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Molecular Detection and Partial Characterization of Begomovirus associated Yellow Mosaic Virus Disease of Pole Bean (*Phaseolus vulgaris* L.) in Southern India

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The present study was conducted to investigate the etiology and molecular relationship of causal virus associated with pole bean yellow mosaic disease and other related viruses. Yellow mosaic disease on pole bean caused by begomovirus was observed to an extent of 60-70 percent in some cultivars grown in experimental plots at Main research station, Hebbal (Bangalore), Karnataka, India. Symptoms like brilliant yellow or golden yellow colour on leaves which was partially or completely yellow. Cultivars express rugosity, rolling of leaves, and stunting of infected plants. The disease was successfully transmitted by whitefly *Bemisia tabaci* G. The causal virus was detected using begomovirus specific degenerate primers. The coat protein gene fragment of 1000 bases was amplified from naturally infected and whitefly inoculated plants. Phylogenetic analysis of the CP gene sequence of the virus with other begomoviruses showed cluster next to French bean yellow mosaic virus (FbYMV) GKVK isolate and Lima bean yellow mosaic virus by sharing 99.7% and 98.7% similarity respectively. Hence, the results clearly indicated that the YMV infecting French bean is a close relative of YMV infecting Pole bean. From now, the yellow mosaic virus infecting pole bean can be called as Pole bean yellow mosaic virus (PbYMV).

Key words: Begomovirus, *Bemisia tabaci* G., coat protein, PCR, leaf rolling, rugosity, yellow mosaic

Pole bean is one of the major vegetables consumed throughout the globe, it is consumed as a immature tender fruits, green grains as vegetable and dry grain. It has some medicinal properties in controlling diabetes, cardiac problems and natural cure for bladder burn. It has got both carminative and reparative properties against constipation and diarrhea respectively[1]. In India, pole bean is extensively grown in Himachal Pradesh, Punjab, Haryana, Uttaranchal, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu. Annually, it is grown in an area of 1.54 million ha with an annual production of 4, 20,000 tonnes with productivity of 3 tonnes per ha[2]. Since the crop has longer pod length, high yielding and long harvest season, produce could fetch better market price as compared to the regular French bean.

Pole bean suffers from fungal, bacterial and viral diseases. Among the fungal diseases ascochyta blight, anthracnose, rust and root rot. The bacterial diseases include angular leaf spot, bacterial blight and common bean mosaic. Yellow mosaic, beangolden mosaic, southern bean mosaic, bean pod mottle and bean leaf rollvirus are the major viral diseases. Among viral diseases, yellow mosaic disease was considered as an important

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constraint in pole bean productivity. Yellow mosaic disease in French bean is widely distributed in the tropical and sub-tropical regions[3]. The estimated crop loss ranged from 40 to 100 per cent depending on the cultivar and time of infection [4-5].

Begomoviruses are the major constraint in successful cultivation of horticultural and agricultural crops throughout the world. The severity and incidence of these viruses in southern India had been increased in the recent years after the introduction of 'B' Biotype *Bemisia tabaci* [6-10]. begomoviruses typically have bipartite ssDNA genomes with essential genes encoded on the DNA-A and DNA-B. Many monopartite begomoviruses have single DNA molecule in tomato, bhendi and cotton with additional satellite molecules called DNA-^{2} which regulates symptom expression[11-12].

In plants or insects, begomoviruses have been visualized as nuclear inclusion bodies through detection tools, such as light microscopy, ultra structural, electron microscopy, serological assays[13-16], DNA hybridization assays[17], Polymerase Chain Reaction (PCR) [18-21] immuno capture PCR[22]. Molecular cloning and DNA sequencing of viral genomes determined virus identification and comparing relationships with other virus isolates [23-26].

All begomoviruses code for coat protein which acts as a protective coat of the virus particle and determine vector transmissibility of the viruses by whitefly vector *B. tabaci*. Thus, the CP gene is highly conserved among begomoviruses originating from the same geographical region and adapted to transmission by local vector population[27-28]. The coat protein sequence have been used to establish provisional species identification owing to the highly conserved nature of the viral CP sequences. The CP primers have been illustrated to amplify a fragment of most, if not all, begomoviruses irrespective of old or new world origin. The rapid detection followed by prediction of provisional species affiliation by comparison with reference begomovirus CP sequences[29-30].

Studies on pole bean yellow mosaic disease symptoms, transmissibility and management were reported[31]. However the etiology and phylogenetic relationship with other begomoviruses were unknown. Hence, in this study we depictedon symptoms, detection and phylogenetic relationship with other important begomoviruses.

MATERIALS AND METHODS

Symptoms and PCR detection of the virus

The culture of the virus was collected from infected pole bean plants grown in MRS, Hebbal (Bangalore). Virus detection and cloning work of viral DNA was carried out in the Department of Plant Pathology, UAS, GKVK, Bangalore, India during 2012-13. Total genomic DNA was extracted from pole bean plants with yellow mosaic symptoms using CTAB method[32]. The extracted genomic DNA was amplified by PCR using gemini virus specific primers[33] which is capable of amplifying the CP region of many begomoviruses.

PCR was carried out in 25 ¼l reaction mixture that contained 2.0 ¼l total DNA extracted from infected pole bean leaf (200-300 ng), 0.2 ¼lTaq DNA polymerase (5U/ ¼l), 2.5 ¼l of 10X PCR buffer (100 mM Tris-HCl, pH 8.3,500 mM KCl, 15 mM $MgCl_2$), 0.5 ¼l of 25mM $MgCl_2$, 2.0 ¼l each primer (10 mM), 2.0 ¼l dNTPs mix (2.5 mM each) and sterile water to make up the volume. The optimal annealing temperature for each primer pair was determined using temperature gradient block (Eppendorf gradient master cycler, Hamburg, Germany). The mixture was subjected to PCR master cycler with 94ºC for 2 min initial denaturation, 94ºC for 30 seconds denaturation, 55ºC for 1 min annealing, 72ºC for 2 min extension, and 72ºC for 10 min final extension with 35 Cycles. Amplified PCR products were separated by electrophoresis on 1% agarose gel and DNA fragments were visualized using ethidium bromide stain and analyzed by alpha imager gel documentation and analysis system. DNA ladder set (1 kb, Cat. No. N3232S, New England Biolabs) included as sized molecular marker. DNA from healthy pole bean plants was used as experimental positive control.

Phylogenetic analysis of coat protein gene of the virus

The 1000bp fragment corresponding to the CP gene of viral DNA was purified using gel extraction kit (Qiagen, GmbH, Hilden, Germany) and cloned in the plasmid pTZ57R/T in T/A cloning kit (Cat#,K 1214, MBI, Fermentas) following manufactures instructions. Clones were sequenced

in both the directions using M13 forward and reverse primers at Chromous Biotech, Bangalore, India. The CP gene sequence of pole bean yellow mosaic virus was compared with other respective viral sequences obtained from the NCBI database (Table 1).

Sequences obtained were identified in terms of closest homology sequence using blast (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Multiple sequence alignments were made by CLUSTAL W. MEGA software (version 4.0) was used for the phylogenetic analysis[34]. Phylogenic trees were constructed by neighbor-joining method.

RESULTS

Disease symptoms

A yellow mosaic disease symptomatically comparable to the disease caused by begomoviruses on other crop plants.The characteristics symptoms observed on naturally infected plants were appears as brilliant yellow or golden yellow colour on leaves which may be partially or completely yellow. The virus infected cultivars showed rugosity, rolling of leaves, pods exhibited blotching, discoloration with reduced size and number (Fig1). The infected plants remained very much stunted when infected at early stages of crop growth and fail to produce any pods.

Fig. 1. Field infected Pole bean plants showing typical yellow mosaic symptom (a) coupled with deformed pods(b)

Details of begomoviruses used in the phylogenetic analysis of coat protein gene nucleotide sequences

Virus species	Abbreviation	NCBI accession No.
French bean yellow mosaic virus isolate Bangalore	FbYMV-[Ban]	KC019306
Lima bean yellow mosaic virus	LbYMY	AM932429
Horse gram yellow mosaic virus	HYMV	AM932427
French bean yellow mosaic virus isolate India	FbYMV-[Ind]	AM932425
Horse gram yellow mosaic virus isolate Lucknow	HYMV-[LK:09]	GU323321
Mung bean yellow mosaic virus isolate Haryana	MYMV-[Haryana]	AY271896
Soybean yellow mosaic virus Isolate Pakistan	SYMV-[Pak]	AY269991
Mung bean yellow mosaic virus	MYMV	AY271892
Mung bean yellow mosaic virus Isolate Thailand	MYMV-[Thi]	AB017341
Mung bean yellow mosaic virus isolate Namakkal	MYMV-[Nam]	DQ865201
Mung bean yellow mosaic virus -Soybean Isolate Madurai	MYMV-[Mad]	AJ421642
Mung bean yellow mosaic virus-Vigna Isolate Maharashtra	MYMV-[MH]	AF314530
Mung bean yellow mosaic virus-Vigna Isolate	MYMV [Vigna]	AJ132575
Urdbean yellow mosaic virus isolate New Delhi	UbYMV-[Delhi]	JQ398669
Mung bean yellow mosaic virus Isolate Pakistan	MYMV-[Pak]	AY269992
Mung bean yellow mosaic virus Isolate Akola	MYMV-[Akola]	AY271893
Soybean yellow mosaic virus Isolate Tamilnadu	SYMV-[TN]	AJ416349
Mung bean yellow mosaic virus isolate Palampur	MYMV-[Palampur]	FN794200
Mung bean yellow mosaic virus -Soybean Isolate India	MYMV-[Ind]	AY049772
Mung bean yellow mosaic virus Isolate Nepal	MYMV-[NP]	AY271895
Mung bean yellow mosaic virus Isolate Varanasi	MYMV-[Varanasi]	AY547317
Mung bean yellow mosaic virus Isolates Bangladesh	MYMV-[BD]	AF314145
Mung bean yellow mosaic virus isolate Indonesia	MYMV-[ID]	JN368438
Mung bean yellow mosaic virus Isolate Indonesia	MYMV-[ID]	JN368434
Mung bean yellow mosaic virus Isolate Indonesia	MYMV-[ID]	JN368437
Mung bean yellow mosaic virus Isolate Indonesia (Bogor)	MYMV-[ID Bogor]	JN368432

Table 2. Percentage nucleotide identities based on coat protein sequences of selected begomoviruses

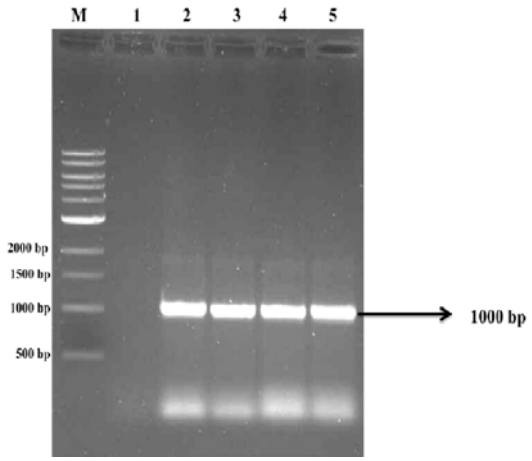
Abbreviation	NCBI accession No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
PbYMV																										
FbYMV-[Ban]	KC019306																									
LbYMY	AM932429	98.4																								
HYMV	AM932427	99.4	98.7																							
FbYMV-[Ind]	AM932425	98.7	98.1	98.9																						
HYMV-[LK:09]	GU323321	95.2	95.3	95.4	95.3																					
MYMV-[Haryana]	AY271896	85.6	85.2	85.7	85.2	85.2																				
SYMV-[Pak]	AY269991	85.4	85.0	85.5	85.0	85.0	96.2																			
MYMV	AY271892	87.2	86.6	87.3	86.6	86.0	93.7	94.4																		
MYMV-[Thi]	AB017341	86.6	86.1	86.8	86.1	85.5	93.5	93.7	98.1																	
MYMV-[Nam]	DQ865201	86.5	86.0	86.6	86.0	85.6	94.7	95.2	96.7	97.2																
MYMV-[Mad]	AJ421642	86.5	86.0	86.6	86.0	85.4	94.8	95.2	96.7	97.2	99.0															
MYMV-[MH]	AF314530	86.9	86.4	87.0	86.4	85.7	94.7	95.2	972	97.8	99.0	99.2														
MYMV [Vigna]	AJ132575	86.5	86.0	86.6	86.0	85.4	94.1	94.5	97.1	97.6	98.7	98.8	99.0													
UbYMV-[Delhi]	JQ398669	82.4	81.6	82.4	81.6	81.1	81.7	81.5	82.5	82.4	82.2	82.4	82.5	82.1												
MYMV-[Pak]	AY269992	81.5	80.7	81.6	80.7	80.2	79.8	79.5	80.4	80.4	80.3	80.4	80.6	80.2	96.2											
MYMV-[Akola]	AY271893	80.3	79.8	80.4	79.8	78.8	78.6	78.6	79.5	79.5	79.4	79.5	79.7	79.3	94.3	97.9										
SYMV-[TN]	AJ416349	81.2	80.4	81.5	80.4	79.9	79.5	79.5	79.8	79.8	79.9	79.8	79.9	79.5	95.8	98.5	96.7									
MYMV-[Palampur]	FN794200	81.1	80.3	81.3	80.3	79.8	79.5	79.8	79.8	79.8	79.9	79.8	79.9	79.5	95.8	98.7	96.8	99.0								
MYMV-[Ind]	AY049772	81.5	81.0	81.7	81.0	80.4	80.2	80.3	81.2	81.2	81.1	81.2	81.3	81.0	94.8	97.8	96.1	96.8	97.0							
MYMV-[NP]	AY271895	80.8	80.2	80.8	80.2	79.3	79.0	78.9	79.7	79.7	79.5	79.8	79.8	79.5	94.8	96.6	95.3	96.6	96.2	95.3						
MYMV-[Varanasi]	AY547317	80.7	80.1	80.7	80.1	79.3	79.1	78.9	79.7	79.7	79.5	79.8	79.8	79.5	94.8	96.8	95.0	96.8	96.5	95.6	99.2					
MYMV-[BD]	AF314145	80.7	80.1	80.8	80.1	78.9	79.5	79.3	80.1	80.1	79.9	80.2	80.2	79.9	94.0	95.9	94.7	96.1	95.7	94.9	95.8	95.8				
MYMV-[ID]	JN368438	80.4	80.3	80.6	80.3	79.7	79.9	80.1	80.1	79.9	79.8	79.8	79.8	79.5	92.8	94.9	93.4	94.8	94.8	93.9	94.0	94.1	96.3			
MYMV-[ID]	JN368434	80.6	80.4	80.7	80.4	79.5	80.3	80.1	80.2	80.2	79.8	80.1	80.1	79.8	93.1	95.2	93.6	94.8	94.8	94.1	94.3	94.4	96.6	99.4		
MYMV-[ID]	JN368437	80.7	80.3	80.8	80.3	79.4	80.4	80.2	80.3	80.3	79.9	80.2	80.2	79.9	93.4	95.4	93.9	95.0	95.0	94.4	94.5	94.7	96.6	99.2	99.4	
MYMV-[ID Bogor]	JN368432	80.6	80.2	80.7	80.2	79.3	80.3	80.6	80.2	80.2	79.8	80.1	80.1	79.8	93.2	95.3	93.7	94.9	94.9	94.4	94.4	94.5	96.5	99.0	99.3	99.6

Isolation of total DNA and PCR amplification

The total viral DNA was extracted by the standard CTAB protocols suggested by[28].PCR tests carried using a set of degenerate primers successfully amplified expected DNA fragment of 1000 bp (Fig 2) from both naturally and whitefly-inoculated pole bean plants. These primers have been used extensively for the identification of begomoviruses in a wide range of crop plants and their vector *Bemisia tabaci* previously[33]. The PCR protocol was slightly modified by altering the PCR conditions as the viral DNA was not amplified when standard PCR protocol suggested by the Wyatt and Brown[29] was adopted. The PCR conditions were slightly modified by changing the annealing temperature and duration. Annealing temperature of 55ºC for 1 minute was found suitable and amplified pole bean yellow mosaic viral DNA, as compared to 55ºC for 2 minute for gemini virus detection by standard PCR protocols suggested by various workers. This modified PCR protocol amplified the gemini virus specific 1000 bp product from infected pole bean samples.

Cloning of PCR products and DNA sequencing

PCR products obtained was eluted from the gel by using gel extraction kit (Cat# 28704) obtained from QIAGEN, GmbH, Hilden, Germany, as per manufacturer's instructions and cloned into the plasmid vector pTZ57R/T using Ins TAclone TM PCR Cloning Kit and transformed into Escherichia coli DH5-a cells (Invitrogen Life Technologies, Carlsbad, CA, USA). Recombinants were screened by blue and white screening method as per standard protocol. Sequencing of 2–3 representative clones was performed with automated sequencing facility at Chromos Biotech (Bangalore, India). In addition purified PCR products were directly used for sequencing.



M- 1 KB marker (New England Biolabs, Cat. No. N3232S)

Fig. 2. A picture of agarose gel electrophoresis illustrating begomovirus-specific PCR products obtained using the coat protein gene primers (Lane 2-5) and Healthy DNA sample (Lane 1)

Phylogenetic analysis

Sequences of PCR products and cloned DNA of PCR products were assembled using Bio Edit software. A database search of homologous sequences was performed by BLAST analysis at NCBI (http://ncbi.nlm.nih.gov/BLAST). BLAST search was carried out at each step for confirmation of the correct orientation and position of sequences. The coat protein gene sequence was then screened for vector contamination using specialized blast programme "VecScreen". The complete coatprotein sequence was subjected to BLAST search. Sequences were compared with other respective viral sequences of the NCBI data base using BLAST and multiple aligned using CLUSTALW2 multiple alignmenttool (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Thephylogenetic distance tree and molecular evolutionary analyses were performed with MEGA4.0 software using the maximum parsimony method with default values and 1000 replications for bootstrap analysis.

Sequence analysis

The coat protein gene of pole bean yellow mosaic virus was sequenced assembled and determined its total length as 920 bp. The actual length of coat protein gene of YMV infecting pole bean was determined by analogy with other YMVs infecting legumes in India as 889 bp including 115 bp pre-coat protein at 52 end and 774 bp core coat protein and the length of the amino acid sequence was determined as 257. The CP gene sequence has been used and illustrated to be useful in begomovirus classification[26]. The CP sequence of the PbYMV was compared with those of selected begomoviruses. Phylogenetic analysis revealed that the pole bean yellow mosaic virus from Bangalore had 99.7% with French bean yellow

mosaic virus (FbYMV) GKVK isolate, 98.7% with Lima bean yellow mosaic virus and shared [Accession No. KC019306] and 99.2% with French bean yellow mosaic virus [Accession No. AM932429] (Fig 4). Based on symptom observation, transmission through *Bemisia tabaci* and PCR detection of the begomovirus-specific DNA products from the infected plants, it is concluded that the disease is caused by a begomovirus.

DISCUSSION

Pole bean is an important vegetable crop grown extensively in the Indian subcontinent and it is severely affected by yellow mosaic disease caused by begomoviruses, the disease causes substantial yield loss by affecting growth and flowering of the infected plants.The estimation of crop loss ranged from 40 to 100 per cent depending

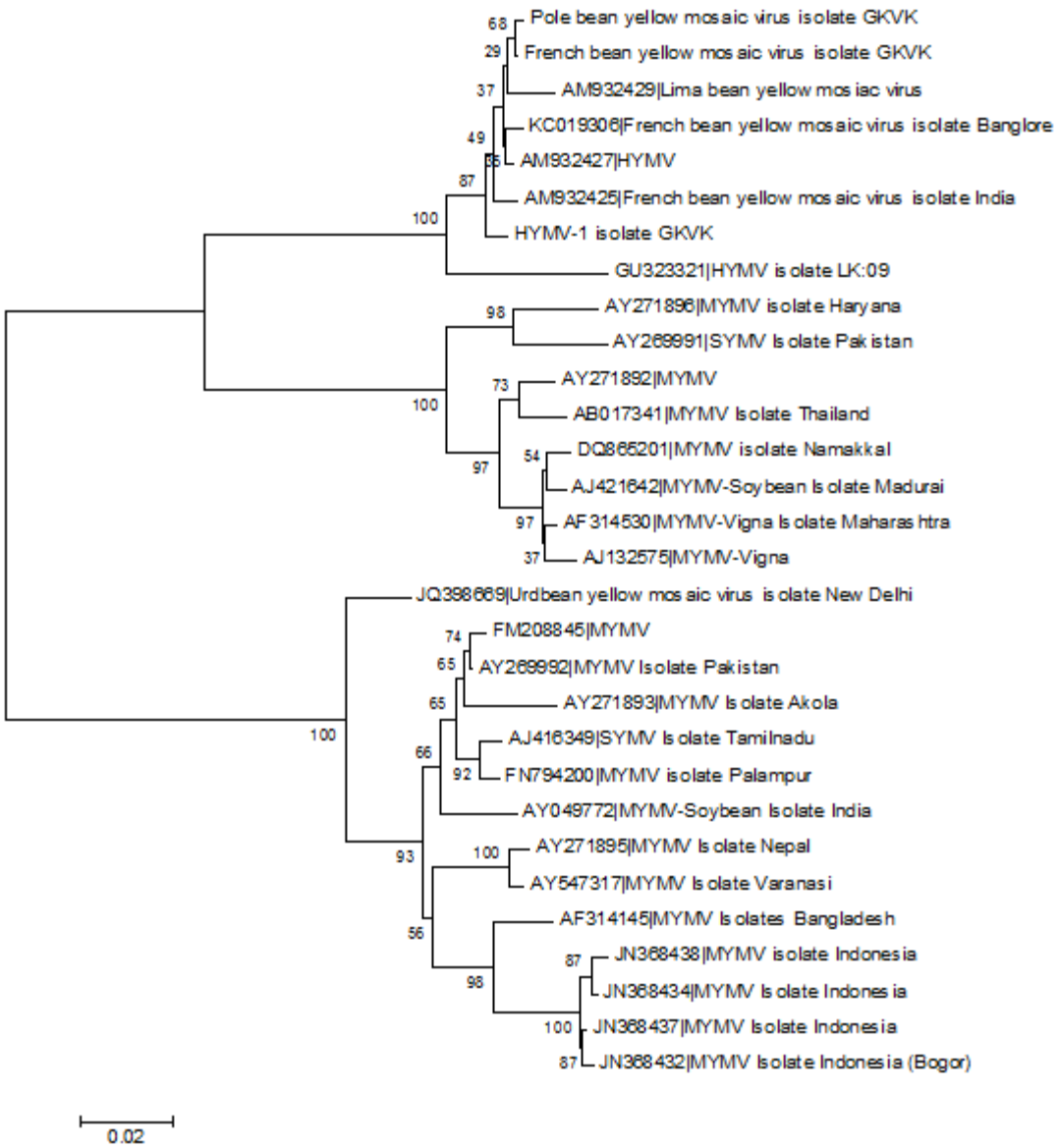


Fig. 3. The most parsimonious phylogenetic tree (1000 boostrap replications) illustrating the relationship of PbYMV with selected begomoviruses (Table 1) based on their CP nucleotide sequences

on the cultivar and time of infection[5].The fast evolution and the recent spread of begomoviruses resulting from new strains/species of viruses through recombination and the increasing abundance of viruliferous whitefly vectors, possibly as a result of enhanced insecticide resistance and climate change are becoming a major challenge for the production of many crops worldwide [35-44].

With this back-drop, we detect and characterized the yellow mosaic virus infecting Pole bean from Bangalore, Karnataka state of India. The genome organization of the virus isolate is typical of begomoviruses originating from the Old World[45-47]. DNA was extracted and Polymerase Chain Reaction was employed to establish association of begomovirus through amplification of geminivirus specific PCR product approximately 1000 bp from DNA-A component of begomoviruses using CP primers. The amplified products were allowed to run on agarose gel, an expected amplified PCR product obtained from coat protein primer (<"1000 bp), and the product was cloned suitably into vector (pTZ57R/T) and transformed by using *E. coli* strain DH5 ±. The positive clones (white clones) were confirmed by colony PCR. Plasmids were isolated and sequenced bi-directionally to ensure sequence identity and reliability of the sequences.

The sequence analyses revealed that the genome sequence isolated from Pole bean shared highest nucleotide identity with an isolates of French bean yellow mosaic virus [GKVK isolate], Lima bean yellow mosaic virus [Accession No. KC019306] and French bean yellow mosaic virus (Bangalore isolate) [Accession No. AM932429] 99.7%, 98.7% and 99.2% respectively. Hence, the results clearly indicated that the YMV infecting French bean is a close relative of YMV infecting Pole bean. From now, the yellow mosaic virus infecting pole bean can be called as Pole bean yellow mosaic virus (PbYMV). The disease assumes greater significance because of the presence of *B tabaci* to feed on pole bean crop over other field crops. Further studies are in progress to determine the epidemiology and sequencing of complete genome of the virus.

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REFERENCES

1. Duke JA. Handbook of legumes of world economic importance. pp 195-200, New York, USA/London, UK: Plenum Press. (1981).
2. Vanitha SM, Chaurasia SNS, Singh PM, Prakash S. Indian Institute of Vegetable Research, Varanasi, *Vegetable Statistics*, 2011; 227-228 pp.
3. Maramorosch K, Muniyappa V. Whitefly transmitted plant disease agents in Karnataka, India. In: International workshop on pathogens transmitted by whiteflies. July 31, (Oxford: Association of Applied Biologist Wellesbourne) 1981.
4. Pierre RE. Observation on golden mosaic of bean (*Phaseolous vulgaris* L.) in Jamaica.In: "Tropical diseases of legumes" (Bird J, Maramoroscheds K.), 1975; pp 35-39.
5. Galvez GE, Cardenas M. Whitefly transmitted viruses. *In*: "Bean production problems" (eds. Schwartz HF, Galvez GE,) pp 261-289, Centro International de Agricultural Tropical, Cali, 1980; Colombia.
6. Banks GK, Colvin RV, Reddy C, Maruthi MN, Muniyappa V *et al*. First report of *Bemisia tabaci* B-biotype in India and an associated Tomato leaf curl virus disease epidemic. *Plant Dis*, 2001; **85**: 231-231.
7. Narayana DSA, Shankarappa KS, Govindappa MR, Prameela HA, Gururaj Rao MR, Rangaswamy KT. Natural occurrence of Jatropha mosaic virus disease in India. *Curr Sci*, 2006; **91**: 584-586.
8. Narayana DSA, Rangaswamy KT, Shankarappa KS, Maruthi MN, Reddy CNL *et al*. Distinct begomovirus closely related to cassava mosaic viruses cause Indian jatropha mosaic disease. *Int J Virol*, 2007; **3**: 1-11.
9. Maruthi MN, Rekha AR, Muniyappa V. Pumpkin yellow vein mosaic disease is caused by two distinct begomoviruses: Complete viral sequences and comparative transmission by an indigenous *Bemisia tabaci* and the introduced B-biotype. *EPPO Bull*, 2007; **37**: 412-419.

10. Mahesh YS, Shankarappa KS, Rangaswamy KT, Prameela HA, Narayana DSA *et al*. Detection and characterization of a begomovirus associated with leaf curl disease of ornamental croton (*Codiaeum variegatum*). *J Hort Sci Biotechnol,* 2010; **85**: 101-105.
11. Navot NE, Pichersky M, Zeidan, Zamir D, Czosnek H. Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. *Virology,* 1991; **185**: 151-161.
12. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K *et al*. Identification of DNA components required for induction of cotton leaf curl disease. *Virology,* 2001; **285**: 243-243.
13. Polston JF, Dodds JA, Perring T. Nucleic acid probes for detection and strains discrimination of cucurbit, geminiviruses. *Phytopathology,* 1989; **79**: 1123-1127.
14. Konate G, Barro N, Fargette D, Swanson MM, Harrison BD. Occurrence of whitefly-transmitted geminiviruses in crops in Burkina Faso, and their serological detection and differentiation. *Ann Applied Biol,* 1995; **126**: 121-129.
15. Hunter WB, Hiebert E, Webb SE, Tsai JH, Polston JE. Location of geminiviruses in whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Plant Dis,* 1998; **82**: 1147-1151.
16. Pico B, Diez MJ, Nuez F. Improved diagnostic techniques for Tomato yellow leaf curl virus in tomato breeding programs. *Plant Dis,* 1999; **83**: 1006-1012.
17. Lotrakul P, Valverde RA, Clark CA, Sim J and De La Torre R. Detection of a geminivirus infecting sweet potato in United States. *Plant Dis*, 1998; **82**: 1253-1257.
18. Deng A, McGrath PF, Robinson DJ, Harrison BD. Detection and differentiation of whitefly transmitted geminiviruses in plants and vector insects by the Polymerase Chain Reaction with degenerated primers. *Ann Appl Biol,* 1994; **125**: 327-336.
19. Mehta P, Wyman JA, Nakhla MK, Maxwell DP. Polymerase chain reaction detection of viruliferous *Bemisia tabaci* (Homoptera: Aleyrodidae) with two tomato infecting geminiviruses. *J Econ Entomol,* 1994; **87**: 1285-1290.
20. Ghanim MS, Morin M, Zeidan, Czosnek H. Evidence for transovarial transmission of tomato yellow leaf curl virus by its vector the whitefly *Bemisia tabaci*. *Virology,* 1997; **240**: 295-303.
21. Rosell CR, Torres-Jerez I, Brown JK. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph and honeydew. *Phytopathology*, 1999; **89**: 239-246.
22. Ramprasad SN, Umaharan P. Detection of begomovirus in clarified plant extracts: A component of standard direct binding and immune capture PCR technique. *Phytopathology,* 2003; **93**: 1153-1157.
23. Rybicki E. A phylogenetic and evolutionary justification for 3 genera of *geminiviridae*. *Arch Virol,* 1994; **139**: 49-77.
24. Padidam M, Beachy RN, Fauquet CM. Classification and identification of geminiviruses using sequence comparisions. *J Gen Virol*, 1995; **76**: 249-263.
25. Paximadis M, Idris AM, Torres-Jerez I, Villarreal A, Rey MEC, Brown JK. Characterization of tobacco geminiviruses in the old and new world. *Arch Virol*, 1999; **144**: 703-717.
26. Brown JK, Idris AM, Torres-Jerez I, Banks GK, Wyatt SD. The core region of the coat protein gene is highly useful in establishing the provisional identification and classification of begomoviruses. *Arch Virol,* 2001; **146**: 1581-1598.
27. McGrath PF, Harrison BD. Transmisson of tomato leaf curl geminiviruses by *Bemisia tabaci*: Effects of virus isolate and vector biotype. *Ann Applied Biol*, 1995; : 307-316.
28. Maruthi MN, Colvin J, Seal S, Gibbson G, Cooper J. Co-adaptation between cassava mosaic geminiviruses and their local vector populations. *Virus Res,* 2002; **86**: 71-85.
29. Wyatt SD, Brown JK. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology,* 1996; **86**: 1288-1293.
30. Harrison BD, Swanson MM, Fargette D. Begomovirus coat protein: serology, variation and functions. *Physiol Mol Plant Pathol,* 2002; **60**: 257-271.
31. Jyothi, V., Nagaraju, N., Padmaja, A. S., and Rangaswamy, K. T., Transmission, Detection and Management of Yellow Mosaic Virus in Polebean (*Phaseolus vulgaris* L.) *J. Mycol. Plant Pathol.,* 2013; **43(2)**: 229 – 236.
32. Lodhi MA, Ye GN, Weeden NF, Reisch BI. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* spp. and *Ampelopsis*. *Plant Mol Biol Rep,* 1994; **12**: 6-13.
33. Naimuddin, Akram M. Detection of mixed infection of begomoviruses in cowpea and their molecular characterization based on CP gene sequences. *J Food Leg,* 2010; **23**: 191-195.
34. Tamura K, Dudley J, Nei M, Kumar S.

MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol*, 2007; **24**: 1596-1599.

35. Polston JF, Anderson PL. The emergence of whitefly transmitted geminiviruses in tomato in the Western Hemisphere. *Plant Disease*, 1997; **81**: 1358–1369.
36. Padidam M, Sawyer S, Fauquet CM. Possible emergence of new geminiviruses byfrequent recombination. *Virology*, 1999; **265**: 218–225.
37. Sanz AI, Fraile A, GarciaArenal F, Zhou XP, Robinson DJ, Khalid S *et al*. Multiple infection, recombinationand genome relationships among begomovirus isolates found in cotton and other plants in *Pak J Gen Virol*, 2000; **81**: 1839–1849.
38. Banks GK, Colvin RV, Reddy C, Maruthi MN, Muniyappa V *et al*. First report of *Bemisia tabaci* B-biotype in India and an associated Tomato leaf curl virus disease epidemic. *Plant Dis*, 2001; **85**: 231-231
39. Varma A, Malathi VG. Emerging geminivirus problems: a serious threat to crop production. *Annuals of Applied Biology*, 2003; **142**: 145–164.
40. Rekha AR, Maruthi MN, Muniyappa V, Colvin J. Occurrence of three genotypic clusters of *Bemisia tabaci* and the rapid spread of the B biotype in south India. *Entomologia Experimentaliset Applicata*, 2005; **117**: 221–233.
41. Seal SE, Vandenbosch F, Jeger MJ. Factors influencing begomovirus evolution and their increasingglobal significance: implications for sustainable control. *Critical reviews in Plant Sciences*, 2006; **25**: 23–46.
42. Shankarappa K S, Rangaswamy KT, Aswathanarayana DS, Rekha AR, Raghavendra N, Reddy, CNL *et al*. Development and optimization of silverleaf assay, protein and nucleic acid based diagnostic techniques for quick and reliable detection and monitoring biotype B of *Bemisia tabaci* in India. *Bulletin of Entomological Research*, 2007; **97**: 503–513.
43. Krishnareddy M. Climate change and virus diseases ofHorticultural crops.In H. P. Singh JP, Singh, Lal SS. (Eds.), Challenges of climate change – Indian Horticulture(pp. 153–165). New Delhi, India: Westville Publishing house. 209P.
44. Venkataravanappa V, Reddy CNL, Jalali S, & Krishna Reddy M. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes*, 2012; **44**; 522–535.
45. Chatterjee A, Ghosh SK. A new monopartite begomovirus isolated from *Hibiscus cannabinus* L. inIndia. *Arch Virol*, 2007; **152**: 2113–2118.
46. Pandey P, Mukhopadhya S, Naqvi AR, Mukherjee SK, Shekhawat GS, Choudhury NR. Molecular characterization of two distinct monopartite begomoviruses infecting tomato in India. *Virology Journal*, 2010; **7**: 337.
47. Kumari P, Singh AK, Chattopadhyay B, Chakraborty S. Molecular characterization of a new species of begomovirus and beta satellite causing leaf curl disease of tomato in India. *Virus Research*, 2010; **152**: 19–29.