First Report of *Sida leaf curl virus* and Associated Betasatellite from Tobacco

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Funding: Funding was provided by National key research and development program (2019YFC1200503). Plant Dis. 106:1078, 2022; published online as <https://doi.org/10.1094/PDIS-05-21-1044-PDN>. Accepted for publication 27 October 2021.

Tobacco (*Nicotiana tabacum* L.) is an economic crop and an important model plant for scientific research in the world. Cigar tobacco, a variety of tobacco, has been planted for industrial production in China since its introduction into the country in 2018 (Wang et al. 2021). In March 2020, symptoms like leaf curling, vein thickening, and enation were frequently observed in cigar tobacco in several plantation areas of 100 ha in Danzhou, Hainan Province, China. It was speculated that a geminivirus was the possible causal agent of the disease because the symptoms resembled those caused by geminiviruses and because of the presence of their insect vectors: whiteflies. Five tobacco leaf samples were collected for DNA extraction, and pooled DNA was subjected to viral metagenomics analysis with Illumina Sequencing Technology (Illumina) at Tiangen Biotech, Beijing. In total, 67,774,552 filtered reads (99.84%) were matched to tobacco genome, and the remaining 110,908 reads (0.16%) were analyzed by BLASTn against GenBank virus Refseq Database with E-value smaller than $1e^{-6}$. Among the virus-matching sequencing data obtained, 65 and 2,058 reads were annotated as genomes of sida leaf curl virus (SiLCV, reference sequence: NC_007638) and its associated betasatellite (SiLCB, reference sequence: NC_007639), accounting for 3 and 95%, respectively. In all, 36 reads matched sweet potato vein clearing virus (reference sequence: NC_015228), and 7 reads matched Emiliana huxleyi virus (reference sequence: JF429838). We speculated that the causal pathogen of this disease might be SiLCV and its associated betasatellite. We

next designed primers SiLCV-DNA-A-F/SiLCV-DNA-A-R (SiLCV-DNA-A-F: GAATTCTTTTCCTCGTCCAGG and SiLCV-DNA-A-R: CGCTTTAAA GACTTGGGCTTT) and SiLCV-β-F/SiLCV-β-R (SiLCV-β-F: ACCGGTGG CGAGCTGGTGTCT and SiLCV-β-R: AATATTAGAACGGTGGCGAGC) to amplify the complete genome of SiLCV and its associated betasatellite, respectively. As expected, all five tobacco DNA samples were PCR positive for the two sets of primers. Thereafter, the amplicons from one sample were fully sequenced by Sanger sequencing at Tiangen Biotech, Beijing, China. The anticipated SiLCV genome with a length 2,760 nucleotides (accession no. MW465952) and its associated betasatellite with a length of 1,369 nucleotides (accession no. MW465953) exhibited the highest sequence identity of 93.5 and 95.6%, respectively, with that of SiLCV isolate 61 (DQ641706) (Ha et al. 2008) and isolate Hn57 (AM050732.1) (Guo and Zhou 2006), respectively. This SiLCV isolate identified in Hainan Province is named SiLCV-HN and its associated betasatellite is named SiLCB-HN. Infectious clones of SiLCV-HN and SiLCB-HN were constructed by ligating two complete genomes of each SiLCV-HN and SiLCB-HN to a binary expression vector pCambia1300, as previously described (Wang et al. 2020). Next, SiLCV-HN alone or coinfiltrated SiLCV-HN and SiLCB-HN were infiltrated into *Nicotiana benthamiana*. At 5 days postinoculation (5 dpi), typical begomovirus symptoms such as severe down-curl on newly emerging leaves were displayed in SiLCV-HN- and SiLCB-HN-coinfiltrated *N. benthamiana* plants. Agroinoculation of *N. benthamiana* plants with SiLCV-HN alone produced severe leaf up-curl symptoms at 12 dpi. Quantitative PCR results showed that the virus titer was higher in SiLCV-HN/SiLCB-HN-coinfected plants in comparison with plants infected with SiLCV-HN individually. Typical virus symptoms and SiLCV DNA could also be detected in a wild tobacco species, *N. glutinosa*, when agroinfiltration-based infection was done, even though at a low infection efficacy. We could not make successful agroinfiltration-based infection of SiLCV in cigar tobacco due to unknown reasons. Nevertheless, our data suggest that SiLCV-HN could infect species from the *Nicotiana* genus and, therefore, poses severe threats to tobacco industry.

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The author(s) declare no conflict of interest.

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Keywords: betasatellite, cigar tobacco, SiLCV, virus metagenomics

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