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Complete Nucleotide Sequence of Tomato Leaf Curl Karnataka Virus and β Satellite Molecule Associated with Leaf Curl Disease on Sunflower in India

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Abstract: Sunflower leaf curl disease caused by begomovirus is one of the newly emerging viral diseases in Nothern Karnataka, Southern India. The disease has attracted lot of attention of the pathologists, as it affects the productivity of sunflower an important oilseed crop in the country. The present study was conducted to characterize the begomovirus causing sunflower leaf curl disease by cloning and sequencing the major genomic components. The Complete DNA-A and betasatellite components of begomovirus isolated from sunflower leaf tissue infected with leaf curl virus collected from Main Agricultural Research Station, Raichur, Karnataka, India was cloned and sequenced. The virus contained the DNA-A and the associated satellite beta DNA components of 2761 and 1373 nucleotides (nt) in length, respectively. The DNA-A molecule shared maximum identity with tomato leaf curl karnataka virus clone IKH12 (ToLCKV-[IKH12]) (HM803118) (97.13%) and clone IKB3 (ToLCKV-[IKB3]) (HM851186) (96.95%) from India. The betasatellite shared a high nucleotide identity (93.6-94.07%) with Potato apical leaf curl betasatellite (PoLCB-[IN-CHI: 05]) and Papaya leaf curl betasatellite (PaLCuB-[IN-CHI:05]) associated with tomato leaf curl disease from India. The results indicate for the first time that a strain of ToLCKV together with beta DNA satellite causing sunflower leaf curl disease (SuLCD) in India.

Key words: Sunflower leaf curl disease, whitefly, begomovirus, polymerase chain reaction, phylogenetic analysis

INTRODUCTION

Sunflower is an important short duration crop grown for its edible oils. It is a crop of choice for farmers due to its wider adaptability, high yield potential shorter duration and profitability. In the world it is cultivated in an area of 20 million ha with production of 3 million tons, whereas in India it is cultivated in 18.85 lac ha with production of 12.52 lac tons. Karnataka is one of the major sunflower growing states in India (Anonymous, 2009).

The sunflower suffers from many fungal, bacterial and a few viral diseases. A sunflower necrosis virus disease caused Tobacco streak virus belonging to the genus *Ilarvirus* (Ramiah *et al*., 2001; Bhat *et al*., 2002a, b; Lavanya *et al*., 2005) is the serious virus disease. Recently, a leaf curl disease associated with a begomovirus has been reported from Northern Karnataka, India (Govindappa *et al*., 2011).

Begomoviruses belong to family geminiviridae which represents the second largest family of plant viruses that infect a wide range of crops, particularly in tropical and subtropical regions. They can be either monopartite or bipartite depending upon the presence of one (DNA-A) or two (DNA-A and DNA-B) genomic components, each of approximately 2.5-2.8 kb size (Stanley, 1983; Fauquet *et al*., 2008). The DNA-A is responsible for replication, gene expression and encapsidation while the DNA-B is responsible for movement of the virus between and within plant cells. The monopartite begomovirus comprises only one component known as DNA-A which is responsible for replication, gene expression, whitefly transmission and systemic

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infection (Stanley *et al*., 2005). Other than genomic components, a satellite molecule, betasatellite which is typically half the length of DNA-A component of begomoviruses (1.35 kb) and required for the induction of typical disease symptoms has been found with the monopartite begomoviruses (Dry *et al*., 1997).

Comparisons between nucleic acid and protein sequences of viral origin along with its structural and biological criteria have long been used to identify and classify plant viruses (Shukla and Ward, 1988). The identification of the begomovirus associated with SuLCD has been mainly on the basis of sequences of the core region of viral Coat Protein (CP) gene (Govindappa *et al*., 2011). Although, the CP sequences are useful for identification of begomovirus (Wyatt and Brown, 1996; Brown *et al*., 2001; Prajapat *et al*., 2011), full length DNA-A sequences are required to determine the taxonomic status and precise identification of the virus. The present work describes the molecular characterization of the begomovirus causing SuLCD in India.

MATERIALS AND METHODS

Virus source: Diseased leaf samples showing leaf curl symptoms were collected from sunflower experimental fields at Main Agricultural Research Station, Raichur, Northern Karnataka, India during 2011 and used for obtaining DNA-A and betasatellite components of the virus by PCR.

DNA extraction, PCR amplification, cloning and sequencing: Total DNA extraction from leaf samples was performed by following the CTAB method as described by Lodhi *et al*. (1994) and Maruthi *et al*. (2002). The abutting primer pair SFLC-F (5^1-TACCAGG ATCCTGTTTGACAACGAGCCTAGCAC-3^1)andSFLC-R (5^1-AT CTGAAGCTTTATTGAACACCTCTCCAAAGT C-3^1) designed based on the 575 nt sequence of the begomovirus core region of the CP gene (Govindappa *et al*., 2011) were used to amplify the full-length DNA-A genome. PCR amplification was carried out in a thermocycler (Eppendorf, Germany) with a final volume of 25 µL reaction containing 2.5 µL 10X PCR buffer, 0.5 µL 25 mM $MgCl_2$, 2.5 mM each dNTPs, 20 mM 1.25 µL each primers, 0.1 µL *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd., Bengaluru, India) and 2 µL template DNA. The DNA was amplified by an initial denaturation of 94°C for 5 min followed by 35 cycles of 94°C for 1 min denaturation, 58°C for 1 min primer annealing, 72°C for the 3 min primer extension and final extension at 72°C for 10 min.

PCR amplification of betasatellite molecule was done by following Briddon *et al*. (2002) protocol. The amplicons obtained using beta 01 and beta 02 primers were electrophoresed through a 1% (w/v) agarose gel in 1X TAE and bands were visualized under UV light after staining with ethidium bromide (0.5 µg mL^{-1}). The bands were later excised from the gel and eluted through Qiagen Gel Extraction kit (Qiagen, Hilder, Germany) and cloned into the plasmid vector pTZ57R/T using the PCR cloning kit (MBI Fermentas, Germany) following the manufacturer's instructions. Plasmid purification was carried out using a Qiagen plasmid miniprep kit (Qiagen, Hilder, Germany). The inserts were sequenced at Chromous Biotech Pvt. Ltd., Bengaluru, India by primer walking.

Analysis of sequence data: The complete nucleotide sequence was initially taken into account for similarity search by using the BLASTn search program according to Altschul *et al*. (1997). Sequence analysis of the complete nucleotide sequences of DNA-A and DNA-beta was aligned with sequences obtained from GenBank databases by using ClustalW provided in MEGA-5 program (Tamura *et al*., 2011).

The phylogenetic analysis of the sequence of full genome (DNA-A) and DNA-beta sequence obtained from the SuLCD infected plant material was carried out together with the known begomoviruses and DNA-beta sequences obtained from gene bank databases. Phylogenetic and molecular evolutionary analyses were performed with MEGA-5 software using the Neighbor Joining method as per Tamura *et al*. (2011).

The expasy proteomic server tool (http://expasy. org/tools) was used for predicting Open Reading Frames (ORFs) and to translate set of protein encoding genes. Gene bank accession numbers of different begomoviruses used for DNA-A and DNA beta molecules sequence comparison and phylogenetic analysis are given in Table 1 and 2, respectively.

RESULTS

Comparison of ToLCKV (Raichure:SF) nucleotide and ORF sequences with other begomoviruses: The specific abutting primer pair SFLC-F/SFLC-R designed based on the sequence of the core region of the CP gene were used to amplify the ~2.8 kb fragment of DNA-A from virus infected leaf samples. The complete nucleotide sequence of the begomovirus was determined to be 2,761 nt (accession No. JX678965). We could not detect the presence of any second genomic component (DNA-B) as revealed by PCR. Analysis of the sequence of DNA-A

Table 1: Genomic DNA -A and protein amino acid identities between ToLCKV (Raichure:SF) and other 18 begomoviruses

Virus	DNA-A[a] (2761 nt)	AV2[b] (118 aa)	AV1[b] (256 aa)	AC1[b] (361 aa)	AC2[b] (134 aa)	AC3[b] (134 aa)	AC4[b] (97 aa)
ToLCKV-[IKH12]	*97.13	*98.30	*99.0	*93.35	*96.26	*97.76	*93.81
ToLCKV-[IKB3]	*96.95	97.45	98.0	*93.07	*96.26	*97.76	*94.84
ToLCV-[Ban-II]	*95.65	*98.30	*99.0	91.41	*96.26	*97.01	86.59
ToLCKV-[Ban]	92.17	90.67	95.0	92.79	*97.01	94.02	88.65
ToLCKV-[Janti]	91.40	95.76	*99.0	86.14	94.02	*97.01	*93.81
ToLCKeV–[K5]	85.11	90.67	95.0	83.61	93.28	94.02	46.39
ToLCND-CTM	82.84	94.91	96.0	72.02	86.56	91.79	22.35
ToLCGuV-[Pune]	84.56	85.21	80.0	90.85	91.79	82.83	*93.81
ToLCND-CTS	84.69	84.34	80.0	89.75	91.04	82.83	92.78
ToLCIV	83.84	87.82	82.0	85.04	94.02	94.77	90.72
ToLCGuV-[Vadodara]	84.45	86.08	80.0	90.85	92.53	82.83	93.81
ToLCGuV-[Nepal]	84.51	84.34	80.0	90.50	91.79	83.58	93.81
ToLCKeV-[K5]	84.20	90.67	94.0	79.10	92.53	93.28	44.32
ToLCBV-AVT1	80.28	70.43	91.0	84.48	85.07	84.32	88.65
ToLCPuV	79.28	72.54	91.0	83.38	86.56	81.34	69.07
ToLCBV-[Ban5]	77.67	67.82	90.0	81.99	84.32	82.83	71.13
TbCSV-[Y35]	77.67	93.04	96.0	85.31	81.34	85.07	79.38
TbCSV-[SC118]	82.88	93.91	96.0	85.04	82.08	85.07	79.38

[a]Nucleotide sequence identity, [b]Predicted amino acid (aa) sequence identity, *Highest percentages of sequence identities

Table 2: Betasatellite DNA molecule and βC1 protein amino acid identities between ToLCB-IN-Raichure: 11 betasatellite molecule and other Betasatellite molecules

Accession No.	Name of begomoviruses	Abbreviation	Beta DNA (%)	βC1 (%)
AY438557	Papaya leaf curl betasatellite [India:Chintapalli: 2003]	PaLCuB-[IN:Chi:03]	*91.32	*94.91
AY244706	Papaya leaf curl betasatellite [India:New Delhi: 2003]	PaLCuB-[IN:ND:03]	*91.83	*94.91
AY230138	Papaya leaf curl betasatellite [India:Jabalpur: 2003]	PaLCuB-[IN:Jab:03]	*91.76	*94.91
EF043234	Potato apical leaf curl betasatellite-[India:Chinthapalli: 2005]	PoLCB-[IN-CHI:05]	*94.07	*96.61
DQ118862	Papaya leaf curl betasatellite-[India:Chinthapalli: 2005]	PaLCuB-[IN-CHI:05]	*93.60	*95.76
HM101175	Tomato leaf curl betasatellite-[India:Varnasi:Pumpkin: 20008:3]	ToLCB-[IN-VAR:08]	*90.20	*90.67
AJ421485	Tobacco curly shoot betasatellite-[China:Yunnan 2: 1999]	TbCSB-[CN:Yn2:99]	48.15	55.93
AJ316033	Tobacco leaf curl betasatellite-[Pakistan:Rahim Yar Khan: 1998]	TbLCB-[PK:RYK:98]	50.95	64.40
AJ421621	Tobacco leaf curl China betasatellite-[China:Yunnan10: 2000]	TbLCB-[CN:Yn10:00]	46.70	66.94
HM101173	Papaya leaf curl betasatellite-[India:Varnasi:Pumpkin: 20008:1]	PaLCB-[IN-VAR:08]	78.39	85.59
AJ316038	Cotton leaf curl Multan betasatellite-[India:Dabwali 2: 1995]	CLCuMB-[IN:Dab2:95]	40.63	26.27
GU984046	Tomato leaf curl Bangalore betasatellite-[India:Bangalore: 2008]	ToLCBB-[IN-BAN:08]	64.30	78.81
AY838894	Tomato leaf curl Maharastra betasatellite-[India:Pune: 2004]	ToLCMaB-[IN-PUNE:04]	68.53	77.96
AY754815	Tomato leaf curl Pune betasatellite-[India:Pune: 2004]	ToLCPuB-[IN-PUNE:04]	68.09	77.11
EU126825	Papaya leaf curl virus betasatellite-[India:New delhi: 2008]	PaLCuB-[IN:ND:08]	51.13	63.55
AJ542499	Zinnia leaf curl betasatellite-[Thailand:Pattaya: 2002]	ZLCuB-[TH:PAT:02]	42.02	53.38
EF417919	Bhendi yellow vein betasatellite-[India:Barrackpore: 2006]	BYVB [IN:Bar:06]	39.66	31.35
AM279669	Chilli leaf curl betasatellite [Pakistan:Takhtbai: 2006]	ChLCB [PK:Tak:06]	54.69	67.79
AY744380	Cotton leaf curl Multan betasatellite-[India:Sirsa: 2004]	CLCuMB [IN:Sir:04]	38.45	27.11
AJ316040	Honeysuckle yellow vein mosaic betasatellite-[United Kingdom:96-2]	HYVMB-[UK:96-2]	36.83	38.79
DQ191161	Cotton leaf curl Multan betasatellite-[India:Bhatinda: 2005]	CLCuMB-[IN:Bha:05]	40.59	26.27
AJ298903	Cotton leaf curl Multan betasatellite-[Pakistan:Faisalabad 1: 1996]	CLCuMB-[PK:Fai1:96]	40.77	26.27
AJ308425	Bhendi yellow vein betasatellite-[India:Muthuppatti: 2000]	BYVB-[IN:Mut:00]	39.09	30.50
AY428768	Tomato leaf curl Bangalore betasatellite-[India:Bangalore: 2003]	ToLCBB-[IN:Ban:03]	66.86	73.72
AY438560	Tomato leaf curl Bangalore betasatellite-[India:Coimbatore: 2003]	ToLCBB-[IN:Coi:03]	64.53	72.03
AY438558	Tomato leaf curl Bangladesh betasatellite-[India:Rajasthan: 2003]	ToLCBDB-[IN:Raj:03]	54.92	67.79
AJ316036	Tomato leaf curl betasatellite-[Pakistan:Rahim Yar Khan: 1997]	ToLCB-[PK:RYK:97]	53.53	63.55

[a]Nucleotide sequence identity, [b]Predicted amino acid (aa) sequence identity, *Highest percentages of sequence identities

from begomovirus associated with SuLCD showed typical features of monopartite begomoviruses infecting tomato from India with maximum identity of 97.13% with ToLCKV-[IKH12]; (HM803118) followed by 96.95 and 95.65% with ToLCKV-[IKB3] (HM851186) and ToLCV-[Ban-II] (U38239), respectively (Table 1). Phylogenetic tree based on alignment of complete DNA-A sequences of the majority of tomato infecting begomoviruses of Indian subcontinent origin and other begomoviruses occurring in Asia shows that begomovirus isolated from the sunflower cluster with Tomato leaf curl Karnataka virus isolates infecting tomato in India (Fig. 1). The begomovirus isolated from sunflower is thus an isolate/strain of ToLCKV, for which we propose the isolate descriptor ToLCKV-(Raichure: Sunflower) and designated as ToLCKV (Raichure:SF).

The virion sense strand of DNA-A encoded two Open Reading Frames (ORFs) (AV1 or CP = 768 nt), AV2 354 nt and four ORFs on complementary strand (AC1 or Rep = 1083 nt; AC2 or Trap = 402 nt; AC3 or REn = 402 nt; and AC4 = 291 nt). The degree of relationship of the nucleotide sequence of DNA-A and the amino acid

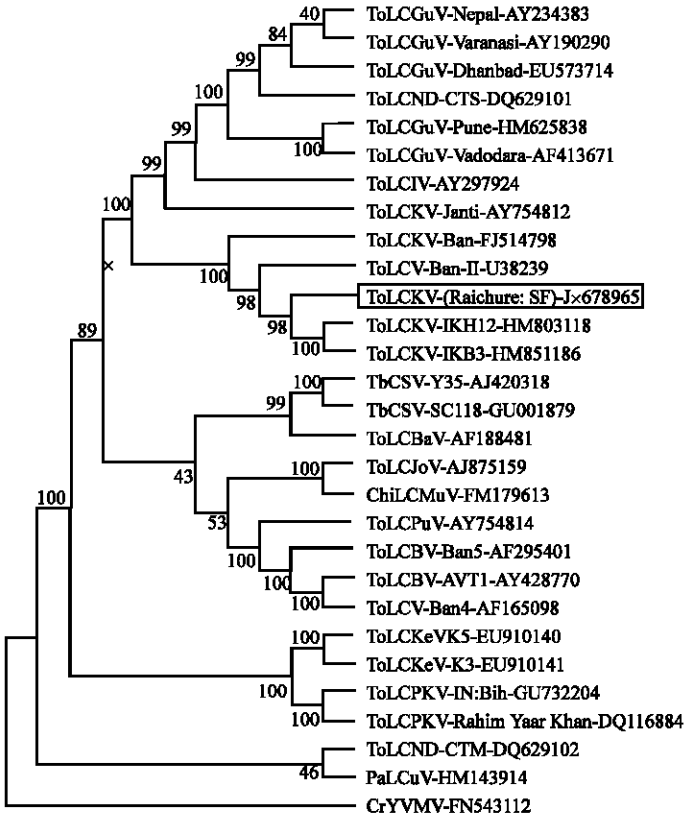

Fig. 1: Phylogenetic tree showing the relationship of ToLCKV-(Raichare: SF)-DNA A with other selected begomoviruses. The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap

sequences of the proteins expressed by the viral genome of the ToLCKV (Raichure: SF) isolate is illustrated in Table 1.

The virion sense AV1 encoded coat-protein shared 99.0% amino-acid identity with ToLCKV-[IKH12], ToLCV-[Ban-II] and ToLCKV-[Janti]. Another virion sense AV2 showed maximum amino acid sequence identity (98.3%) with ToLCKV-[IKH12] and ToLCV-[Ban-II]. Comparison of ORFs showed that AC1 encoded Rep protein shared 93.35% amino acid identity with ToLCKV-[IKH12] (HM803118). ORF AC2 had maximum (97.01%) identity with ToLCKV-[Ban] followed by ToLCKV-[IKH12] ToLCKV-[IKB3]) (HM851186) and ToLCV-[Ban-II] (96.26%). ORF AC3 showed maximum similarity (97.76%) with ToLCKV-[IKH12] and ToLCKV-[IKB3] (96.95%). ORF AC4 had maximum amino acid similarity (94.84%) with ToLCKV-[IKB3] (Table 1).

Molecular relationship of DNA β associated with ToLCKV (Raichure: SF) and its phylogenetic relationship with other satellite molecules: The full-length DNA betasatellite was amplified using universal primer set (Briddon *et al.*, 2002) and the sequence was determined. Sequence analysis demonstrated that the DNA-β satellite isolated in the present investigation has structural features shared by other DNA-β molecules, including an adenine-rich region, a Satellite-Conserved Region (SCR) with and one conserved βC1 ORF. The sequence consisted of 1,373 nt (accession No. JX678964) with a functional ORF (βC1) (204-557 nt) in complementary-sense DNA. The βC1 protein of isolate composed of 118 amino acid residues.

The full-length nucleotide sequence of the ToLCKV (Raichure: SF) betasatellite obtained in the present study showed 36.83-94.07% identity with the other betasatellites compared in this study (Table 2). In phylogenetic analysis of betasatellite DNA associated with SuLCD branch with PaLCuB-[IN:Chi:03] and clustered with some of the betasatellites molecules associated with begomovirus causing disease of tomato and potato occurring in the Indian sub-continent (Fig. 2).

Maximum nucleotide sequence identity (94.07%) was with Potato apical leaf curl beta (PoLCB-[IN-CHI:05]) followed by Papaya leaf curl beta-[India:Chinthapalli:2005]

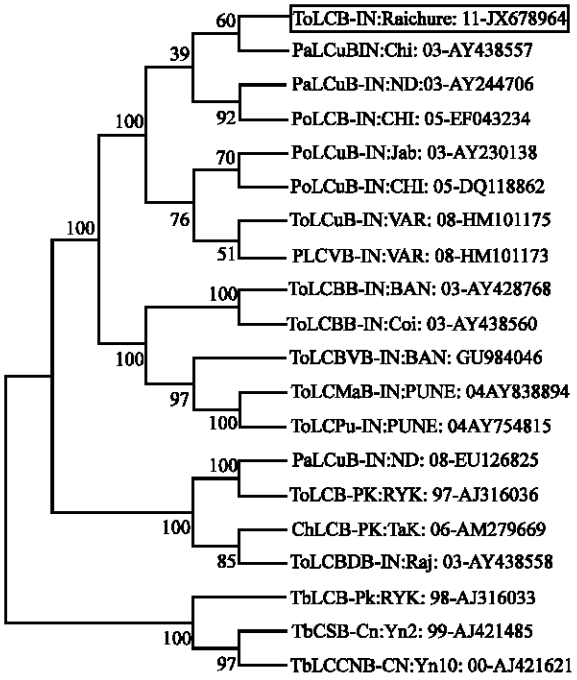

Fig. 2: Phylogenetic tree showing the relationship of ToLCB-IN-Raichure: 11 DNA betasatellite with other selected begomoviruses betasatellites. The tree was constructed using the neighbor-joining method with 1,000 bootstrap replications

(PaLCuB-[IN-CHI:05] (93.6%) and betasatellites associated with Tomato leaf curl disease (Table 2). It was therefore, designated as ToLCB-[India:Raichure:11] and abbreviated as ToLCB (IN:Rai:11].

The present isolate also shared maximum amino acid sequence identity (95.76-96.61%) of βC1 with PoLCB-[IN-CHI:05] and PaLCB-[IN:CHI:05] (Table 2).

DISCUSSION

The complete nucleotide sequence of DNA-A and the associated satellite beta DNA from SuLCD isolate collected from Raichur, India was determined for precise identification of the associated begomovirus. The genome organization of virus causing sunflower leaf curl was typical of begomovirus with monopartite genome containing two ORFs (AV1 or CP = 768 nt and AV2 354 nt) in virion sense strand and four ORFs (AC1 or Rep = 1083 nt; AC2 or Trap = 402 nt; AC3 or REn = 402 nt; and AC4 = 291 nt) on complementary strand. Phylogenetic analysis based on the DNA-A component isolated from SuLCD infected sunflower, the virus clustered with isolate of Tomato leaf curl Karnataka virus clone IKH12 (ToLCKV-[IKH12]; (HM803118) and clone IKB3 (ToLCKV-[IKB3]) (HM851186) and shared sequence identities of 97.13 and 96.95%, respectively. PCR detection, cloning and sequencing results of DNA A component of SuLCD are in support of studies on tomato leaf curl virus by Muniyappa *et al*. (2000).

Majority of members of the geminiviridae, genus begomovirus have a genome comprising of two similar sized DNA components (DNA A and DNA B). DNA A encodes a replication associated protein, coat protein and proteins that participate in the control of replication and gene expression. DNA B encodes proteins required for nuclear trafficking and cell to cell viral DNA transport. In contrast only a single genomic component (DNA A) has been isolated for several begomoviruses including Tomato yellow leaf curl virus, Tomato leaf curl virus and cotton leaf curl virus. It is evident that the single genomic component is sufficient for maintainance of the disease in the host (Briddon *et al*., 2000; Kheyr-Pour *et al*., 1991).

The DNA β satellite molecule isolated from SuLCD infected plants had the structural features of a typical DNA β molecule with 1373 nt with a functional ORF (βC1) in complimentary sense DNA including an adenine rich region and a satellite conserved region similar to that of other begomoviruses including ToLCV Karnataka isolates. A database search revealed that DNA β shows homology of 93.6% to a satellite DNA associated with ToLCKV. However, maximum nucleotide sequence identity of 94.07 per cent was with Potato apical leaf curl beta (PoLCB-[IN-CHI:05]). Association of circular DNA β of ~1300 nt have been reported with many monopartite begomoviruses (Saunders *et al*., 2000, 2004) and demonstrated that the satellite, one protein that plays a major role in symptom development and is essential for disease progression.

The genomes of the three ToLCV isolates from Karnataka, Southern India, have been cloned and sequenced (Chatchawankanphanich *et al*., 1993; Hong and Harrison, 1995). The ToLCVs from Southern India known to possess a single genomic component as DNA-A associated with alpha and betasatellite DNA molecules. There is no citation of these 3 isolates possessing DNA-B component (Hong and Harrison, 1995).

The presence of *Bemisia tabaci* B biotype in south India (Banks *et al*., 2001; Rekha *et al*., 2005; Shankarappa *et al*., 2007) has raised serious concern as the biotype has the ability to alter the epidemiology of begomoviruses in India. Emergence of new begomoviruses and their epidemics in the subcontinent has been attributed to the spread of B biotype *B. tabaci*. (Narayana *et al*., 2006, 2007; Maruthi *et al*., 2007; Mahesh *et al*., 2010).

This is the first report on genome sequence analysis of begomovirus associated with SuLCD. The sequence information of the viral genes can be used for developing virus specific nucleic acid specific detection methods and to develop transgenic sunflower against the virus disease.

Studies are needed on epidemiology, virus transmission characters, developing infectious clones of the virus. The construction of infectious clones of DNA-A and DNA β associated with sunflower leaf curl disease to prove Koch's postulate is in progress.

CONCLUSION

The data strongly suggest that ToLCKV strain is the causal agent of Sunflower leaf curl virus disease. Furthermore, to our knowledge, this study is the first report of the complete genomic sequence of a begomovirus from sunflower in Southern India.

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REFERENCES

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res., 25: 3389-3402.

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

Banks, G.K., C.J. Reddy, R.V.C. Maruthi, M.N. Muniyappa and V. Venkatesh *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 and M. Ramiah, 2002a. Detection of *Tobacco streak virus* from sunflower and other crops by reverse transcription polymerase chain reaction. Ind. Phytopathol., 55: 216-218.

Bhat, A.I., R.K. Jain, V. Chaudhary, M. Krishna Reddy, M. Ramiah, S.N. Chattannavar and A. Varma, 2002b. 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., S. Mansoor, I.D. Bedford, M.S. Pinner and P.G. Markham, 2000. Clones of cotton leaf curl geminivirus induce symptoms atypical of cotton leaf curl disease. Virus Genes, 20: 17-24.

Briddon, R.W., S.E. Bull, S. Mansoor, I. Amin and P.G. Markham, 2002. Universal primers for the PCR-mediated amplification of DNA β: A molecule associated with some monopartite begomoviruses. Mol. Biotechnol., 20: 315-318.

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 for establishing the provisional identification and classification of begomoviruses. Arch. Virol., 146: 1581-1598.

Chatchawankanphanich, O., B.T. Chiang, S.K. Green, S.J. Singh and D.P. Maxwell, 1993. Nucleotide sequence of a geminivirus associated with tomato leaf curl from India. Plant Dis., 77: 1168-1168.

Dry, I.B., L.R. Krake, J.E. Rigden and M.A. Rezaian, 1997. A novel subviral agent associated with a geminivirus: The first report of a DNA satellite. Proc. Natl. Acad. Sci. USA., 94: 7088-7093.

Fauquet, C.M., R.W. Briddon, J.K. Brown, E. Moriones, J. Stanley, M. Zerbini and X. Zhou, 2008. Geminivirus strain demarcation and nomenclature. Arch. Virol., 153: 783-821.

Govindappa, M.R., I. Shankergoud, K.S. Shankarappa, W.A.R.T. Wickramaarachchi, B.A. Reddy and K.T. Rangaswamy, 2011. Molecular detection and partial characterization of begomovirus associated with leaf curl disease of sunflower (*Helianthus annuus*) in Southern India. Plant Pathol. J., 10: 29-35.

Hong, Y.G. and B.D. Harrison, 1995. Nucleotide sequences from tomao leaf curl viruses from different countries: Evidence for three geographically separate branches in the evolution of the coat protein of whitefly-transmitted geminiviruses. J. General Virol., 76: 2043-2049.

Kheyr-Pour, A., M. Bendahmane, V. Matzeit, G.P. Accotto, S. Crespi and B. Gronenborn, 1991. Tomato yellow leaf curl virus from Sardinia is a whitefly-transmitted monopartite geminivirus. Nucleic Acids Res., 19: 6763-6769.

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.

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., 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.

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.

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.

Prajapat, R., A. Marwal, A. Sahu and R.K. Gaur, 2011. Phylogenetics and *in silico* docking studies between coat protein of Mimosa yellow vein virus and Whey α-lactalbumin. Am. J. Biochem. Mol. Biol., 1: 265-274.

Ramiah, 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.

Rekha, A.R., M.N. Maruthi, V. Muniyappa and J. Colvin, 2005. Occurrence of three genotypic clusters of *Bemisia tabaci* (Gennadius) and the rapid spread of the B-biotype in South India. Entomol. Exp. Applied, 117: 221-233.

Saunders, K., A. Norman, S. Gucciardo and J. Stanley, 2004. The DNA β satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (βC1). Virology, 324: 37-47.

Saunders, K., I.D. Bedford, R.W. Briddon, P.G. Markham, S.M. Wong and J. Stanley, 2000. A unique virus complex causes *Ageratum* yellow vein disease. Proc. Natl. Acad. Sci. (USA)., 97: 6890-6895.

Shankarappa, K.S., K.T. Rangaswamy, D.S. Aswathanarayana, A.R. Rekha and N. Raghavendra *et al*., 2007. Development of silver leaf assay, protein and nucleic acid-based diagnostic techniques for the quick and reliable detection and monitoring of biotype B of the whitefly, *Bemisia tabaci* (Gennadius). Bull. Entomol. Res., 97: 503-513.

Shukla, D.D. and C.W. Ward, 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the Potyvirus group. J. Gen. Virol., 69: 2703-2710.

Stanley, J., 1983. Infectivity of the cloned geminivirus genome requires sequences from both DNAs. Nature, 305: 643-645.

Stanley, J., D.M. Bisaro, R.W. Briddon, J.K. Brown and C.M. Fauquet *et al*., 2005. Geminiviridae. In: Virus Taxonomy. Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball (Eds.). Elsevier/Academic Press, London, ISBN: 9780080575483, pp: 301-326.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.

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.