Documentation of viruses infecting lablab (*Lablab purpureus* L.) beans in Tamil Nadu, India

ABSTRACT

Field surveys were conducted in lablab-bean growing areas of Tamil Nadu to document the occurrence and distribution of virus diseases. Samples collected from lablab bean field showed symptoms like bright yellow mosaic patches and puckering on leaves. The total DNA and RNA were isolated from the symptomatic samples separately. The DNAs were subjected to polymerase chain reaction using *Begomovirus* universal primers and the positive samples amplified 1200 bp amplicon. Similarly, the isolated RNAs were subjected to reverse transcription PCR using *Potyvirus* universal primers and which amplified 350 bp in preliminary confirmation of viruses. The amplified products were sequenced and the DNA sequences were compared with the sequences available in the NCBI database and the phylogenetic tree was constructed using Mega 11 software. The occurrence of viruses *viz., Bean common mosaic virus* (BCMV) and *Dolichos yellow mosaic virus* (DoYMV) were documented. The results indicated a higher incidence of *Begomovirus,* DoYMV with a mean incidence of 86 per cent followed by the *Potyvirus*, BCMV with an incidence of around 70 per cent.

Keywords: Lablab bean; Dolichos yellow mosaic virus; Bean common mosaic virus; phylogeny

1. INTRODUCTION

"Lablab-bean (*Lablab purpureus* L.) is one of the multipurpose leguminous vegetable crops cultivated throughout India. It is widely cultivated in dry tropical parts of South-Central America, Asia, Africa, West Indies and China" (Suruthi *et al*., 2018). "India occupies around 2.3 lakh ha area under lablab bean cultivation with of 6.43 lakh tonnes vegetables bean production with a productivity of around 11.71 tonnes ha-1. Within India, lablab bean is cultivated to a large extent in Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh and Maharashtra. In Tamil Nadu, lablab-bean occupies an area of 0.70 lakh hectares with a production of around 0.35 lakh tonnes and productivity of around 0.5 tonnes ha-1. The lablab-bean crop is grown both during kharif and rabi. Lablab bean is grown for fresh green pods used as vegetables, dry seed are used for culinary purposes and the other plant parts as fodder for livestock. The green pods are rich in proteins (22.4–31.3%) and carbohydrates (55%) and can be used as substitute for expensive animal proteins. The pods also contain vitamins A, C and minerals such as copper, potassium, magnesium, iron, and phosphorus, are fibre-rich and are suitable for human nutrition" (Adnan *et al.,* 2021). The lablab-bean is affected by many fungal, bacterial and viral diseases. Among them, the viral diseases becoming a major threat for lablab-bean production. Viral diseases in Lablab-beans such as *Dolichos yellow mosaic virus* (*Begomovirus*), *Tobacco streak virus* (*Ilarvirus*) (Bhaskara et al.,2013); *Dolichos enation mosaic virus* (*Tobamovirus*); *Peanut stunt virus* (*Cucumovirus*); *Clover yellow mosaic virus* (*Potyvirus*); *Bean common mosaic virus* (*Potyvirus*); *Bean common mosaic necrosis virus* (*Potyvirus*); *Cowpea mosaic virus* (*Comovirus*) (Odedara *et al.,*2008) are reported.

The yellow mosaic disease is a devastating disease that significantly reduces yield. Capoor and Verma (1950) first reported "the *Dolichos yellow mosaic virus* and it was transmitted by whitefly (*Bemisia tabaci*)". "Dolichos yellow mosaic virus (DoYMV) belonging to the genus *Begomovirus*; family *Geminiviridae* and consists of circular single-stranded DNA genomic component encapsidated in twinned or geminate, icosahedral particles (18 × 30 nm size)" (Stanley *et al.,*2005). **"**The DNA A component encodes for transcription activator protein, coat protein on the viral strand, replication enhancer protein on the complementary strand" (Hanley et al., 1999). The DNA B component encodes for protein in viral sense strand and facilitating intra and intercellular movement.

Bean common mosaic virus (BCMV) was first reported by McDonald and Kulkarni in 1973 confirmed its true identity. Bean common mosaic virus was transmitted through sap, seeds, pollens and aphids (*Aphis craccivora)* (Kapil *et al*., 2011 and Kennedy *et al*., 1962,)**.** Bean common mosaic virus is belonging to the genus *Potyvirus*; family *Potyviridae*. It is a helical flexous rod-shaped virus measuring about 750nm length and 15nm diameter in size encapsidating a positive sense single stranded RNA genome of about 10 kb size (Bhadramurthy *et al*., 2009). The present study was undertaken to understand the prevalence and distribution of viruses in lablab-bean in Tamil Nadu.

2. MATERIALS AND METHODS

2.1 Survey area and sample collection

A systematic field survey was conducted in major lablab-bean growing districts *viz.,* Krishnagiri, Dharmapuri, Erode, Salem, Coimbatore and Theni of Tamil Nadu during Rabi season of 2022. The samples were collected from the plants showing the characteristic symptoms like bright golden speck on the leaf lamina followed by complete yellowing of leaves and pods, puckering and downward curling of leaves. The virus disease incidence was recorded and calculated by the following formula (Wheeler, 1969)

$$\text{Per cent disease incidence} = \frac{\text{Number of virus infected plants}}{\text{Total number of plants observed}} \times 100$$

About two to three young symptomatic leaves were collected per plant and placed in a separate zip-loc bags and labelled. Samples were carried to the laboratory in a cool box and the collected samples were processed within 24h of collection.

2.2 Total genomic DNA Isolation

For the detection of Begomovirus, total DNA was extracted from symptomatic leaves by the GEM-CTAB method using 2 per cent β - mercapto ethanol (Rouhibakhsh *et al*., 2008). The buffer containing 2% CTAB, 1M Tris HCL, 0.5 M EDTA and 5M NaCl was prepared and adjusted the pH 8.0 then autoclaved the buffer. “β-mercaptoethanol was added to the buffer prior to the isolation procedure. 100mg of infected leaf sample was taken in pre chilled pestle and mortar and ground by 1ml of the buffer then transferred into 2 ml of Eppendorf tube and kept for incubation at @ 65°C for 30 mins. During incubation the tubes were intermittently mixed for homogenous the plant sample. After incubation, 0.8 volumes of chloroform:iso-amyl alcohol was added to the tubes and mixed gently by inversion until formation of emulsion. The tubes were centrifuged in a refrigerated centrifuge at 10,000rpm for 10 min at 4°C. The supernatant was collected into a new tube without disturbing the layer. Added 0.6 volume of ice-cold isopropanol to the tubes and incubated at -20°C overnight. After overnight incubation, the tubes were centrifuged at 10,000 rpm for 10 min. The supernatant was decanted and pellet was washed twice with 70% ethanol. After ethanol wash pellet was kept at room temperature to evaporate the ethanol completely from the DNA”. [33] The DNA pellet was dissolved in 50µl of double distilled sterile water and stored at -20°C for further use.

2.3 PCR based detection using universal primers for begomovirus

The DNA was subjected to Polymerase Chain Reaction using the universal primer pair of *begomovirus* i.e., PALr772 and PALc1960 (Rojas *et al.,* 1993) to confirm the association of a begomovirus. The reaction mixture containing 5µl of taq DNA polymerase, optimized buffer and DNTP mixture in a ready to use master mix (2X smart Prime 2X PCR Master Mix-Red), 1µl of forward primer, reverse primer and template DNA, the final volume was adjusted to 10µl using double sterile distilled water. “The reaction was programmed with temperature profile followed was initial denaturation at 94°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 55° C for 2 minutes and elongation at 72°C for 3 minutes, the final extension step was carried out at 72°C for 10 minutes. The PCR product was kept in hold at 4°C in thermocycler (Eppendorf, Hamburg, Germany)”. [33]

The PCR products were run on the 1.2% agarose gel using 1X TAE buffer and staining the gel with ethidium bromide. The gel image was viewed under alpha imager (Alpha Innotec, USA) and size of the amplicons were determined with 1 kb DNA ladder.

2.4 Total genomic RNA Isolation

Total RNA was extracted from symptomatic young leaf tissue of lablab-bean samples collected from different locations of Tamil Nadu by Trizol method (Chomczynski and Sacchi, 1987). Ground 100 mg of leaf tissue by using liquid nitrogen in pre autoclaved pestle and mortar and added 1ml of trizol reagent for homogenized leaf tissue. The content was transferred into new sterile 1.5 ml eppendorf

tube and incubated at room temperature for 2 min. The centrifuge was pre cooled at 4°C for 5 mins and centrifuged the content at 10,000 rpm for 15 min. Transferred the aqueous layer into new tube and added 250µl of chloroform. Mixed gently by inversion until the formation of emulsion and centrifuged the tube at 10,000 rpm for 15 mins at 4°C. The supernatant was added into the new tube and added 2M sodium chloride and isopropyl alcohol then incubated in -20°C for 15 mins. After incubation, the tubes were centrifuged at 12,000 rpm for 15 min to obtain nucleic acid pellet. The obtained pellet was washed twice with 70% ethanol to remove the salts. After the ethanol wash, tubes were kept open in room temperature for the ethanol to evaporate completely from the RNA. Pellet was dissolved in 30µl of double sterile water and isolated RNAs were stored at - 40°C for further use.

2.5 Reverse transcription - PCR (RT-PCR) analysis

The total RNA isolated from the plant samples were used for RT-PCR. First strand cDNA synthesis kit (iScript cDNA synthesis Kit) was used according to the manufacturer's instructions to synthesis the cDNA. Reaction mixture contained 5X iScript Reaction mix - 4 µl; iScript Reverse Transcriptase - 1 µl; Nuclease-free water - 11 µl; RNA template - 4 µl was added. The sample were then incubated for 5 min at 25°C followed by reverse transcription for 20 min at 46°C and RT inactivation for 1 min at 95°C using Thermocycler (Eppendorf, Hamburg, Germany). The cDNA was used in PCR for amplification of a portion of the virus genome using universal primers for preliminary confirmation for the presence of *Potyvirus*. The universal degenerative primers of potyvirus i.e., NIb2F and NIb3F (Zheng *et al*., 2008) was used. "The reaction mixture was added to sterile PCR tube containing 5µl DNTP mixture in a ready-to-use master mix, 1µl of each forward primer, reverse primer and template DNA, the final volume was adjusted to 10µl using double sterile distilled water. The temperature profile followed was initial denaturation at 94°C for 3 minutes followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing at 45°C and extension at 72°C for 60 seconds, the final extension step was carried out at 72°C for 7 minutes. The PCR product was kept in hold at 4°C. The polymerase chain reaction was carried out in the Mastercycler nexus thermal cycler. The primer set was expected to amplify the DNA fragment of ~350 bp. After the PCR reaction the samples were made to run on 1.2% agarose gel using 1X TAE buffer after staining the gel with ethidium bromide. The gel was imaged under an alpha imager to know the amplification of the sample to the desired size to confirm the presence of the virus". [33]

Table 1. Details of primers used and anticipated amplicon sizes

S.No	Name of the Primer	Sequence 5'to 3'	Annealing Temperature(°C)	Anticipated Amplicon Size	Reference
1	PALr772	5'GGNAARATHTGGGATGGA3'	55	~1.5Kb	(Rojas *et al*., 1993)
2	PALc1960	5'ACNGGNAARACNATGTGGGC3'			
3	NIb2F	5'GTITGYGTIGAYGAYTTYAAYAA'3	45	~350bp	(Zheng *et al*., 2008)
4	NIb3F	5'TCIACIACIGTIGAIGGYTGNCC'3			

2.6 Sequencing, sequence analysis and phylogeny

The sequence amplified for viruses using universal primers from different locations were sequenced by sanger sequencing. The sequences obtained were searched in NCBI-BLAST to know the sequence similarity with other Begomoviruses and potyviruses, respectively. Nucleotide sequence alignments and assembling were done with Matrix tool, Bio Edit software and the sequences were deposited in NCBI BLAST and the accession numbers were obtained. Sequence Demarcation Tool 1.2 (SDT v 1.2) (Muhire *et al*., 2014) was used to determine the per cent identity matrix among the DoYMV and BCMV sequence. Using MEGA 11 Software, a phylogenetic tree was constructed based

on the sequences available in the NCBI database and with 1000 replications to generate the phylogenetic trees of the viruses and the evolutionary were also estimated.

3. RESULTS

3.1 Occurrence of virus diseases in lablab beans in Tamil Nadu

Lablab bean is the one of the major vegetables grown in the Tamil Nadu. A systematic roving field survey was conducted to assess the virus incidence in lablab-bean growing areas in Tamil Nadu (Table 2). The maximum per cent disease incidence of DoYMV was observed on lablab-bean in Kenathukadavvu village of Coimbatore district (86.0%) and the least incidence was observed in Sellappavpalayam village of Erode district (35%). The maximum per cent disease incidence of BCMV was observed in Adagapadi village of Dharmapuri district (70%) and the least incidence was observed in Thalaivasal of Salem district (15%). The affected plants showing bright yellow mosaic patches and puckering on leaves (Fig 1). The reason behind the variations in the disease incidence and severity might be due to variation in the climatic conditions, source of inoculum and vector population.

Table 2. Per cent disease incidence of virus diseases on lablab-beans in Tamil Nadu

S.No	District	Location	GPS Co-ordinates	Per cent disease incidence	
				DoYMV	BCMV
1	Dharmapuri	Molayanur	11.958014°N/78.349312°E	47	63
		Attukkarampatti	12.148162°N/78.113654°E	56	45
		Pappinaickanahalli	12.158683°N/78.118644°E	82	65
		Adagapadi	12.145842°N/78.107982°E	59	70
2	Coimbatore	Kenathukadavvu	10.853045°N/77.112827°E	86	33
		Thondamuthur	10.997338°N/76.825037°E	76	46
		Vellimalaipattinam	10.988935°N/76.78559°E	82	53
		Narasimmapuram	10.999008°N/76.820955°E	65	27
3	Salem	Thalaivasal	11.5867°N/78.7583°E	71	15
		Thoppampalayam	11.463069°N/77.146177°E	56	41
4	Erode	Sellappavpalayam	11.32062°N/77.148772°E	35	34
		Erode	11.42241°N/77.156168°E	42	25
		Pasur	11.283055°N/77.120756°E	58	18
5	Krishnagiri	Paiyur	12.371688°N/78.219949°E	63	20
		Malaipaiyur	12.371688°N/78.219949°E	45	32
		Krishnagiri	12.371688°N/78.219949°E	40	18
6	Theni	Chinnavoalur	9.784573°N/77.370381°E	69	28
		Pusanampatti	9.812212°N/77.388889°E	73	54

	Chinnamanur	9.826475°N/77.382644°E	68	47

(a) (b)

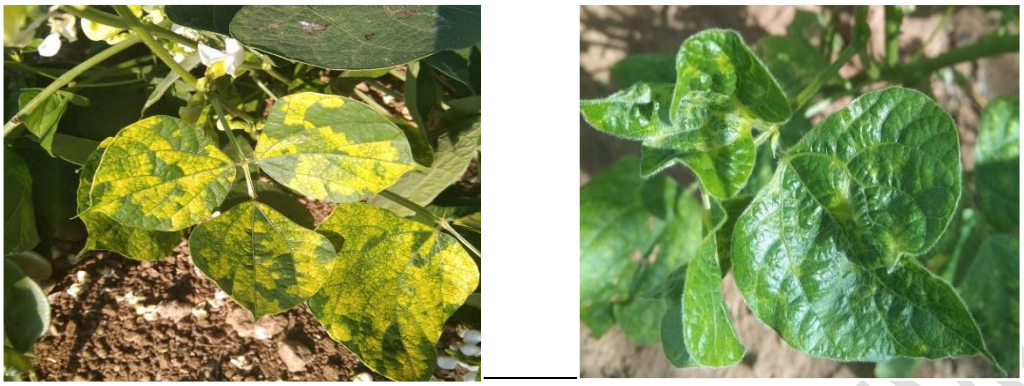

Fig 1. (a) Symptom of Dolichos yellow mosaic virus (DoYMV); (b) Symptom of Bean common mosaic virus (BCMV)

3.2 Detection of viruses by PCR/RT-PCR

All the samples were subjected to nucleic acid-based detection using PCR for begomoviruses and RT-PCR for the potyviruses. The isolated DNAs were diluted and subjected for amplification using Roja's primer and showed an amplicon at ~1.2 Kb with its expected size (Fig 2). The isolated RNA was converted to complementary DNA (cDNA) and subjected for amplification using NIb2F/ NIb3R primer pair yielded an expected amplicon of the size ~350 bp (Fig 2 and 3). Most of the samples (86.0%) were found infected with Begomoviruses (Dolichos yellow mosaic virus) and followed by potyvirus (70.0%) (Bean common mosaic virus).

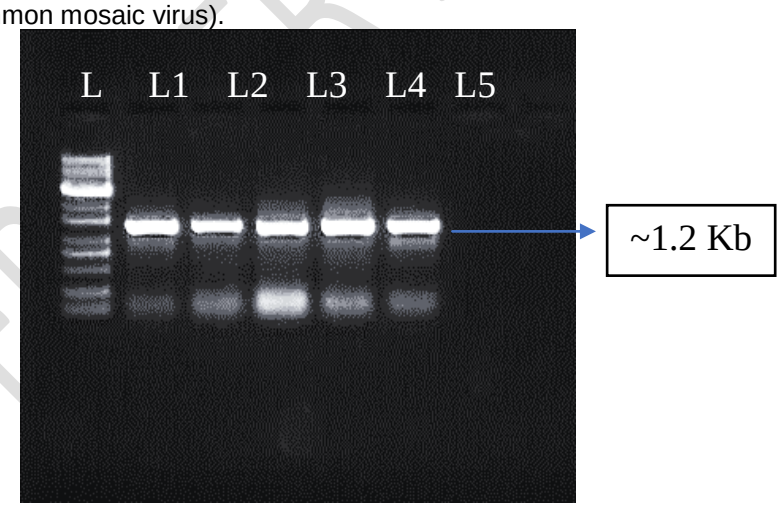

Fig 2. Agarose gel electrophoresis of infected plant genomic DNA amplified with universal primers of begomovirus Lane L- 1Kb ladder, Lane1- (Thondamuthur, Coimbatore) Sample1, Lane2- (Dharmapuri) Sample2, Lane3- (Theni) Sample3, Lane4- (Erode) Sample4, Lane5- Positive control (Tomato leaf curl virus), L6- Negative control (Healthy lablab-bean leaf)

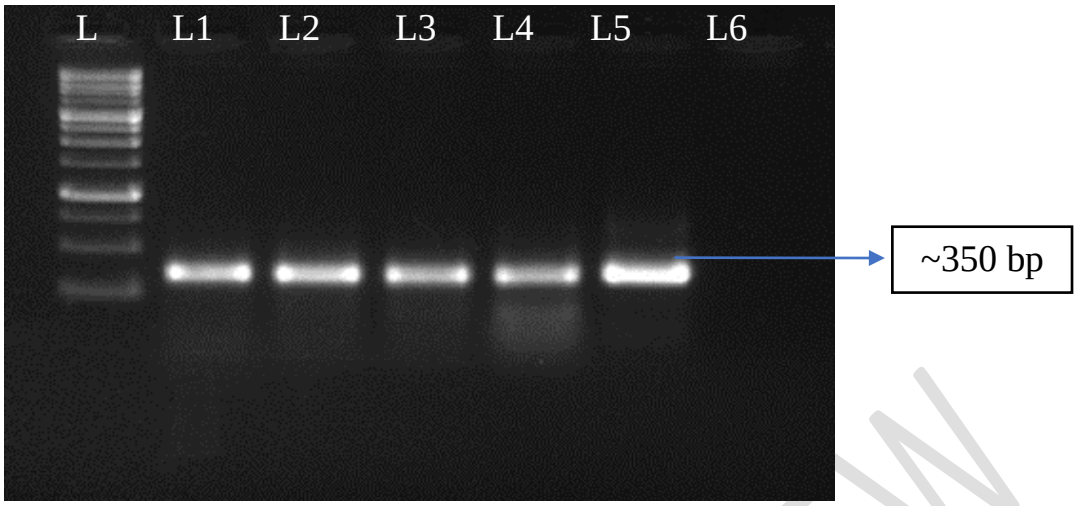

Fig 3. Agarose gel electrophoresis of infected plant genomic RNA amplified with universal primers of potyvirus Lane L -1Kb ladder, Lane1- (Coimbatore) Sample1, Lane2- (Dharmapuri) sample2, Lane3- (Krishnagiri) Sample 3, Lane 4- (Theni) Sample 4, Lane 5-Positive control (Papaya ring spot virus, PRSV), Lane 6- Negative control (Healthy lablab bean leaves).

3.3 Sequence and Phylogenetic analysis of DoYMV

Sequences from representative samples of each virus was analyzed using BLASTn tool. The present DoYMV sequence shared 97% identity with Dolichos yellow mosaic virus isolate TM1 segment DNA-A of Tamil Nadu. The sequence of DoYMV was analysed and the sequence was submitted to the NCBI. Phylogeny revealed the grouping of DoYMV with similar sequence and had close relationship with India DoYMV isolate along with Bangladesh isolate (Fig. 4). The nucleotide identity matrix generated using the SDT tool was determined for the DoYMV isolates of Tamil Nadu with other reported DoYMV sequences from different countries (Fig. 5).

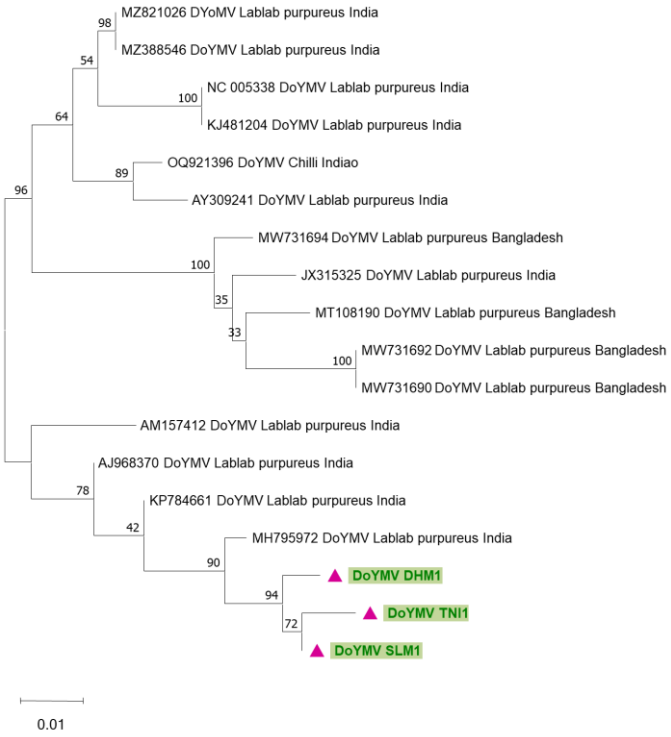

Fig 4. Phylogenetic relatedness of DoYMV infecting lablab-bean with previously reported isolates.

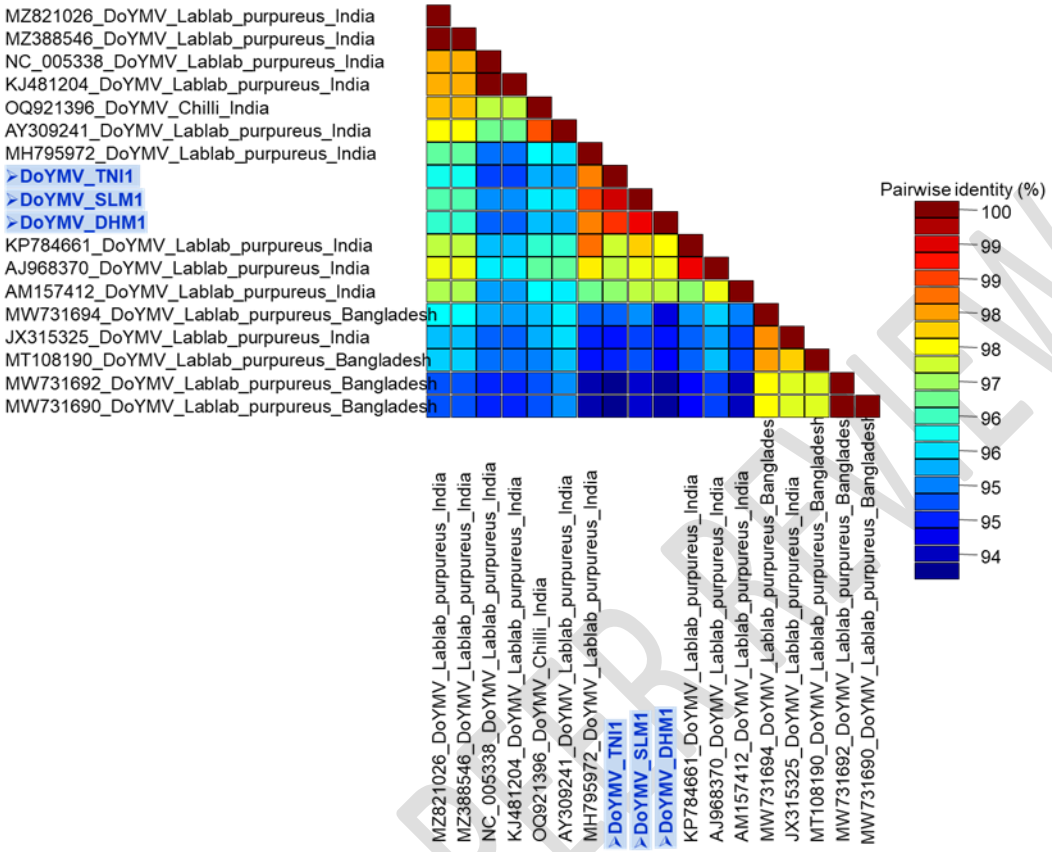

Fig 5. Heat map sequence identity matrix based on DoYMV isolates with other isolates reported early

3.4 Sequence and Phylogenetic analysis of BCMV

BCMV sequence shared an identity of 95% with Bean common mosaic virus isolate BD18-03 of China. The sequence of BCMV were analysed and sequence was submitted to the NCBI (OR545585 and OR545586). Phylogeny revealed the grouping of BCMV with similar sequence and had close relationship with Bangladesh BCMV Lablab bean (MW620817) (Fig. 6). The nucleotide identity matrix generated using the SDT tool was determined for the BCMV isolates of Tamil Nadu with other reported BCMV sequences from different countries (Fig. 7).

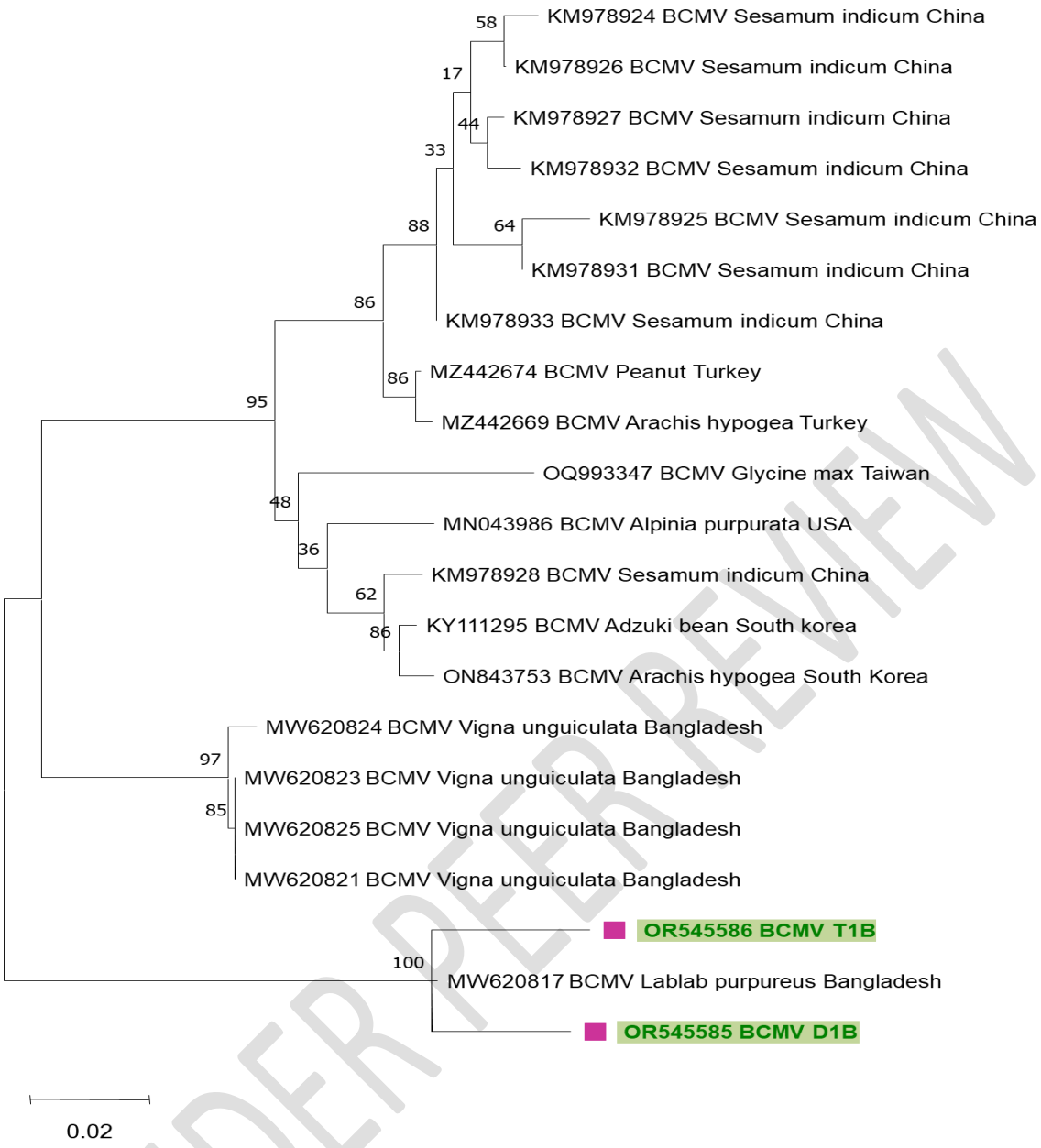

Fig 6. Phylogenetic relatedness of BCMV infecting lablab-bean with previously reported isolates.

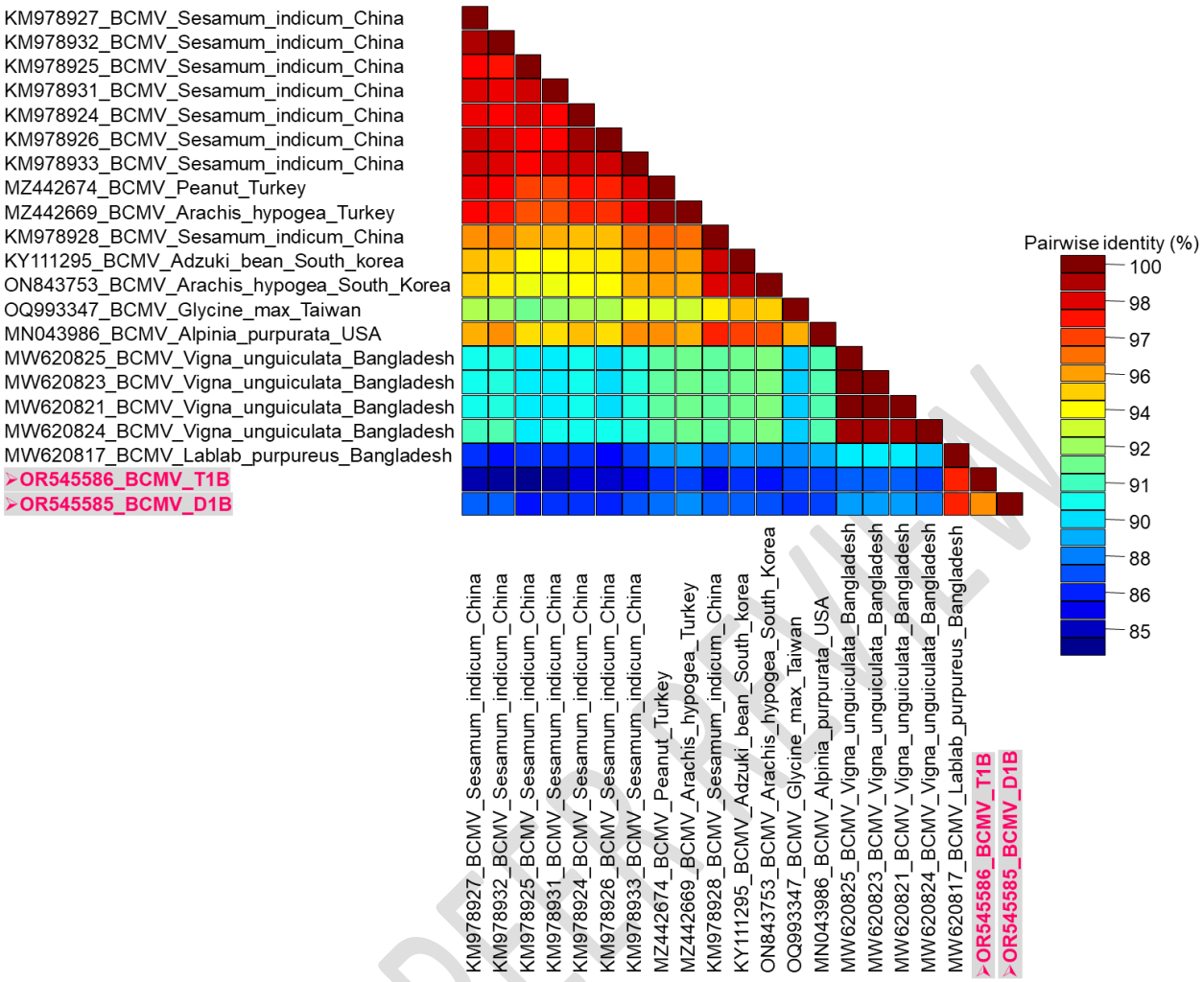

Fig 7. Heat map sequence identity matrix based on BCMV isolates with other isolates reported early

4. DISCUSSION

The present study was conducted to understand the prevalence of viruses infecting lablab-bean in Tamil Nadu. The field survey was conducted in the lablab-bean growing areas of Tamil Nadu and disease incidence was calculated. Lablab-bean is severely affected by the yellow mosaic disease in south India and incidence has been increased from 15% in 1990s to 40% in 2000 (Maruthi *et al*., 2006). In our study, more than 86.0% incidence of Dolichos yellow mosaic virus was observed. A similar survey has been conducted by Satya and Alice (2019) and the disease incidence due to DoYMV ranged from 86-90%. Since, it was first recorded by Capoor and Varma in 1950 the characterization, etiology, epidemiology, diagnostics and host plant resistance of DoYMV have been reported by several scientists (Maruthi *et al*., 2005, 2006; Akram *et al*., 2015; Ramakrishnan *et al.,* 1972: Raj *et al.,* 1988; Yaraguntaiah and govindu, 1964). Infected plant showed characteristic yellow mosaic symptoms similar to Mungbean yellow mosaic virus (MYMV), but DoYMV infect only Dolichos bean does not infect cowpea, black gram, green gram, soybean and French bean (Maruthi *et al*., 2006). DoYMV was biologically and also genetically different from other yellow mosaic virus (Swanson *et al*.,1992). DoYMV is highly specific to lablab-bean and it is not transmitted to other legume hosts (Capoor and varma, 1950).

Followed by DoYMV, lablab-bean was affected by BCMV with an incidence of 70%. First report of BCMV in India was done by (Udayashankar *et al.,* 2011) in lablab bean and reported the incidence ranged from 1 to 10%. The BCMV was reported in Vannila planifolia (Bhadramurthy and Bhat 2009), Vigna unguiculata and in Phaseolus vulgaris (Sachchidananda *et al.,* 1973). The symptoms observed

during survey are similar to those described by Udayashankar *et al.,* (2011). The common symptoms produced by BCMV in lablab-bean were characterized by twisting of leaves, vein clearing, puckering, uneven leaf lamina, mosaic and mottling which were similar as per report of Basavaraja and Keshava murthy (1992). This virus is known to affect numerous leguminous weed plant as well as crops like soybean, mungbean, and yambean (Latha *et al.,* 2017). However, BCMV failed to infect chickpea, horse gram, pigeonpea, moth bean, cucumber, chilli, pumpkin and tomato (Sangeeta *et al.,* 2022). Out of 25 samples, 23 samples show approximately ~1.1 bp amplification and isolates shared 93-100% match with other DoYMV isolates, 60-63 % match with other begomovirus infecting grain legumes, 58% match with Indian cassava mosaic virus, 57-58 % match with Bean golden mosaic virus (Satya and Alice, 2019). The three isolates of DoYMV were examined and shows highest levels of sequence similarity, ranging from 92.5% to 95.3% with DoYMV isolates of North India and Bangaladesh. Similarly, 99.1% of matches with DoYMV-[Ban1] and DoYMV-[Ban2]. These isolates were found to have the closest genetic resemblance to begomoviruses that infect mungbeans (MYMV and MYMIV), sharing a sequence similarity of 61.6% to 64.4%. Additionally, they demonstrated similar levels of similarity to non-legume viruses found in the Indian subcontinent (Maruthi *et al*., 2005). The coat protein gene sequence of Antalya isolates shared the highest identity rates between 91.22 per cent and 94.71 per cent. The phylogenetic analyses showed that BCMV-Antalya 1 and Antalya 10 are best isolate clustered with the England isolate (AY112735) and Turkish isolate (KT766179), respectively (Kyrychenko *et al*., 2019). The comparison of BCMV-Hebbal sequence with corresponding sequence of other BCMV isolates showed 94.90 per cent similarities with 5'UTR isolates of soybean variant of China (KJ807806). Whereas sequence 3'UTR with sequence of BCMV isolates showed 94.90 percent identity with the NL 1 isolate (AY112735) of England (Manjunatha *et al*., 2015).

5. CONCLUSION

The diseased samples collected from different lablab-bean growing areas of Tamil Nadu showed the characteristic symptoms of DoYMV and BCMV. The DNA isolated from diseased samples using GEM-CTAB method and PCR successfully amplified the expected size ~1.2 Kb of Roja's primer indicating the target region of DNA-A. The RNA isolated by using TRIzol method and RT-PCR was performed a universal primer pair NIb2F/NIb3R amplified at ~350 bp. PCR products were sequenced to confirm their identity. The phylogenetic tree revealed the similarity with Dolichos yellow mosaic virus isolate TN-TM1 segment DNA-A (accession no; MH795972) and Bean common mosaic virus isolate BD18-03**(**accession no; MW620818). The disease severity may lead to lower production of flowers and pods, thereby it leads to reduce the yield.

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