Tobacco Leaf Curl Puer Virus: a Novel Monopartite Begomovirus Infecting *Nicotiana Tabacum* in China

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Abstract

Begomoviruses (family Geminiviridae) cause serious diseases in many economic crops and weeds globally. This study characterized a begomovirus isolated from a tobacco plant with leaf curl in Puer, Yunnan Province, China. Analysis of the viral genome obtained from a diseased *Nicotiana tabacum* plant showed a novel monopartite begomovirus. The DNA-A-like sequence (2741 nt) has the typical Old World monopartite begomovirus genome organization, sharing the highest nucleotide sequence identity (81.2%) with that of Tomato yellow leaf curl Vietnam virus (TYLCVV). According to the current International Committee on Taxonomy of Viruses (ICTV) taxonomic criteria, this is a new species of begomovirus for which the name “Tobacco leaf curl Puer virus” is proposed.

Main Text

Viruses in the family Geminiviridae can cause grievous economic losses to many crops worldwide [1, 2]. The geminivirus genome consists of one (monopartite) or two (bipartite) circular single-stranded DNA molecules that are encapsidated within geminated icosahedral particles [3]. Geminiviruses are classified into nine genera based on their host ranges, insect vectors, and genome structures:

Becurtovirus, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus*, and *Turncurtovirus*. *Begomovirus* is the largest genus in this family, with more than 400 members [4]. Under natural conditions, begomoviruses are transmitted only by whiteflies of the *Bemisia tabaci* complex [5], and are known as whitefly-transmitted geminiviruses [6].

Tobacco leaf curl disease (TLCD) caused by geminiviruses is one of the most important diseases in tropical and subtropical tobacco planting areas [7, 8], causing huge losses to the tobacco industry. More than 10 viruses cause TLCD in Asia, all belonging to *Begomovirus* [7-17]. Tobacco is the most important economic crop in Yunnan Province, China [18]. Here we describe a novel monopartite begomovirus infecting *N. tabacum* in China.

In November 2020, leaves from eight tobacco plants (T1–T8) with severe leaf curl and dwarfing suspected being TLCD were collected from two tobacco fields in Puer, Yunnan Province, China. Total DNA was extracted from eight symptomatic and one non-symptomatic tobacco samples using the CTAB method [19]. PCR assays were performed using *Begomovirus* universal primer pair PA/PB [20]. PCR fragments of the expected size (0.5 kb) were obtained from all symptomatic samples, cloned into pMD19-T vector (Takara, China), and sequenced independently. However, attempts to amplify the satellite DNA component with specific primers were unsuccessful [21]. Sequence analysis results showed that they possessed 99% nucleotide sequence identity with each other. BLASTn results indicated the presence of a novel begomovirus. To obtain the full genome sequence of the new begomovirus, total DNA from sample T4 was subjected to rolling-circle amplification (GE Healthcare, UK). The ~2.7 kb viral DNA fragments were then digested with *Bam*HI endonuclease (Takara, China) and cloned into pGD vector previously digested with *Bam*HI [22]. The products were transformed into *Trans1*-T1 competent cells (TransGen, China). The recombinant plasmids were then sequenced (Tsingke, China). The DNA

sequences were assembled, edited, and analyzed using DNAMAN 8.0 (LynnonBiosoft, Canada). A BLASTn similarity search of the obtained sequence was used to identify sequences with the highest similarity in the GenBank database (<http://www.ncbi.nlm.nih.gov>). The DNA-A like sequences were used for pairwise sequence comparisons and phylogenetic and recombination analysis using SDT ver. 1.2, MEGA-X, and RDP4, respectively [23-25].

A BLASTn (GenBank) search revealed a monopartite begomovirus in *N. tabacum* sample T4. The 2741 nt DNA-A-like sequence (GenBank acc. no. MZ465370) from the tobacco plant had features typical of Old World begomoviruses, encoding six open reading frames (ORFs). The DNA-A-like sequence shared the highest nucleotide sequence identity (81.2%) with Tomato yellow leaf curl Vietnam virus (TYLCVV) isolate TYLCVV-DX1 (EU189150) from Vietnam. SDT analysis revealed < 86% sequence identity with DNA-A sequences of 22 begomoviruses selected from the initial BLASTn analysis (Fig. 1). In accordance with the begomovirus species demarcation criteria (91% nucleotide sequence identity of DNA-A and DNA-A-like components) [4, 26], this begomovirus isolate associated with *N. tabacum* is a previously undescribed, monopartite begomovirus species, for which we propose the name “Tobacco leaf curl Puer virus” (TbLCPeV).

Phylogenetic trees were constructed using the maximum likelihood (ML) method with 1000 bootstrap replications for the selected begomovirus (Fig. 2). The ML tree confirmed that TbLCPeV clustered with two Old World begomoviruses from China: Ageratum yellow vein virus (JQ804985) and Pepper leaf curl Yunnan virus (KU975395) (Fig. 2). Recombination analysis of TbLCPeV was performed using the program RDP4 with the default settings. The begomovirus sequences that were used to construct the ML tree (except Sida golden yellow vein virus, which was used as an outgroup) were used for the RDP analysis. A potential recombination event was detected by seven methods (RDP, GENECONV, Bootscan, MaxChi, Chimaera, SiScan, and 3Seq) involving the AC3 ORF and part of AC1 ORF (nt 1053–1889; *P*-value, 3.75×10^{-35} for the Bootscan method) (see Supplementary Table S1). The recombinant fragment of the genome (837 bp) was close to the TYLCVV-DX1 isolate (EU189150; 96.06% nucleotide sequence identity). The non-recombination fragment of the genome (1904 bp) was related to an “unknown parent”. Although we did not obtain any DNA satellites, their existence needs to be verified using high-throughput sequencing.

This study provides evidence that TbLCPeV is a new monopartite begomovirus that is genetically distinct from the reported begomoviruses causing TLCD in Asia. Further studies need to examine the pathogenicity, host range, and geographic extent of this novel begomovirus.

Declarations

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Compliance with ethical standards

Human and animal studies

This study contains no human participants or animals.

Conflict of interest

The authors declare that there is no conflict of interest.

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Figures

Figure 1

Color code matrix of pairwise sequences of Tobacco leaf curl Puer virus (TbLCPeV) and closely related begomoviruses obtained from the GenBank database

Figure 2

Maximum likelihood phylogenetic tree of TbLCPeV and the most closely related Old World begomoviruses was constructed in MEGA-X with 1000 bootstrap replications. Sida golden yellow vein virus (SiGYVV) segment DNA-A was used as the outgroup. The Begomovirus characterized here is in bold font. The bar below the tree represents nucleotide substitutions per site. All the sequences used to generate the phylogenetic tree were obtained from the GenBank database. The begomoviruses used in

the study are as follows: ALCV, Ageratum leaf curl virus; AYVCNV, Ageratum yellow vein China virus; AYVV, Ageratum yellow vein virus; BIYVV, Blechnum yellow vein virus; CLCuV, Cotton leaf curl virus; HUYSMV, Hedyotis uncinella yellow mosaic virus; MiYLCV, Mimosa yellow leaf curl virus; PaLCuCNV, Papaya leaf curl China virus; PeLCYNV, Pepper leaf curl Yunnan virus; PLCuV, Premna leaf curl virus; SiGYVV, Sida golden yellow vein virus; SiYMGV, Sida yellow mosaic virus; SiYVVNV, Sida yellow vein Vietnam virus; StaLCV, Stachytarpheta leaf curl virus; TbLCPuV, Tobacco leaf curl Pusa virus isolate; ToLCCeV, Tomato leaf curl Cebu virus; ToLCJV, Tomato leaf curl Java virus; TLMV, Tomato leaf curl Malaysia virus; ToLCPatV, Tomato leaf curl Patna virus isolate; ToLCPV, Tomato leaf curl Philippines virus; ToLCTWV, Tomato leaf curl Taiwan virus; TYLCVV, Tomato yellow leaf curl Vietnam virus; TYLCYNV, Tomato yellow leaf curl Yunnan virus

Supplementary Files

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