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 crops. In this study, we characterized a begomovirus isolated from a tobacco plant with leaf curl in Puer, Yunnan Province, China. Analysis of the viral genome obtained from a symptomatic *Nicotiana tabacum* plant showed that it belonged to a novel monopartite begomovirus. The genome (2741 nt) shared the highest nucleotide sequence identity (83.43%) with that of tomato yellow leaf curl Vietnam virus (TYLCVV). Based on the current taxonomic criteria of the International Committee on Taxonomy of Viruses, this virus, for which the name “tobacco leaf curl Puer virus” is proposed, represents a new species of begomovirus.

Viruses in the family *Geminiviridae* cause significant economic losses to many crops [1]. Geminiviruses are classified into 14 genera based on their host range, insect vector, and genome structure [2]. *Begomovirus* is the largest genus in this family, with more than 400 species [3].

In November 2020, leaves from eight tobacco plants (T1–T8) with severe leaf curl and dwarfing, as well as one plant lacking symptoms, were collected from two tobacco fields in Puer, Yunnan Province, China. Total DNA was extracted from the leaf samples using the CTAB method [4]. PCR assays were performed using the begomovirus universal primer pair PA/PB [5]. Amplicons of the expected size (0.5 kb) were obtained from samples extracted from all symptomatic leaves, but not from non-symptomatic leaves. PCR products were cloned in the vector pMD19-T (Takara, China) and sequenced. Attempts to amplify possible betasatellites present in samples, using specific primers, were unsuccessful [6].

The sequences of the 0.5-kb fragments from eight plants showed >99% nucleotide sequence identity. BLASTn results indicated the presence of a 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 resulting amplified DNA was then digested with *Bam*HI (Takara, China) and cloned into the vector pGD [7]. The products were used to transform *Escherichia coli* Trans1-T1 competent cells (TransGen, China). Three positive clones were selected for sequencing (Tsingke, China). The DNA sequences were assembled, edited, and analyzed using DNAMAN 8.0 (LynnonBiosoft, Canada). A sequence alignment showed the three sequences to be identical (100% identity).

A BLASTn search of the GenBank database revealed a begomovirus in *N. tabacum* sample T4. The 2741-nt genome sequence (MZ465370) had features typical of Old World begomoviruses, encoding six open reading frames (ORFs) (Fig. 1A). The DNA-A-like sequence shared the highest nucleotide sequence identity (83.43%) with tomato yellow leaf curl Vietnam virus (TYLCVV) isolate TYLCVV-DX1 (EU189150) from Vietnam in SDT analysis (Supplementary Fig. S1) [8]. In accordance with the begomovirus species demarcation criteria (91% nucleotide sequence identity of DNA-A and DNA-A-like components) [3, 9], this begomovirus isolate associated with *N. tabacum*...
is a previously undescribed, monopartite begomovirus for which we propose the name “tobacco leaf curl Puer virus” (TbLCPeV).

Phylogenetic trees were constructed using the maximum-likelihood (ML) method for the selected begomoviruses (Fig. 2) using MEGA X [10]. The ML tree confirmed that TbLCPeV clustered with two 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 [11]. 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, involving part of the C3 ORF and part of Rep (C1) ORF (Fig. 1B and Supplementary Table S1). The recombinant fragment (837 bp) was most closely related to TYLCVV-DX1 (EU189150), with 96.06% nucleotide sequence identity. The major parent, donating 1904 bp, could not be identified. Although no betasatellite was identified, high-throughput sequencing should be used to confirm this.

This study provides evidence that TbLCPeV is a new monopartite begomovirus that is genetically distinct from the reported begomoviruses causing TLCD. Further studies are needed to examine the pathogenicity, host range, and geographic extent of this novel begomovirus.
Fig. 2  ML tree of TbLCPeV and the most closely related begomoviruses, constructed in MEGA X with 1000 bootstrap replications. The segment DNA-A of SiGYVV was used as an outgroup. The begomovirus characterized here is indicated in bold font. The bar below the tree represents nucleotide substitutions per site. All of the sequences used to generate the phylogenetic tree were obtained from the GenBank database.

Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05267-9.

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Declarations

Conflict of interest  The authors declare that there is no conflict of interest.

Human and animal studies  This study did not involve human participants or animals.

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