Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 10 (1): 29-35, 2011
ISSN 1812-5387 / DOI: 10.3923/ppj.2011.29.35

Molecular Detection and Partial Characterization of Begomovirus Associated with Leaf Curl Disease of Sunflower (*Helianthus annuus*) in Southern India

[1]M.R. Govindappa, [1]I. Shankergoud, [2]K.S. Shankarappa, [3]W.A.R.T. Wickramaarachchi,
[1]B. Anjeneya Reddy and [3]K.T. Rangaswamy
[1]AICRP on Sunflower, Main Agricultural Research Station,
University of Agricultural Sciences, Raichur-584101, India
[2]Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, India
[3]Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore-560065, India

Abstract: The present study was conducted to investigate the etiology, mode of transmission and molecular relationship of the causal virus associated with Sunflower leaf curl disease with other related viruses. Leaf curl disease on sunflower caused by a begomovirus was observed to an extent of 40% for the first time on some hybrids grown in experimental plots at Main Research Station (MRS), Raichur, Karnataka, Southern India. Symptoms like curling, malformation of leaves, leaf thickening, leaf enations and severe stunting were observed on infected plants. The disease was successfully transmitted by whitefly, *Bemisia tabaci*. The experimentally inoculated plants produced symptoms similar to that of naturally infected plants. The causal virus was detected using two sets of begomovirus specific degenerate primers. The core Coat Protein (CP) gene fragment of 575 bases was amplified from naturally as well as whitefly inoculated plants. Phylogenetic analysis of the core CP gene sequence of the virus with those of other begomoviruses clustered next to Tomato Leaf Curl Karnataka Virus isolate Lucknow (ToLCKV-(Luc) (Accession No. EU604297.2) and Tomato Leaf Curl Virus-Bangalore II (ToLCBV-(Ban2) (Accession No. EU604297.2) and shared 97.5% nucleotide identities. Thus, its exact taxonomic status will require sequencing of the complete ssDNA viral genome. The begomovirus infecting sunflower, which we tentatively called Sunflower Leaf Curl Virus (SuLCV).

Key words: Begomovirus, core coat protein, polymerase chain reaction, sunflower leaf curl disease, whitefly, vein thickening, enations

INTRODUCTION

Sunflower is one of the major edible oils consumed throughout the globe. The oil is used for culinary purposes, the preparation of vanaspati and the manufacture of soaps and cosmetics. World sunflower area accounts for 20 million hectares and production around 30 million tons. In India, sunflower is cultivated in around 18.85 lakh hectares (10% of the world sunflower area) and production is around 12.52 lakh tons (4% of the world sunflower production). The major sunflower producing states are Karnataka (54.86%), Andhra Pradesh (20.83%) and Maharashtra (14.58%) (Anonymous, 2009).

Sunflower crop is affected by several fungal, bacterial and viral diseases. Among the viral diseases affecting the crop, sunflower necrosis virus disease caused by Tobacco streak virus belongs to the genus *Ilarvirus* (Bhat *et al*., 2002; Ramaiah *et al*., 2001; Lavanya *et al*., 2005) and bud necrosis caused by tospovirus (Jain *et al*., 2000) are the major production constraints.

Begomoviruses are the major constraint in successful cultivation of horticultural and agricultural crops throughout the world. The severity and incidences of these viruses in Southern India have increased in the recent years after the introduction of B Biotype *Bemisia tabaci* (Banks *et al*., 2001; Narayana *et al*., 2006, 2007; Maruthi *et al*., 2007; Mahesh *et al*., 2010; Shivakumar, 2010). Begomoviruses typically have bipartite ssDNA genomes with essential genes encoded on the DNA-A and DNA-B. Many monopartite Begomoviruses that have single DNA molecule are also reported from tomato, bhendi and cotton (Navot *et al*., 1991; Briddon and Markham, 2000) with additional satellite molecules called DNA-β which regulates symptom expression (Briddon *et al*., 2001).

Begomoviruses have been detected in plants or insects by tools, such as visualization of nuclear inclusion bodies by light microscopy, ultrastructural localization of virions in plant cell by transmission electron microscopy,

Corresponding Author: K.T. Rangaswamy, Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore-560065, India

serological assays (Polston *et al*., 1989; Konate *et al*., 1995; Hunter *et al*., 1998; Pico *et al*., 1999), DNA hybridization assays (Lotrakul *et al*., 1998), Polymerase Chain Reaction (PCR) (Deng *et al*., 1994; Mehta *et al*., 1994; Ghanim *et al*., 1997; Lotrakul *et al*., 1998; Pico *et al*., 1999; Rosell *et al*., 1999), immunocapture PCR (Ramprasad and Umaharan, 2003). Molecular cloning and DNA sequencing of viral genomes have become the tools of choice, allowing virus identification and comparing relationships with other virus isolates (Rybicki, 1994; Padidam *et al*., 1995; Paximadis *et al*., 1999; Brown *et al*., 2001).

All begomoviruses code for coat protein which act as the protective coat of the virus particle and determine vector transmitability 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 populations (McGrath and Harrison, 1995; Maruthi *et al*., 2002). Smaller fragments comprising the core Coat Protein gene (core CP), a partial 575-579 Base Pair (BP) sequence of the Coat Protein gene (Brown *et al*., 2001), or the complete CP sequence have also been used to establish provisional species identification owing to the highly conserved nature of the viral CP sequence. The core CP primers have been illustrated to amplify a fragment for most, if not all, begomoviruses irrespective of Old or New World origin, making possible the rapid detection followed by prediction of provisional species affiliation by comparison with reference begomovirus core CP sequences (Wyatt and Brown, 1996; Brown *et al*., 2001; Harrison *et al*., 2002).

Sunflower leaf curl disease observed for the first time was illustrated to be caused by begomovirus based on its symptoms, transmissibility by whitefly vector *B. tabaci* and PCR tests. However the etiology and phylogenetic relationship with other begomoviruses were unknown. In this work we are reporting symptoms, virus transmission, detection of begomovirus and phylogenetic relationship with other begomoviruses.

MATERIALS AND METHODS

Symptoms and virus transmission tests: The culture of the virus was collected from sunflower plants grown in experimental plots at MRS, Raichur. Experiment to transmit putative begomovirus from sunflower was carried out during November 2009 using the whitefly vector *Bemisia tabaci* collected from colony that has been maintained on cotton plants at MRS, Raichur. Whiteflies were released on infected sunflower 24 h virus acquisition, which were then used to inoculate 8-10 old healthy sunflower seedlings enclosed in poly vinyl cages. The seedlings were inoculated using 20 viruliferious whiteflies for 48 h. The inoculated plants were then sprayed with a systemic insecticide (Confidor 0.05%) and maintained in an insect proof cage for 2 months for symptom development.

PCR detection of the virus: Virus detection and cloning work of viral DNA were carried at the Department of Plant Pathology, UAS, GKVK, Bangalore, India during December 2009-February 2010. Total DNA was extracted from the sunflower plants with leaf curl symptoms using CTAB method (Lodhi *et al*., 1994; Maruthi *et al*., 2002). Two sets of begomovirus group specific universal primers viz. Deng A and B primers (Deng *et al*., 1994) and AV 494 and AC 10483 (Wyatt and Brown,1996), which are capable of universally amplifying the core CP region of many begomoviruses were used for viral detection.

PCR was carried out in 25 μL reaction mixture containing 6.0 mM Tris HCl, 2.5 mM dNTPs, 20 μM primers, 2.5 units of Taq DNA polymerase (Bangalore Genei Pvt Ltd., Bangalore) and 10-15 μg of DNA. PCR were performed in thermocycler (Eppendorf, Cambridge, UK) according to Wyatt and Brown (1996). The PCR protocol consisted of 2 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at 61°C, 2 min at 72°C and finally 10 min at 72°C. Amplified PCR products were separated by electrophoresis on 1% agarose gel and DNA fragments were visualized using ethidium bromide stain and analysed by alpha imager gel documentation and analysis system. DNA ladder set (1 Kb, MBI Fermentas, Germany) was included as sized molecular marker. DNA from healthy plants and double distilled water were used as a experimental controls.

Phylogenetic analysis of the core coat protein gene of the virus: The 575 bp fragment corresponding to core CP gene of viral DNA was purified using gel extraction kit (Qiagen, Germany) and cloned in the plasmid pTZ57R/T in T/A cloning kit (MBI Fermentas) following manufactures instructions. Clones were sequenced in both directions using universal M13 forward and reverse primers at Chromous Biotech, Bangalore, India. The core CP gene sequence of sunflower leaf curl virus were compared with that of reference begomoviruses obtained from the EMBL data base (Table 1).

Sequences obtained were identified in terms of closest homology sequence using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments with the respective reference strains sequences were made by CLUSTAL W. MEGA software (version 4.0) was used for the phylogenetic analysis (Tamura *et al*., 2007). Phylogenetic trees were constructed by the Neighbour Joining method.

Table 1: Details of begomoviruses used in the phylogenetic analysis of core Coat Protein gene nucleotide sequences

Virus species	Abbreviation	NCBI accession No.
Tomato leaf curl Karnataka virus isolate Lucknow	ToLCKV-[Luc]	EU604297.2
Tomato leaf curl virus-Bangalore 2	ToLCBV-[Ban2]	U38239.1
Tomato leaf curl Rajasthan virus	ToLCRV	DQ339117.1
Tomato leaf curl New Delhi virus isolateToLCND-CTM	ToLCND-CTM	DQ629102.1
Cotton leaf curl Bangalore virus	CLCuBV	AY705380.1
Tomato leaf curl Kerala virus isolateToLCV-K3	ToLCV-K3	EU910141.1
Tomato leaf curl Bangalore virus-Cotton [Fatehabad]	ToLCBVC-[Fat]	AY456684.1
Tomato leaf curl Bangalore virus-[Ban5]	ToLCBV-[Ban5]	AF295401.1
Tomato leaf curl Bangalore virus-[India:Kerala 2]	ToLCBV-[Ker]	DQ852623.2
Tomato leaf curl virus-Bangalore 1	ToLCBI	Z48182.1
Tomato leaf curl Bangalore virus-[Kolar]	ToLCBV-[Kolar]	AF428255.1
Chilli leaf curl virus-[Multan]	ChiLCV-[Mul]	AF336806.1
Pepper leaf curl virus isolate Varanasi	PepLCV-[Var]	EF190217.1
Tomato leaf curl Pune virus-[India:Pune:2005]	ToLCBV-[Pun]	AY754814.1
Mesta yellow vein mosaic virus [India:Srikakulam:2008]	MeYVMV-[Srikakulam]	FJ159271.1
Cotton leaf curl Kokhran virus	CLCuKV	GU385878.1
Sida yellow vein virus-[Barrackpore]	SiYVV[Barr]	EU184016.1
Cotton leaf curl virus-(Burewala), clone KoT	CLCuBV-[KoT]	AM774305.1
Papaya leaf curl virus complete genome	PaLCuV-[Pak]	AJ436992.1
Jatropha mosaic India virus-[Bangalore]	JMIV-[Ban]	AM296493.1
Jatropha mosaic India virus-[Dharwad]	JMIV-[Dha]	AM296494
Ageratum yellow vein virus-[Pakistan]	AgYVV-(PK)	AJ810825
Cotton leaf curl Rajasthan virus-[Abohar]	CLCuRV-[Abo]	AY795606
East African cassava mosaic Zanzibar virus	EACMZV	AF422174
Indian cassava mosaic virus-[Trivandrum]	ICMV-[Tri]	AF423180
Bean golden yellow mosaic virus-[Mexico]	BGYMV-[MX]	AF173555
Chilli leaf curl virus-[Pakistan]	ChiLCuV-[Pak]	DQ114477
Crotalaria juncea leaf curl virus-[Lucknow]	CrJLCuV-[Luc]	EF119337
Cotton leaf curl Kokhran virus-[Faisalabad1]	CLCuKV-[Fai1]	AJ496286
Cotton leaf curl Multan virus-[Bhatinda]	CLCuMV-[Bha]	DQ191160
Pepper leaf curl Bangladesh virus	PepLCBV	AF314531

RESULTS AND DISCUSSION

Disease symptoms, incidence and virus transmission: A leaf curl disease symptomatologically comparable to the diseases caused by begomoviruses on other crop plants was first noticed on sunflower hybrid 'Sun breed-275' upto 40% disease incidence in the fields of MRS, University of Agricultural Sciences, Raichur, Northern Karnataka, India during rabi season of 2009. The characteristics symptoms observed on naturally infected plants were marginal cupping, upward curling, vein thickening and enations on the leaves (Fig. 1a,b). Emerging leaves illustrated yellow discoloration and severe reduction in leaf size. The infected plants remained very much stunted when infected at early stages of crop growth and did not produce earheads.

The disease was successfully transmitted to healthy sunflower plants using whitefly vector *B. tabaci*. Healthy sunflower seedlings inoculated with viruliferous whiteflies (twenty adults/plant) developed symptoms similar to those of naturally infected plants in the field. Leaf cupping from the margins and upward curling were the first symptoms observed after 15 days of virus inoculation. Affected leaves subsequently developed vein thickening and enations in about 25 to 30 days (Fig. 2). Transmission of the virus to an extent of 80-100% was achieved with twenty adult whiteflies, which were given 24 h AAP and 48 h IAP. The results were comparable that of begomovirus like Tomato Leaf Curl Virus (Muniyappa *et al*., 2000) and Pumpkin Yellow Vein Mosaic Virus (Muniyappa *et al*., 2003; Maruthi *et al*., 2007) which required 5-15 adult viruliferous whiteflies to get 100% transmission and developed symptoms in 20-30 as compared to 8-10 days in Tomato Leaf Curl Virus and Pumpkin Yellow Vein Mosaic Virus. The differences in the nature of host tissue could be the probable reason for more incubation period in sunflower. Pumpkin and tomato being fleshy/tender tissues support high virus multiplication within a short period.

PCR detection of the virus: PCR tests carried using two sets of degenerate primers successfully amplified expected DNA fragments of c. 520 bp (Fig. 3, lane, 10) and 575 bp from both naturally-and whitefly-inoculated sunflower plants (Fig. 3). This is the first report on detection of a begomovirus from sunflower crop. These primers in combination or alone have been used extensively for the identification of begomoviruses in a wide range of crop plants and their vector *B. tabaci* previously (Deng *et al*., 1994; Maruthi *et al*., 2006; Narayana *et al*., 2007; Sharma *et al*., 2009; Mahesh *et al*., 2010).

Comparison of core coat protein gene sequences: In order to know the relationship of the begomovirus causing

Fig. 1: Field-infected sun flower plants with typical marginal upward leaf curling and vein thickening (a) coupled with the enation on abaxial leaf surface (b)

Fig. 2: Whitefly-inoculated sunflower seedling illustrating prominent vein thickening and enations on abaxial leaf surface

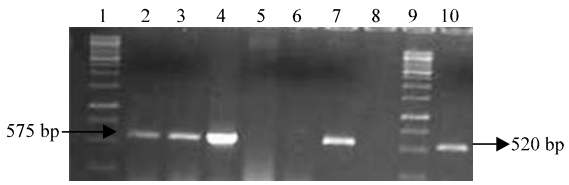

Fig. 3: A picture of agarose gel electrophoresis illustrating begomovirus-specific PCR products obtained using the core coat protein gene primers (Lanes 1-7) and Deng primers (Lane 10). Lanes 1, 9: 1kb DNA; lanes 2, 10: Field-infected sunflower sample; lane 3: Whitefly-inoculated sample, lanes 4, 7: Croton leaf curl sample used as a control; lane 5: healthy sunflower sample, lane 6: Water control, lane 8: Blank

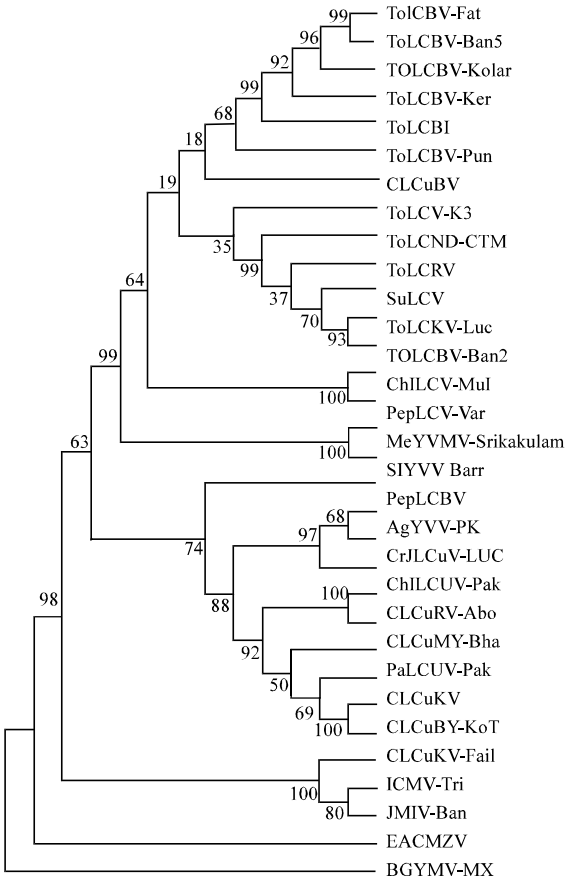

Fig. 4: The most parsimonious phylogenetic tree illustrating the relationship of SuLCV with selected begomoviruses (see Table 1) based on their core CP nucleotide sequences. The number at each node indicates ≥50% percentage bootstrap values for 500 replications

Table 2: Percentage nucleotide identities based on core CP sequences of selected begomoviruses

Abbreviation (name)	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	26	27	28	29	30	31	32
SuLCV																																
ToLCKV-[Luc]	97.5																															
ToLCBV-[Ban2]	97.5	99.3																														
ToLCRV	94.3	95.7	96.0																													
ToLCND-CTM	93.5	94.7	94.7	92.6																												
CLCuBV	91.4	92.0	91.9	90.3	89.2																											
ToLCV-K3	89.6	90.0	90.0	88.0	86.9	87.1																										
ToLCBVC-[Fat]	90.1	90.1	90.9	88.7	87.4	90.2	88.0																									
ToLCBV-[Ban5]	90.1	90.5	90.9	88.7	87.4	90.2	88.0	100																								
ToLCBV-[Ker]	89.9	90.3	90.7	88.7	88.7	88.8	86.2	97.1	97.1																							
ToLCBI	89.8	90.2	90.2	88.3	87.8	89.1	88.5	95.3	95.3	93.2																						
ToLCBV-[Kolar]	89.8	90.0	90.4	88.1	87.6	90.4	87.6	98.8	98.8	96.7	94.7																					
ChiLCV-[Mul]	89.3	90.0	90.0	87.9	87.4	89.0	86.5	87.1	87.1	87.1	88.0	87.3																				
PepLCV-[Var]	89.3	90.5	90.5	88.4	88.0	89.2	85.8	87.2	87.2	87.0	87.2	88.0	97.5																			
ToLCBV-[Pun]	88.0	88.6	88.4	86.2	85.3	88.6	85.2	90.6	90.6	89.6	89.6	90.2	88.4	88.1																		
MeYVMV-[Srikakulam]	86.2	86.3	86.3	85.1	83.8	86.7	83.8	84.2	84.2	84.9	83.8	85.6	85.6	85.0	83.5																	
CLCuKV	85.0	86.0	86.0	87.0	87.0	85.0	84.0	84.0	84.0	83.0	83.1	84.0	84.8	85.0	83.1	81.5																
SiYVV (Barr)	85.0	85.0	86.0	85.0	84.0	87.2	84.0	79.0	85.6	86.0	85.0	85.6	85.6	85.0	84.5	93.0	81.5															
CLCuBV-[KoT]	85.1	86.0	86.0	86.1	87.0	84.0	84.0	86.0	83.2	83.0	82.0	84.0	84.0	85.2	83.0	83.0	97.9	82.0														
PaLCuV-[Pak]	85.1	86.0	86.0	85.8	87.0	84.0	84.0	83.0	84.2	84.3	83.0	84.0	84.0	84.1	83.0	83.0	96.0	82.0	96.0													
JMIV-[Ban]	83.0	82.0	82.0	82.0	81.0	80.0	80.0	84.0	79.0	80.0	79.0	80.0	80.0	80.0	80.0	81.0	82.0	79.0	81.0	82.0												
JMIV-[Dha]	81.0	81.0	81.0	81.0	80.0	81.0	80.0	80.0	80.0	80.0	80.0	79.0	81.0	81.0	80.0	81.0	81.0	80.0	81.0	81.0	94.0											
AgYVV-[PK]	82.0	82.0	82.0	82.0	83.0	82.0	81.0	81.0	81.0	82.0	80.0	81.0	81.0	81.0	81.0	82.0	89.0	82.0	89.0	90.0	81.0	81.0										
CLCuRV-[Abo]	84.0	84.0	84.0	84.0	83.0	81.0	81.0	81.0	81.0	81.0	80.0	81.0	82.0	82.0	80.0	82.0	93.0	81.0	94.0	91.0	80.0	79.0	85.0									
EACMZV	73.0	72.0	72.0	72.0	72.0	73.0	73.0	72.0	72.0	72.0	72.0	73.0	74.0	74.0	73.0	75.0	75.0	74.0	75.0	76.0	72.0	72.0	75.0	73.0								
ICMV-[Tri]	81.0	82.0	82.0	81.0	82.0	81.0	80.0	81.0	81.0	82.0	81.0	82.0	81.0	81.0	81.0	80.0	82.0	79.0	81.0	82.0	93.0	92.0	81.0	79.0	72.0							
BGYMV-[MX]	73.0	72.0	73.0	72.0	72.0	73.0	75.0	72.0	72.0	72.0	72.0	72.0	74.0	74.0	71.0	72.0	73.0	72.0	74.0	74.0	73.0	73.0	73.0	72.0	71.0	75.0						
ChiLCuV-[Pak]	84.0	85.0	85.0	84.0	86.0	83.0	84.0	82.0	82.0	82.0	82.0	83.0	84.0	84.0	83.0	83.0	90.0	82.0	90.0	92.0	83.0	82.0	92.0	86.0	75.0	83.0	73.0					
CrJLCuV-[Luc]	84.0	84.0	84.0	85.0	86.0	83.0	84.0	81.0	81.0	82.0	81.0	82.0	82.0	83.0	82.0	83.0	92.0	81.0	92.0	93.0	83.0	81.0	92.0	88.0	76.0	82.0	75.0	93.0				
CLCuKV-[Fai1]	85.0	86.0	86.0	86.0	87.0	84.0	84.0	83.0	83.0	83.0	82.0	84.0	84.0	85.0	83.0	83.0	97.0	82.0	100	96.0	81.0	81.0	89.0	94.0	75.0	81.0	74.0	90.0	91.8			
CLCuMV-[Bha]	84.0	84.0	84.0	84.0	83.0	81.0	81.0	81.0	81.0	81.0	80.0	81.0	82.0	82.0	80.0	82.0	93.0	81.0	94.0	91.0	80.0	79.0	85.0	100	73.0	79.0	72.0	86.0	88.0	94.0		
PepLCBV	84.0	84.0	84.0	83.0	84.0	83.0	82.0	83.0	83.0	84.0	82.0	83.0	83.0	84.0	82.0	80.0	88.0	80.0	88.0	88.0	84.0	83.0	86.0	84.0	74.0	83.0	72.0	86.4	86.1	87.8	84.0	

1: SuLCV, 2: ToLCKV-[Luc], 3: ToLCBV-[Ban2], 4: ToLCRV, 5: ToLCND-[CTM], 6: CLCuBV, 7: ToLCV-[K3], 8: ToLCBVC-[Fat], 9: ToLCBV-[Ban5], 10: ToLCBV-[Ker], 11: ToLCBI, 12: ToLCBV-[Kolar], 13: ChiLCV-[Mul], 14: PepLCV-[Var], 15: ToLCBV-[Pun], 16: MeYVMV-[Srikakulam], 17: CLCuKV, 18: SiYVV (Barr), 19: CLCuBV-[KoT], 20: PaLCuV-[Pak], 21: JMIV-[Ban], 22: JMIV-[Dha], 23: AgYVV-[PK], 24: CLCuRV-[Abo], 25: EACMZV, 26: ICMV-[Tri], 27: BGYMV-[MX], 28: ChiLCuV-[Pak], 29: CrJLCuV-[Luc], 30: CLCuKV-[Fai1], 31: CLCuMV-[Bha], 32: PepLCBV

SuLCVD, the 575 bp PCR fragment of the core CP region of the virus was cloned and sequenced. The core CP gene sequence has been used and illustrated to be useful in begomovirus classification (Wyatt and Brown, 1996; Brown *et al*., 2001). The core CP sequence of the SuLCV was compared with those of selected begomoviruses. Phylogenetic analysis revealed that the sunflower leaf curl virus isolate from Raichur had 97.5% nucleotide identity with Tomato Leaf Curl Virus isolate Lucknow (ToLCKV-(Luc) (Accession No. EU604297.2) and Tomato Leaf Curl Virus-Bangalore II (ToLCBV-(Ban2) (Accession No. EU604297.2) (Fig. 4, Table 2) Based on symptom observations, transmission studies of the virus through *B. 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. The disease assumes greater significance because of the preference of *B. tabaci* (15-20 adults per plant) to feed on sunflower crop over other field crops. Further studies are in progress to determine the virus-vector transmission relationship, host range, epidemiology and sequencing of the complete genome of the virus.

REFERENCES

Anonymous, 2009. Sunflower, annual report, 2009-10. All India Co ordinated Research Project. Directorate of Oil Seed Research, Rajendranagar, Hyderbad, pp: 225.

Banks, G.K., J. Colvin, R.V.C. Reddy, M.N. Maruthi and V. Muniyappa *et al*., 2001. First report of the *Bemisia tabaci* B-biotype in India and an associated Tomato leaf curl virus disease epidemic. Plant Dis., 85: 231-231.

Bhat, A.I., R.K. Jain, V. Chaudhary, M. Krishna Reddy, M. Ramiah, S.N. Chattannavar and A. Varma, 2002. Sequence conservation in the coat protein gene of Tobacco streak virus isolates causing necrosis in cotton, mungbean, sunflower and sunn-hemp in India. Indian J. Biotechnol., 1: 350-356.

Briddon, R.W. and P.G. Markham, 2000. Cotton leaf curl disease. Virus Res., 71: 107-121.

Briddon, R.W., S. Mansoor, I.D. Bedford, M.S. Pinner and K. Saunders *et al*., 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology, 285: 234-243.

Brown, J.K., A.M. Idris, I. Torres-Jerez, G.K. Banks and S.D. Wyatt, 2001. The core region of the coat protein gene is highly useful in establishing the provisional identification and classification of begomoviruses. Arch. Virol., 146: 1581-1598.

Deng, D., P. F. Mcgrath, D.J. Robinson and B.D. Harrison, 1994. Detection and differentiation of whitefly-transmitted geminiviruses in plants and vector insects by the polymerase reaction with degenerate primers. Ann. Applied Biol., 125: 327-336.

Ghanim, M., S. Morin, M. Zeidan and H. Czosnek, 1997. Evidence for transovarial transmission of tomato yellow leaf curl virus by its vector the whitefly *Bemisia tabaci*. Virology, 240: 295-303.

Harrison, B.D., M.M. Swanson and D. Fargette, 2002. Begomovirus coat protein: Serology, variation and functions. Physiol. Mol. Plant Pathol., 60: 257-271.

Hunter, W.B., E. Hiebert, S.E. Webb, J.H. Tsai and J.E. Polston, 1998. Location of geminiviruses in whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). Plant Dis., 82: 1147-1151.

Jain, R.K., A.I. Bhat, A.S. Byadgi, N. Nagaraju and S. Harvir *et al*., 2000. Association of a tospovirus with sunflower necrosis disease. Curr. Sci., 79: 1703-1705.

Konate, G., N. Barro, D. Fargette, M. M. Swanson and B.D. Harrison, 1995. Occurrence of whitefly-transmitted Geminiviruses in crops in Burkina Faso and their serological detection and differentiation. Ann. Applied Biol., 126: 121-129.

Lavanya, N., R. M. Ramiah, A. Sankaralingam, P. Renukadevi and R. Velazhahan, 2005. Identification of hosts for ilarvirus associated with sunflower necrosis disease. Acta Phytopathol. Entomol. Hungarica, 40: 31-34.

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch, 1994. A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*. Plant Mol. Biol. Rep., 12: 6-13.

Lotrakul, P. R.A. Valverde, C.A. Clark, J. Sim and R. De La Torre, 1998. Detection of a geminivirus infecting sweet potato in United States. Plant Dis., 82: 1253-1257.

Mahesh, Y.S., K.S. Shankarappa, K.T. Rangaswamy, H.A. Prameela and D.S.A. Narayana *et al*., 2010. Detection and characterisation of a begomovirus associated with leaf curl disease of ornamental croton (*Codiaeum variegatum*). J. Hort. Sci. Biotechnol., 85: 101-105.

Maruthi, M.N., A.R. Rekha and V. Muniyappa, 2007. 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., 37: 412-419.

Maruthi, M.N., A.R. Rekha, M.R. Govindappa, J. Colvin and V. Muniyappa, 2006. A distinct begomovirus causes Indian dolichos yellow mosaic disease. Plant Pathol., 55: 290-290.

Maruthi, M. N., J. Colvin, S. Seal, G. Gibbson and J. Cooper, 2002. Co-adaptation between cassava mosaic Gemini viruses and their local vector populations. Virus Res., 86: 71-85.

McGrath, P.F. and B.D. Harrison, 1995. Transmisson of tomato leaf curl Geminiviruses by *Bemisia tabaci*: Effects of virus isolate and vector biotype. Ann. Applied Biol., 126: 307-316.

Mehta, P., J.A. Wyman, M.K. Nakhla and D.P. Maxwell, 1994. Polymerase Chain Reaction detection of viruliferous *Bemisia tabaci* (Homoptera: Aleyrodidae) with two tomato infecting Gemini viruses. J. Econ. Entomol., 87: 1285-1290.

Muniyappa, V., H. M. Venkatesh, H. K. Ramappa, R.S. Kulkarni and M. Zeidan *et al*., 2000. Tomato leaf curl virus from Bangalore (ToLCV-Ban4): Sequence comparison with Indian ToLCV isolates, detection in plants and insects and vector relationships. Arch. Virol., 145: 1583-1598.

Muniyappa, V., M.N. Maruthi, C.R. Babitha, J. Colvin, R.W. Briddon and K.T. Rangaswamy, 2003. Characterisation of pumpkin yellow vein mosaic virus from India. Ann. Applied Biol., 142: 323-331.

Narayana, D.S.A., K.S. Shankarappa, M.R. Govindappa, H.A. Prameela, M.R. Gururaj Rao and K.T. Rangaswamy, 2006. Natural occurrence of Jatropha mosaic virus disease in India. Curr. Sci., 91: 584-586.

Narayana, D.S.A., K.T. Rangaswamy, K.S. Shankarappa, M.N. Maruthi and C.N.L. Reddy *et al*., 2007. Distinct begomovirus closely related to cassava mosaic viruses cause Indian jatropha mosaic disease. Int. J. Virol., 3: 1-11.

Navot, N., E. Pichersky, M. Zeidan, D. Zamir and H. Czosnek, 1991. Tomato yellow leaf curl virus: A whitefly transmitted Gemini virus with a single genomic molecule. Virology, 185: 151-161.

Padidam, M., R.N. Beachy and C.M. Fauquet, 1995. Classification and identification of geminiviruses using sequence comparisions. J. Gen. Virol., 76: 249-263.

Paximadis, M., A.M. Idris, I. Torres-Jerez, A. Villarreal, M.E.C. Rey and J.K. Brown, 1999. Characterization of tobacco Geminiviruses in the old and new world. Arch. Virol., 144: 703-717.

Pico, B., M.J. Diez and F. Nuez, 1999. Improved diagnostic techniques for Tomato yellow leaf curl virus in tomato breeding programs. Plant Dis., 83: 1006-1012.

Polston, J.F., J.A. Dodds and T. Perring, 1989. Nucleic acid probes for detection and strains discrimination of cucurbit, geminiviruses. Phytopathology, 79: 1123-1127.

Ramaiah, M., A.I. Bhat, R.K. Jain, R.P. Pant, Y.S. Ahlawat, K. Prabhakar and A. Varma, 2001. Isolation of an isometric virus causing sunflower necrosis disease in India. Plant Dis., 85: 443.2-443.2.

Ramprasad, S.N. and P. Umaharan, 2003. Detection of begomovirus in clarified plant extracts: A component of standard direct binding and immune capture PCR technique. Phytopathology, 93: 1153-1157.

Rosell, C.R., I. Torres-Jerez and J.K. Brown, 1999. Tracing the geminivirus-whitefly transmission pathway by polymerase chain reaction in whitefly extracts, saliva, hemolymph and honeydew. Phytopathology, 89: 239-246.

Rybicki, E.P., 1994. A phylogenetic and evolutionary justification for 3 genera of *Geminiviridae*. Arch. Virol., 139: 49-77.

Sharma, A., K.S. Shankarappa, K.T. Rangaswamy, R.K. Dubey and M. N. Maruthi, 2009. First record of duranta leaf curl, a begomovirus associated disease in India. Ind. J. Virol., 20: 88-89.

Shivakumar, 2010. Studies on zinnia leaf curl virus disease. M.Sc. Thesis, University of Agricultural Sciences, Bangalore, India.

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Wyatt, S.D. and J.K. Brown, 1996. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology, 86: 1288-1293.