First Report of Squash Leaf Curl China Virus Infecting Tomato in China

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Squash leaf curl China virus (SLCCNV) is a species in the genus *Begomovirus* with a bipartite genome. It is transmitted by the whitefly *Bemisia tabaci* and infects cucurbit crops in various parts of the Old World (Wu et al. 2020). In 2020, tomato plants with curled, distorted, and yellow leaves were found in a greenhouse in Shouguang, Shandong Province, China. Leaves with these symptoms were collected from 11 plants and the total RNA was extracted with TRIzol reagent (Invitrogen, U.S.A.). Five RNA extracts of the highest quality were combined and a small RNA library was generated by the company BGI-Shenzhen (China). About 22,338,920 clean reads (18 to 28 nt) were acquired and assembled into larger contigs with the software Velvet 1.0.5. These were further compared against nucleotide sequences in the NCBI databases with BLASTn searches. Not unexpectedly, there were many assembled contigs with high identities (90 to 100% identity) with known tomato-infecting viruses, including 241 contigs matching tomato chlorosis virus, 26 contigs matching southern tomato virus, and 4 contigs matching tomato yellow leaf curl virus. However, 12 contigs had high identities (90 to 100%) with the genomic DNA-A of SLCCNV, and 9 other contigs had high identities (90 to 100%) with the genomic DNA-B of SLCCNV. To verify the presence of SLCCNV in tomato plants, two sets of primer pairs were designed according to the specific contigs assembled from derived small interfering RNAs (vsiRNAs). The primer pairs A742-F/A742-R (5'-GTAATACGAGCATCCGCACGGTAG-3'/5'-CGTGGAGGGCGACAAACAGCTAACG-3') and B539-F/B539-R (5'-GCTACTTTCAAGGACGAAGAAGAGG-3'/5'-CGACATAGATTCTGTCGGTGGGC-3') directed the amplification of 742 bp and 539 bp for DNA-A and DNA-B fragments, respectively, from the total genomic DNA of the 11 tomato samples. The DNA-A and DNA-B of SLCCNV were both detected from all of the tomato samples. After sequencing, the 742-bp PCR products shared 100% nucleotide sequence identity with the DNA-A of SLCCNV isolate GDXW (MW389919), whereas the PCR-amplified 539-bp fragments shared 100% nucleotide sequence identity with the DNA-B of SLCCNV isolate GDXW (MW389920). The full lengths of DNA-A and DNA-B components were amplified with back-to-back primers A-F/A-R (Wu et al. 2020) and B-F/B-R (5'-GATAACACGTCTCATTGACCCGC-3'/5'-GAGACGTGTTTATCAATATGGACG-3'), respectively. The amplified fragments were further cloned into the PCE2TA/Blunt-Zero vector (Vazyme Biotech, China). After sequencing, the complete sequence of DNA-A was 2,736 nt (MZ682117), while the DNA-B was 2,718 nt (OK236348). The phylogenetic relationships of the DNA-A and DNA-B components were determined using MEGA 7 based on the full-length sequences of DNA-A and DNA-B, respectively (Kumar et al. 2016). Results showed that the DNA-A formed an independent cluster and was mostly related to the GDHY (MW389917) in the phylogenetic tree constructed using the neighbor-joining method, while the DNA-B formed an independent cluster and was mostly related to the SLCCNV isolates BLDG (MW389928) and GDBL (MW389922). The nt identities of DNA-A were also calculated with SDT v1.2 by comparison with other begomovirus sequences from the initial BLASTn analysis (Muhire et al. 2014), showing that the virus shared 99.4% sequence identity with SLCCNV isolate GDHY (MW389917). According to the current demarcation threshold for begomoviruses recommended by the International Committee on Taxonomy of Viruses (91% nt identity) (Brown et al. 2015), this virus is a distinct strain of SLCCNV, designated SLCCNV-SDSG. To the best of our knowledge, this is the first report of a natural infection of SLCCNV on tomato in China. SLCCNV has caused serious problems in cucurbit production in some areas, so it is important to investigate if tomato plays a role in the disease biology by serving as a reservoir host.

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References

- Brown, J. K., et al. 2015. Arch. Virol. 160:1593.
- Kumar, S., et al. 2016. Mol. Biol. Evol. 33:1870.
- Muhire, B. M., et al. 2014. PLoS One 9:e108277.
- Wu, H.-J., et al. 2020. J. Integr. Agric. 19:570.

The author(s) declare no conflict of interest.

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