted in NCBI database. Sequence homology studies of coat protein revealed 98.3% similarity at nucleotide level and 99.6% at amino acid level with MYMIV-IN-Raichur-Rg-MF693401. Movement protein sequence shared 98% at nucleotide level and 95.2% at amino acid level with MYMIV-IN-Raichur-Bg-MT027037. The YMV-IN-Kadapa-Gg isolate shared 98.3 to 90.8% nucleotide similarity with *Mungbean yellow mosaic India virus* isolates from NCBI database hence it was confirmed that the begomovirus associated with Yellow mosaic disease of green gram at Kadapa district of Andhra Pradesh is MYMIV isolate.

Keywords: MYMV, MYMIV, HgYMV, DoYMV

Introduction
Green gram (*Vigna radiata* (L.) Wilczek) is an excellent source of high quality protein (25%) having high digestibility. It is consumed as whole grain as well as "Dal" in a variety of ways in our food. Nutritional value of 100 gm green gram seed has 16 grams of fibre, 24 grams of protein, 189 mg of Magnesium, 367 mg of Phosphorus and 1246 mg of Potassium. Total area under green gram cultivation in India is about 51.30 lakh ha with 30.85 lakh tonnes production and 601 kg/ha productivity. The major green gram producing states were Rajasthan followed by Madhya Pradesh, Maharashtra, Karnataka and Gujarat. In Andhra Pradesh 1.05 lakh ha area is under green gram cultivation with 0.81 lakh tonnes production and 772 kg/ha productivity during 2020-2021year (https://www.indiastat.com/data/agriculture/moong-green-gram).
The main reason for the low yield is the susceptibility of the crop to biotic factors like insects, weeds and diseases caused by fungus, virus or bacteria. Yellow mosaic disease is one of the most devastating biotic factor accounting generally up to 32-78% loss and up to 100% under early stage infection (Singh *et al*., 2019) [1]. Currently, there are four begomoviruses associated with Yellow mosaic disease of pulses which are *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Horsegram yellow mosaic virus* (HgYMV), *Dolichus yellow mosaic virus* (DoYMV) (Qazi *et al*., 2007) [2]. These begomoviruses are bipartite with DNA-A segment coding for 7 ORFs (Pre coat protein, Coat protein, Replication enhancer protein (REn), Replication-associated protein, Transcription activator protein (TrAP)) and DNA-B segment coding for 2 ORFs (Nuclear shuttle protein and Movement protein). In general, MVMV is the major isolate infecting mungbean crop in western and southern India, Thailand and Indonesia; whereas, MYMIV isolate in central, eastern and northern India, Pakistan, Bangladesh, Nepal and Vietnam (Malathi and John, 2009) [3].

Materials and Methods
Yellow mosaic infected and healthy green gram leaves were collected from Kadapa district of Andhra Pradesh during *Rabi* season of 2021-22 (Fig. 1). Total DNA was isolated from both diseased and healthy leaf tissue by CTAB method. The quality and quantity of isolated DNA was measured with nanodrop spectrometer. The viral presence was confirmed by PCR amplification with viral specific primers (Table. 1). PCR reaction was performed in a 25 µl of final volume 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, 53/55 °C for 45s annealing, 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 0.8% 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) [4]. 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 (Tamura *et al*., 2007) [5].

Fig 1: Green gram plants showing typical yellow mosaic disease symptoms

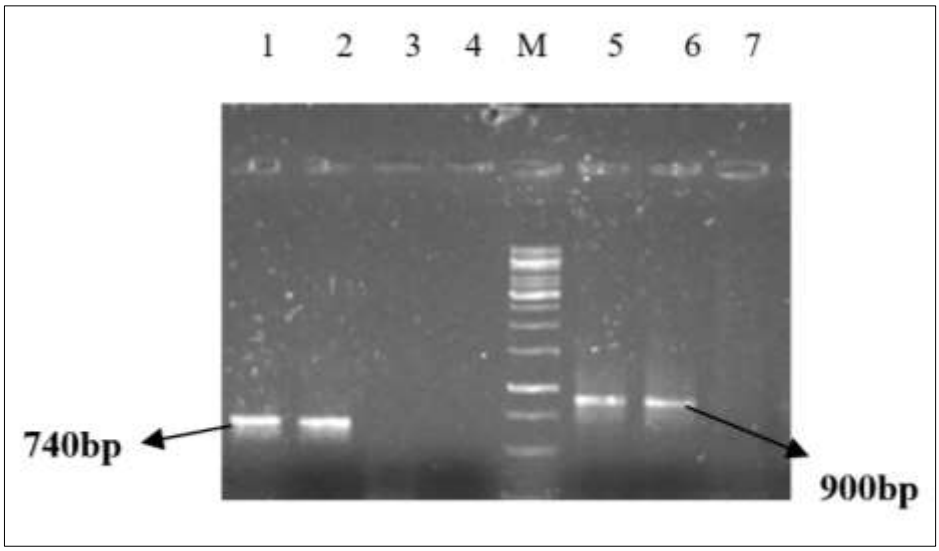
Table 1: List of primers used for detection of Yellow mosaic virus infecting green gram

Primer Name	Nucleotide Sequence (5'→3')	Start	Stop	Specific to	Product Size (bp)	Annealing Temp	Reference
MYMIV A1F	TGGGTACCCTTATCTTTAG	54	73	Pre coat and coat protein	838	53 °C	Designed in this study
MYMIV A1R	ATATTGGCCACCAGTAA	875	892				
MYMIV A2F	GTACAGCTACTGTGAAGA	810	828	Replication enhancer protein	742	53 °C	Designed in this study
MYMIV A2R	GATCTCGAATTGATCTAACC	1532	1552				
MYMV MP-F	ATGGAGAATTATTCAGGCGCA	-		Movement protein	900	55 °C	Naimuddin *et al.* (2011) [11]
MYMV MP-R	TTACAACGCTTTGTTCACATT						

Results and Discussion

DNA isolation and PCR confirmation

The quantity and quality of total DNA isolated by CTAB method ranged from 1800-2000 ng/µl and 1.8 to 2 of 260/280 nm ratio respectively. DNA diluted to 100 ng/µl was used as template for PCR reaction. The amplicon size of 800, 700 and 900 bp (Fig. 2) was observed with MYMIV A1, MYMIV A2, MYMV-MP primers respectively. PCR products were sequenced, assembled and analyzed using the Bioedit software. The nucleotide sequence of both MYMIV A1 and MYMIV A2 products were joined and resulted in 1400 bp sequence which codes for Pre/Coat protein and AC3/ Replication enhancer protein. The sequences were submitted to NCBI database and accession numbers OQ732574 (Pre/Coat protein and AC3/ Replication enhancer protein) and OP677560 (Movement protein) were obtained.



Lane M: 1Kb plus ladder
Lane 1-2 and 5-6: DNA samples from YMV infected green gram leaves.
Lane 3-4, 7: DNA samples from healthy green gram leaves.

Fig 2: Detection of MYMIV in infected green gram leaf samples by PCR amplification of coat protein and movement protein gene.

Sequence analysis

Sequence homology studies were performed with already available sequences downloaded from NCBI database. Coat protein gene sequence shared 90.8-98.3% nucleotide identity with MYMIV followed by 79.7-79.9% with MYMV, 80.2-81.3% with HgYMV and 70.6% with DoYMV. The coat protein gene shared 93.3-99.6% at amino acid level with MYMIV followed by MYMV (86%), HgYMV (85%) and DoYMV (80.9%). Coat protein gene has been traditionally used for identification and classification of plant viruses. If nucleotide similarity at coat protein or complete DNA-A sequence ranging from 94-100%, then it will be considered as variant as per strain demarcation criteria (Fauquet *et al*. 2008) [6]. Hence it is confirmed that the begomovirus associated with Yellow mosaic disease of green gram at Kadapa district in Andhra Pradesh is *Mungbean yellow mosaic India virus*.

AC3/ Replication enhancer gene sequence shared 87.6-99.2% similarity at nucleotide level with MYMIV followed by 84.6-85.6% (MYMV) then 84.4-85.1% (HgYMV) and 60.1% (DoYMV). At amino acid level AC3 gene shared 74.6-98.4% similarity with MYMIV followed by 70% (MYMV), 69% (HgYMV) and 36% (DoYMV) (Table. 2). Movement protein sequence shared 93.1-98% similarity with MYMIV; 79.6-80.8% with MYMV; 79% with HgYMV; 67% with DoYMV at nucleotide level. At amino acid level movement protein gene shared 82.6-95.2% with MYMIV; 55% with MYMV; 51% with HgYMV; 30% with DoYMV indicting major closeness to MYMIV isolates (Table. 3).

Table 2: Percentage identity of coat protein of YMV-IN-Kadapa-Gg with other begomoviruses

S. No.	Isolate	Coat Protein		AC3/REn		1400 bp (Accession No: OQ732574)	
		Nucleotide level	Amino Acid level	Nucleotide level	Amino Acid level	Nucleotide level	Amino Acid level
1	MYMIV-IN-Raichur-Cp-MW814709	0.974	0.984	0.992	0.984	0.977	0.944
2	MYMIV-IN-Raichur-Bg-MN698277	0.974	0.984	0.992	0.984	0.976	0.942
3	MYMIV-IN-Raichur-Rg-MF693401	0.983	0.996	0.930	0.858	0.97	0.935
4	MYMIV-IN-Pakisthan-Gg-AJ512495	0.98	0.992	0.913	0.826	0.963	0.924
5	MYMIV-Bangladesh-Gg-AF314145	0.954	0.968	0.928	0.857	0.949	0.883
6	MYMIV-IN-East Godavari-Bg-MT312254	0.956	0.976	0.933	0.849	0.948	0.883
7	MYMIV-Pakistan-Sb-MN885468	0.908	0.933	0.876	0.746	0.907	0.778
8	MYMV-IN-Tirupati-Gg-OQ732573	0.798	0.867	0.856	0.703	0.811	0.630
9	MYMV-IN-Tirupati-Bg-OQ615924	0.799	0.863	0.856	0.700	0.810	0.632
10	MYMV-IN-Kanpur-Gg-MW736055	0.799	0.867	0.858	0.708	0.808	0.629
11	MYMV-IN-Hyderabad-Gg-MN698273	0.797	0.863	0.846	0.677	0.808	0.623
12	MYMV-IN-Dharward-Gg-MN602425	0.798	0.863	0.849	0.685	0.806	0.623
13	HgYMV-IN-Dharwad-Gg-MW791246	0.811	0.856	0.851	0.695	0.827	0.627
14	HgYMV-IN-Bangalore-Cp-AM932427	0.813	0.856	0.844	0.687	0.825	0.629
15	HgYMV-IN-coimbatore-Hg-AJ627904	0.812	0.852	0.849	0.687	0.823	0.622
16	HgYMV-Srilanka-Fb-GU323321	0.802	0.832	0.844	0.679	0.817	0.624
17	DoYMV-IN-Kanpur-Do-NC005338	0.706	0.809	0.601	0.360	0.653	0.376

Table 3: Percentage identity of movement protein of YMV-IN-Kadapa-Gg with other begomoviruses

S. No.	Isolate	Nucleotide Level	Amino acid Level
1	MYMIV-IN-Raichur-Bg-MT027037	0.980	0.952
2	MYMIV-IN-Meghlaya-Gg-KU950431	0.966	0.905
3	MYMIV-IN-Guwahati-Gg-OK431080	0.969	0.916
4	MYMIV-IN-Bhubaneswar-Gg-OK431084	0.965	0.908
5	MYMIV-Indonesia-So-JN368446	0.948	0.871
6	MYMIV-IN-New Delhi-Gg-MW791230	0.947	0.850
7	MYMIV-IN-Hisar-Gg-MW791227	0.947	0.858
8	MYMIV-IN-Bg-NC_004609	0.931	0.826
9	MYMIV-IN-Coimbatore-Gg-MW717980	0.932	0.836
10	MYMV-IN-Hyderabad-Gg-MN698274	0.808	0.553
11	MYMV-IN-Dharward-Gg-MN602420	0.807	0.559
12	MYMV-IN-Coimbatore-Bg-KA27-AF262064	0.802	0.555
13	MYMV-IN-New Delhi-Bg-JQ398670	0.800	0.532
14	MYMV-IN-Tirupati-Gg-OP442513	0.796	0.537
15	HgYMV-IN-Tiupati-Hg-OQ508785	0.789	0.508
16	HgYMV-IN-Tiupati-Rg-OQ659401	0.790	0.519
17	DoYMV-IN-NC_024779	0.674	0.300

The Phylogenetic tree was constructed from of coat protein and movement protein nucleotide sequence of Yellow mosaic virus from green gram host in Andhra Pradesh with other begomoviruses downloaded from NCBI database. It formed four unique clusters representing MYMV, HgYMV, MYMIV and DoYMV (Fig. 3 & 4). YMV-IN-Kadapa-Gg-OQ732574 (Pre/Coat protein and AC3/ Replication enhancer protein) formed unique clade with MYMIV-IN-Raichur-Rg-MF693401 & MYMIV-IN-Pakisthan-Gg-AJ512495 and OP677560 (Movement protein) with MYMIV-IN-Raichur-Bg-MT027037.

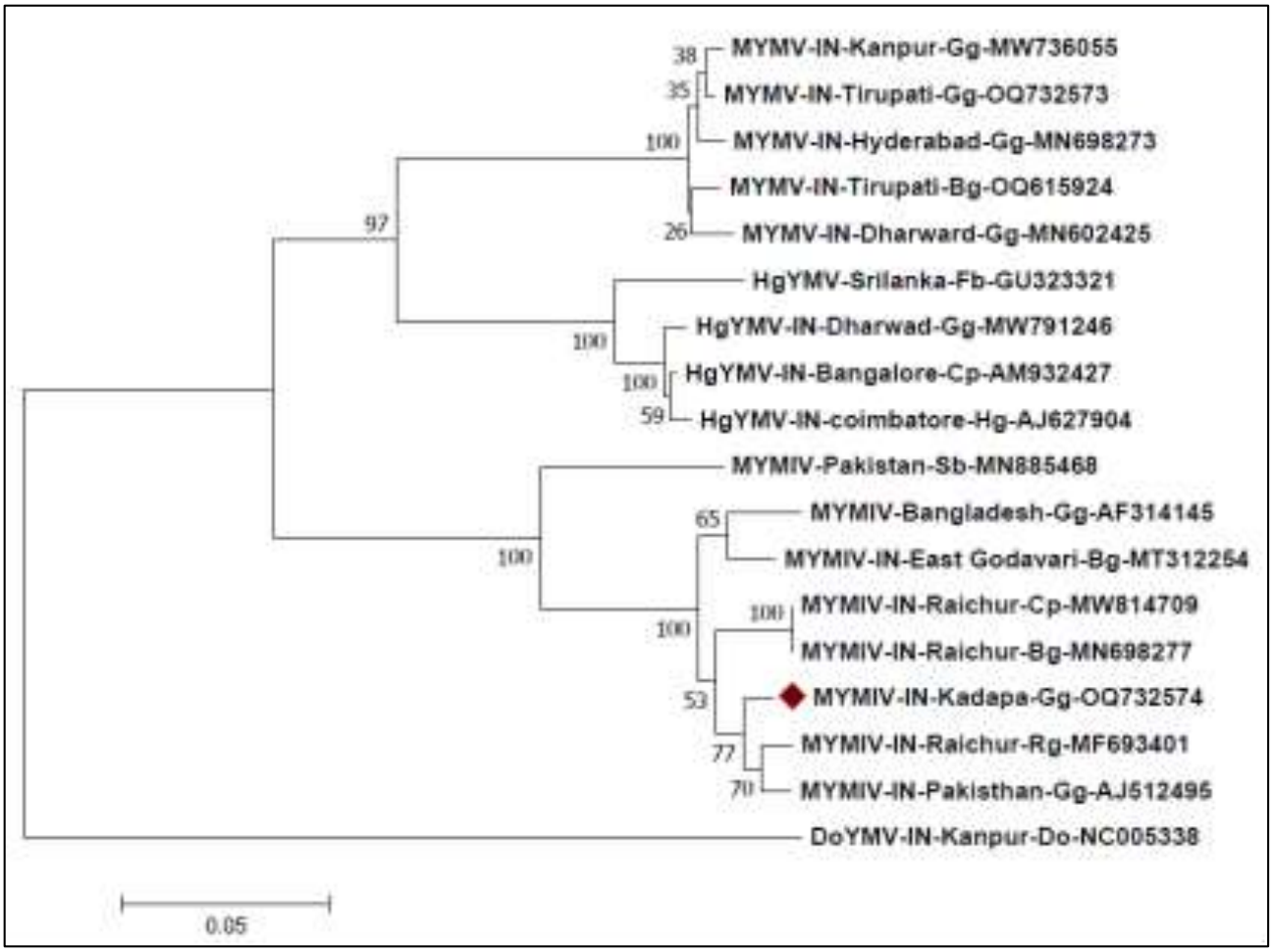


Fig 3: Phylogenetic tree (1000 boot strap replications) derived from of coat protein nucleotide sequence of Yellow mosaic virus from green gram host in Andhra Pradesh with other begomoviruses. Abbreviations and accession numbers of begomoviruses sequences used were given in table 2.

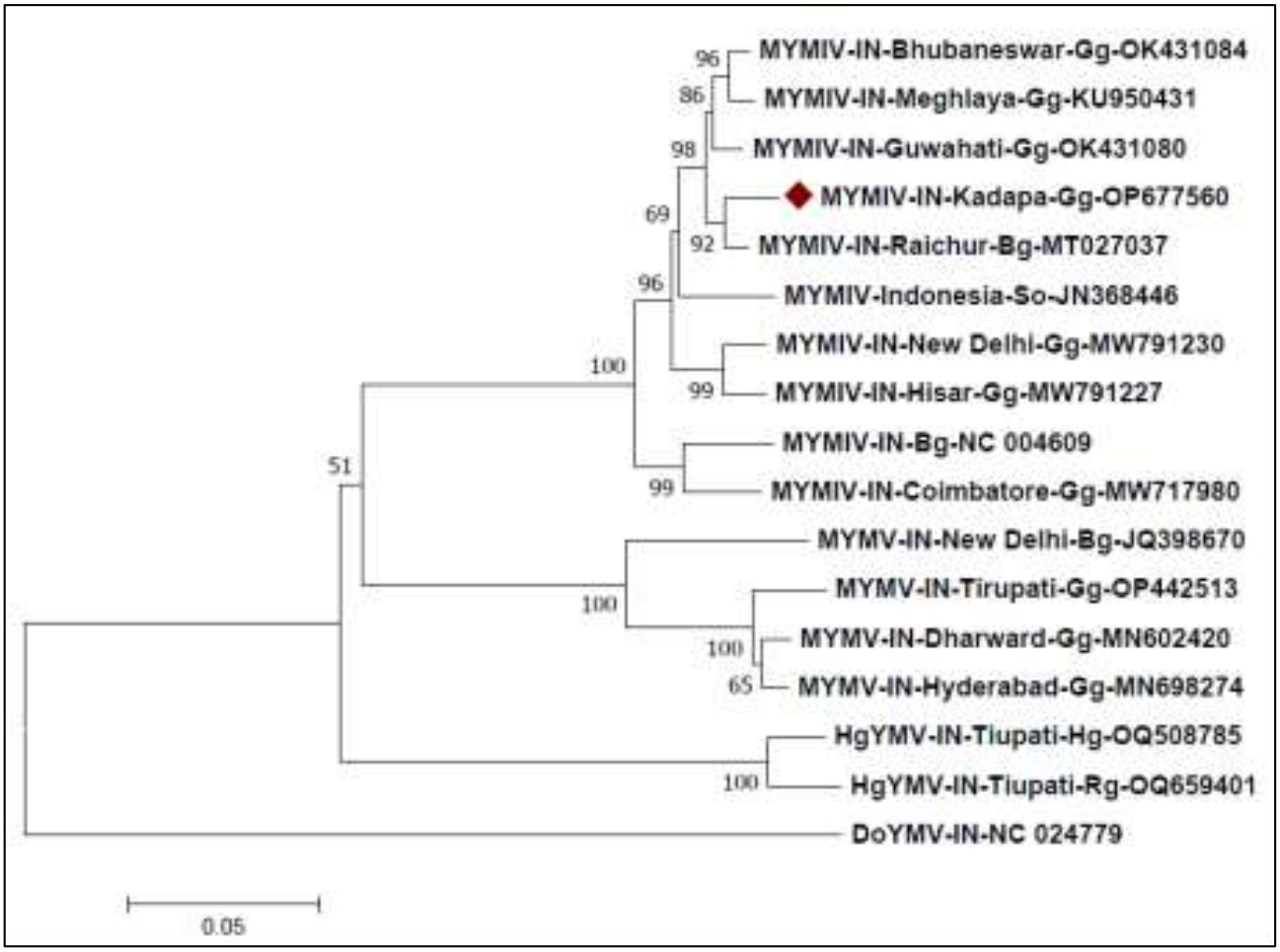


Fig 4: The phylogenetic tree constructed from comparison of movement protein nucleotide sequence of yellow mosaic virus from green gram host in Andhra Pradesh with other begomoviruses. Abbreviations and accession numbers of begomoviruses sequences used were given in table 3.

Madhumitha *et al*., 2019 [7] conducted survey on five different mungbean growing localities in Coimbatore district of Tamil Nadu to explore the species of begomoviruses (MYMV or MYMIV) prevalent in Tamil Nadu. Based on molecular analysis of coat protein and movement protein gene sequence it was concluded that, MYMV is associated with green gram grown in Coimbatore district of Tamil Nadu. Based on complete DNA-A genome sequence and phylogeny Ambarish *et al*., 2022 [8] concluded that, YMV isolates infecting green gram from Karnataka were closely clustered with MYMV with greater than 95% nucleotide similarity. Reddy *et al*., 2015 [9] reported association of MYMIV with blackgram in Andhra Pradesh based on coat protein sequence data. The *Yellow Mosaic Virus* infecting blackgram and green gram in Telangana and Andhra Pradesh is closely related to *MYMIV* rather than *MYMV* or other begomoviruses was reported by Bhanu (2014) [10].

Conclusions

From above results it is clear that begomovirus associated with yellow mosaic disease of green gram from Kadapa region of Andhra Pradesh is closely related to MYMIV rather than MYMV.

References

1. Singh CM, Singh P, Pratap A, Pandey R, Purwar S, Douglas CA, *et al*. Breeding for enhancing Legumovirus resistance in mungbean: current understanding and future directions. Agronomy. 2019 Oct 10;9(10):622.
2. Qazi J, Ilyas M, Mansoor S, Briddon RW. Legume yellow mosaic viruses: genetically isolated begomoviruses. Molecular plant pathology. 2007 Jul;8(4):343-8.
3. Malathi VC, John P. Mungbean yellow mosaic viruses. Desk encyclopedia of plant and fungal virology. Eds. M. Van Regenmortal and B. Mahy (London: Academic press). 2009;217-226.
4. Hall TA. Bio Edit: A user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series. 1999;41:95-98.
5. Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 2007;24(8):1596-1599.
6. Fauquet CM, Briddon RW, Brown JK, Moriones E, Stanley J, Zerbini M *et al*. Geminivirus strain demarcation and nomenclature. Archives of Virology. 2008;153:783-821. doi: 10.1007/s00705-008-0037-6.
7. Madhumitha B, Eraivan AK, Aiyanathan A, Sudha M. Coat protein based characterization of Mungbean yellow mosaic virus in Tamil Nadu. Journal of Pharmacognosy and Phytochemistry. 2019;8(2):2119-2123.
8. Ambarish S, Kalleshwaraswamy CM, Venkataravanappa V. Biological and molecular characterization of Mungbean yellow mosaic virus (MYMV) and its vector,

Bemisia tabaci cryptic species in green gram. Indian Phytopathology. 2022;76(1):223-232. https://doi.org/10.1007/s42360-022-00567-9.
9. Reddy BVB, Obaiah S, Prasanthi L, Sivaprasad Y, Sujitha A, Krishna TG. Mungbean yellow mosaic India virus is associated with yellow mosaic disease of black gram (*Vigna mungo* L.) in Andhra Pradesh, India. Archives of Phytopathology and Plant Protection. 2015;48(4):345-353.
10. Bhanu SH. Molecular characterization and development of diagnostic methods for detection of yellow mosaic virus infecting pulses. M. Sc. (Ag.) Thesis. Acharya N G Ranga Agricultural University, Hyderabad, India; c2014. http://krishikosh.egranth.ac.in/handle/1/87151.
11. Naimuddin K, Akram M, Sanjeev G. Identification of *Mungbean yellow mosaic India virus* infecting *Vigna mungo var. silvestris* L. Phytopathologia Mediterranea. 2011;50:94-100.