Disease Note

Diseases Caused by Viruses

First Reports of Beet Curly Top Virus, Citrus Yellow Vein-Associated Virus, and Hop Latent Viroid in Industrial Hemp (*Cannabis sativa*) in Washington State

Sridhar Jarugula,[1] Camille Wagstaff,[2] Arunabha Mitra,[1,3] David W. Crowder,[2] David R. Gang,[2] and Rayapati A. Naidu[1,†]

[1] Department of Plant Pathology, Washington State University, Prosser, WA 99350
[2] Molecular Plant Science Program, Washington State University, Pullman, WA 99164
[3] Microbiology and Plant Pathology, University of California, Riverside, CA 92521

Funding: This research was supported in part by Washington State University and the USDA-NIFA Hatch Project (WNP00006). Plant Dis. 107:2897, 2023; published online as https://doi.org/10.1094/PDIS-12-22-2981-PDN. Accepted for publication 14 February 2023.

In 2021 and 2022, virus-like symptoms were observed in several cultivars of industrial hemp (*Cannabis sativa*) in two fields in central Washington, U.S.A. Affected plants had a range of symptoms at different developmental stages, with young plants having severe stunting with shortened internodes and reduced flower mass. Young leaves of infected plants also showed light green to total yellowing and twirling with twisted margins. Infections of older plants caused fewer foliar symptoms that consisted of mosaic, mottling, and mild chlorosis on a few branches with tacoing of older leaves. To assess if symptomatic hemp plants were infected with beet curly top virus (BCTV) as reported earlier (Chiginsky et al. 2021; Giladi et al. 2020), symptomatic leaves were collected from 38 plants, and the extracted total nucleic acids were tested by PCR to amplify a 496-base pair (bp) fragment specific to BCTV coat protein (CP) using the primers BCTV2-F (5′-GTGGATCAATTTCCAG-ACAATTATC-3′) and BCTV2-R (5′-CCCATAAGAGCCATATCA-AACTTC-3′) (Strausbaugh et al. 2008). BCTV was found in 37 of the 38 plants. To further assess the virome of symptomatic hemp plants, total RNA was extracted from symptomatic leaves of four plants using Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO) and subjected to high-throughput sequencing on an Illumina NovaSeq platform in paired-end mode (University of Utah, Salt Lake City, UT). The raw reads (33 to 40 million per sample) were trimmed based on quality and ambiguity, and resulting paired-end reads of ≈142 bp in length were assembled de novo into a pool of contigs (CLC Genomics Workbench 21, Qiagen). Virus sequences were identified through BLASTn analysis in GenBank (https://www.ncbi.nlm.nih.gov/blast). One contig of 2,929 nucleotides (nt) obtained from one sample (accession no. OQ068391) showed 99.3% identity with the BCTV-Wor strain reported from sugar beet in Idaho (accession no. KX867055; Strausbaugh et al. 2017). Another contig of 1,715 nt from the second sample (accession no. OQ068392) shared 97.3% identity with the BCTV-CO strain (accession no. KX867022). Two contig sequences of 2,876 nt (accession no. OQ068388) and 1,399 nt (accession no. OQ068389) obtained from the third and fourth samples showed 97.2 and 98.3% identity, respectively, with citrus yellow vein-associated virus (CYVaV, accession no. MT893740.1) reported in industrial hemp from Colorado (Chiginsky et al. 2021). Contigs of a 256-nt sequence (accession no. OQ068390) obtained from the third and fourth samples also showed 99 to 100% identity with hop latent viroid (HLVd) sequences in GenBank (accessions OK143457 and X07397). These results indicated single infections of BCTV strains and coinfection of CYVaV and HLVd in individual plants. To confirm the agents, symptomatic leaves were collected from 28 randomly selected hemp plants and tested by PCR/RT-PCR using primers specific to BCTV (Strausbaugh et al. 2008), CYVaV (Kwon et al. 2021), and HLVd (Matoušek et al. 2001). Amplicons specific to BCTV (496 bp), CYVaV (658 bp), and HLVd (256 bp) were detected in 28, 25, and 2 samples, respectively. BCTV CP sequences obtained by Sanger sequencing from seven samples showed 100% sequence identity with BCTV-CO and BCTV-Wor strains in six and one samples, respectively. Similarly, sequences of CYVaV- and HLVd-specific amplicons showed 100% identity with corresponding sequences in GenBank. To the best of our knowledge, this is the first report of two strains of BCTV (BCTV-CO and BCTV-Wor), CYVaV, and HLVd infecting industrial hemp in Washington State.

References:

Chiginsky, J., et al. 2021. Front. Agron. 3:778433.
Giladi, Y., et al. 2020. Plant Dis. 104:999.
Kwon, S.-J., et al. 2021. Front. Microbiol. 12:683130.
Matoušek, J., et al. 2001. Virology 287:349.
Strausbaugh, C. A., et al. 2008. Phytopathology 98:1212.
Strausbaugh, C. A., et al. 2017. Plant Dis. 101:1373.

The author(s) declare no conflict of interest.

***e*-Xtra**

Keywords: field crops, pathogen detection, viruses and viroids

[†]Indicates the corresponding author.
R. A. Naidu; naidu.rayapati@wsu.edu