Diseases Caused by Viruses

First Report of Cotton Leafroll Dwarf Virus Infecting *Hibiscus syriacus* in South Korea

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Three cotton leafroll dwarf virus (CLRVDV; genus *Polerovirus*, family *Solemoviridae*) genotypes have recently been identified (Ramos-Sobrinho et al. 2021; Tabassum et al. 2021). This virus is widespread in the United States (Aboughanem-Sabanadzovic et al. 2019; Tabassum et al. 2020; Thiessen et al. 2020) and has also been reported to infect chickpea (*Cicer arietinum*) in Uzbekistan (Kumari et al. 2020). As well, CLRVDV was detected from 23 weed species (16 families), including *Hibiscus sabdariffa* (Hagan et al. 2019; Sedhain et al. 2021). From June to September 2019, virus-like symptoms, including mild leaf stunting, crinkling, and deformation, were observed in multiple plants ($n = 14$) in several provinces of South Korea. To characterize the associated viruses, pooled leaf tissues from all 14 samples were used for total RNA isolation, followed by paired-end high-throughput sequencing (HTS) on the Illumina NovaSeq 6000 platform (Macrogen, South Korea). A total of 614,424,952 trimmed and high-quality reads were assembled into 506,024 contigs using Trinity de novo transcriptome assembly. The resulting contigs were compared with viral sequences in the GenBank database using BLASTx analysis. Several viral contigs were identified, including cucumber mosaic virus, apple stem pitting virus, apple stem grooving virus, cherry virus A, and CLRVDV. The CLRVDV contig of 5,800 nucleotides (nt) with an average coverage of 307× shared 92.1% identity (query coverage: 99%) with the

CLRVDV isolate CN-S5 (KX588248). To confirm CLRVDV infection and to obtain its complete genome sequence, total RNA was extracted from each of the 14 samples and used for reverse transcription (RT)-PCR with six overlapping sets of primers designed from the HTS contig. The expected product sizes were obtained only for the *Hibiscus syriacus* L. (family: Malvaceae) sample showing foliar mild vein-clearing symptoms on the leaves. All RT-PCR products were cloned using the RBC TA Cloning vector (Taipai, Taiwan) and at least five positive clones per cloned DNA fragment were sequenced. The 5' and 3' termini sequences were determined as described previously (Zhao et al. 2016). The complete genome of CLRVDV isolate SK (OK073299) was determined to be 5,862 nt, and it shared 89 to 91% complete genome identity with 12 other CLRVDV isolates based on pairwise comparisons. Maximum likelihood phylogenetic analysis based on the complete genome and P3-CP aa sequences showed that CLRVDV-SK is more closely related to CN-S5. In the fall of 2021, additional *H. syriacus* samples ($n = 18$) with mild chlorosis, blistering, and crinkling symptoms were collected from two provinces of South Korea and tested by RT-PCR using the primers CLRVDV-SK-101-120 For and CLRVDV-SK-1021-1040 Rev targeting a region of the ORF0. Two of 18 samples (11.1%) tested positive for CLRVDV. The 16 negative samples only showed symptoms of mild yellowing. The RT-PCR products were cloned and sequenced. In pairwise comparisons, the obtained sequences (OM339522-23) were 95.85 and 96.06% identical to the corresponding sequences of CLRVDV isolate SK. This is the first report of CLRVDV occurrence in *H. syriacus* in South Korea to the best of our knowledge. Our findings will assist further studies on the epidemiology and sustainable management of diseases caused by CLRVDV.

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