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Complete Nucleotide Sequence of Tomato Leaf Curl Karnataka Virus and \( \beta \) 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 Northern 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; Elhat 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
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; Prajapati 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'-TACCAGGATCTCTGGTTTGACAAACGCCTAGCAC-3') and SFLC-R (5'-ATCTGAAGGTTTATTGAAACCTCTCCTCAAGT-3') 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 MgCl₂, 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 Tol.CKV (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
from begomovirus associated with SuLCO and showed typical features of monopartite begomoviruses infecting tomato from India with maximum identity of 97.13% with ToLCV-[IKH12], (HM803118) followed by 96.95 and 95.65% with ToLCV-[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 ToLCV-[IKH12], ToLCV-[IKB3], ToLCV-[Ban-I], ToLCV-[Ban-II], ToLCV-[Kan], ToLCV-[Kes], ToLCND-CTM, ToLCGSSV-[Pore] and ToLCND-CTS. ToLCV-[Kes] and ToLCVG-[Kes] are the main betasatellite molecules and other ToLCB-1N-II. The DNA-A and DNA-B proteins of betasatellite are the main proteins that are responsible for the development of disease symptoms.

Table 2: Betasatellite DNA molecular and protein amino acid identities between ToLCB-1N-II. The DNA-A and DNA-B proteins of betasatellite are the main proteins that are responsible for the development of disease symptoms.

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Name of begomoviruses</th>
<th>Abbreviation</th>
<th>DNA-A (%)</th>
<th>DNA-B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AY343857</td>
<td>Papaya leaf curl betasatellite-[India:Chintapalli:2003]</td>
<td>PaLCuB-[IN-Chi:03]</td>
<td>91.32</td>
<td>94.91</td>
</tr>
<tr>
<td>AY244706</td>
<td>Papaya leaf curl betasatellite-[India:New Delhi:2003]</td>
<td>PaLCuB-[IN-ND:03]</td>
<td>91.85</td>
<td>94.91</td>
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<tr>
<td>AY230138</td>
<td>Papaya leaf curl betasatellite-[India:Jabalpur:2003]</td>
<td>PaLCuB-[IN-Jab:03]</td>
<td>91.76</td>
<td>94.91</td>
</tr>
<tr>
<td>EF043234</td>
<td>Potato topical leaf curl betasatellite-[India:Chintapalli:2005]</td>
<td>PoLCB-[IN-Chi:05]</td>
<td>94.07</td>
<td>96.61</td>
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<tr>
<td>DQ118862</td>
<td>Papaya leaf curl betasatellite-[India:Chintapalli:2005]</td>
<td>PaLCuB-[IN-Chi:05]</td>
<td>91.60</td>
<td>95.76</td>
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<td>AJ316033</td>
<td>Tobacco leaf curl betasatellite-[Pakistan:Rawal Yar Khan:2008]</td>
<td>ToLCB-[PK-RYK:08]</td>
<td>50.95</td>
<td>64.60</td>
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<td>AJ316038</td>
<td>Tomato leaf curl betasatellite-[India:Dalhousi:2002]</td>
<td>ToLCB-[IN-Da:02]</td>
<td>49.63</td>
<td>68.27</td>
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<td>GU384046</td>
<td>Tomato leaf curl bangalore betasatellite-[India:Bangalore:2008]</td>
<td>ToLCB-[IN-BAN:08]</td>
<td>64.30</td>
<td>78.81</td>
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<td>AT838994</td>
<td>Tomato leaf curl maharashtra betasatellite-[India:Panse:2004]</td>
<td>ToLCB-[IN-PUN:04]</td>
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<td>77.96</td>
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<td>AT838915</td>
<td>Tomato leaf curl Panse betasatellite-[India:Panse:2004]</td>
<td>ToLCB-[IN-PUN:04]</td>
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<td>AMZ749660</td>
<td>chilli leaf curl betasatellite-[Pakistan:Tukhabhi:2006]</td>
<td>ChLCB-[PK-Tch:06]</td>
<td>48.69</td>
<td>67.79</td>
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<td>AY744480</td>
<td>Cotton leaf curl betasatellite-[India:Sierra:2008]</td>
<td>CLoMB-[IN-Sir:04]</td>
<td>38.45</td>
<td>27.11</td>
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<td>AY423878</td>
<td>Tomato leaf curl bangalore betasatellite-[India:Bangalore:2003]</td>
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<td>AY428560</td>
<td>Tomato leaf curl bangalore betasatellite-[India:Chimbolute:2003]</td>
<td>ToLCB-[IN-Coi:03]</td>
<td>64.53</td>
<td>72.03</td>
</tr>
</tbody>
</table>

* Nucleotide sequence identity, * Predicted amino acid (aa) sequence identity, * Highest percentages of sequence identities
sequences of the proteins expressed by the viral genome
of the ToLCVV (Raichure: SF) isolate is illustrated in
Table 1.

The virion sense AV1 encoded coat-protein shared
99.0% amino-acid identity with ToLCVV-[Ikh12],
ToLCVV-[Ban-II] and ToLCVV-[Janti]. Another virion
sense AV2 showed maximum amino acid sequence
identity (98.3%) with ToLCVV-[Ikh12] and ToLCVV-[Ban-
II]. Comparison of ORFs showed that AC1 encoded
Rep protein shared 93.35% amino acid identity with
ToLCVV-[Ikh12] (HM803118). ORF AC2 had maximum
(97.01%) identity with ToLCVV-[Ban] followed by
ToLCVV-[Ikh12] ToLCVV-[IKB3] (HM851186) and
ToLCVV-[Ban-II] (96.26%). ORF AC3 showed maximum
similarity (97.76%) with ToLCVV-[Ikh12] and
ToLCVV-[IKB3] (96.95%). ORF AC4 had maximum amino
acid similarity (94.84%) with ToLCVV-[IKB3] (Table 1).

Molecular relationship of DNA β associated with
ToLCVV (Raichure: SF) and its phylogenetic
relationship with other satellite molecules: The
full-length DNA betasatellite was amplified using
universal primer set (Bridden 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 [BC1 ORF. The sequence consisted of 1,373 nt
(accession No. JX678964) with a functional ORF (BC1)
(204-557 nt) in complementary-sense DNA. The BC1
protein of isolate composed of 118 amino acid residues.

The full-length nucleotide sequence of the ToLCVV
(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 StLCD branch with
PaLCB-[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 (ToLCB-[IN-CHI:05])
followed by Papaya leaf curl beta-[India:Chinthapalli:2005]

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 maintainence of the disease in the host (Bridgdon et al., 2000; Kheyr-Pour et al., 1991).

The DNA β satellite molecule isolated from SuLCV infected plants had the structural features of a typical DNA β molecule with 1373 nt with a functional ORF (BC1) 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 ToLCV. However, maximum nucleotide sequence identity of 94.07 per cent was with Potato apical leaf curl beta (PaLCb-[IN-CHI:05]). Association of circular DNA β of ~1300 nt have been reported with many monopartite begomoviruses (Saurkards 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 SunLC. 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


