Disease Notes

Diseases Caused by Viruses

First Report of the Cycas Necrotic Stunt Virus Infecting *Cnidium officinale* in South Korea

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This article does not contain any studies with human participants or animals performed by any of the authors.
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Funding: This research was supported by grants from the National Institute of Forest Science, Republic of Korea (Project no. FG0700-2018-02-2198), Rural Development Administration, Republic of Korea (Project no. PJ0137412018), and the KRIBB Research Initiative Program, funded by the Ministry of Science and ICT, Republic of Korea. Also, we are thankful for the office of Research and Innovation at the Mongolian National University of Education. Plant Dis. 104:3275, 2020; published online as https://doi.org/10.1094/PDIS-01-20-0092-PDN. Accepted for publication 26 June 2020.

The *Cnidium officinale* plant, which belongs to the Apiaceae family, has been widely used as a side dish and a traditional medicine to treat a variety of diseases in South Korea as well as other East Asian countries (Kumar et al. 2013). Recently, two distinct viruses, Cnidium vein yellowing virus-1 (CnVYV-1) and CnVYV-2 (family *Secoviridae*) (Yoo et al. 2015) and Cnidium virus X (family *Alphaflexiviridae*) (Honma et al. 2019) have been reported in *C. officinale* in South Korea and Japan, respectively. In May 2018, 43 individual *C. officinale* plants showing symptoms of leaf mosaic, vein clearing, and mild mottling were observed in the garden of the National Institute of Forest Science in Seoul, South Korea. Total RNA was extracted from a pool of 43 samples using the easy-spin total RNA extraction kit (iNtRON Biotechnology, South Korea), and ribosomal RNA was removed using Ribo-Zero rRNA Removal Kits (Plant Leaf) (Epicentre, Madison, WI) before complementary DNA library construction. A library was generated using an Illumina TruSeq RNA sample prep kit (Illumina, San Diego, CA) and sequencing performed by the Illumina HiSeq4000 system at Macrogen (Daejeon, Korea). In total, 97,718,248 high-quality reads were obtained after trimming, which were assembled into 75,798 contigs. The contigs were analyzed using the BLASTn and BLASTx searches to identify sequences in the high-throughput sequencing (HTS) data similar to the reference genome sequences in GenBank, which showed that the *C. officinale* plants were infected with several previously known plant viruses, including CnVYV-1, CnVYV-2, and cucumber mosaic virus. In addition, we identified 12 contigs with lengths of 238 to 4,180 bp that showed query coverage of 94 to 100% and 79.71 to 89.07% nucleotide (nt) identities with RNA1 and RNA2 segments of cycas necrotic stunt virus (CNSV) isolates in the GenBank database. To further confirm HTS data and detect the presence of CNSV, reverse transcription polymerase chain reaction (RT-PCR) was performed using virus-specific primer sets of CNSV RNA1 (F1, 5′-AATGCTTATGTTTTCGGAGAAAGAG-3′; R1, 5′- GTTGGTACATATGGGTCCAATC-3′) and CNSV RNA2 (F2, 5′-GTGCCGCACTATTAATTTTACTC-3′; R2, 5′-ATTTTTCCCTTTTGGTG TAGTGAA-3′). The expected size RT-PCR fragments, 1,334 and 442 bp, were amplified from RNA extracted from one *C. officinale* sample (no. 7) with vein-clearing symptom out of the 43 samples tested. The RT-PCR products were directly sequenced by Sanger sequencing, and the partial sequences of the amplicons corresponding to CNSV RNA-1 and RNA-2 have been deposited at the GenBank database under the accession numbers MN905738 and MN905739, respectively. BLASTn analysis demonstrated that RT-PCR products shared 86.80% (RNA1) and 84.20% (RNA2) nt identities with CNSV RNA1 (GenBank accession BK010916) and RNA2 (GenBank accession BK010917), respectively. CNSV, a member of the genus *Nepovirus* in the family *Secoviridae,* was first detected in cycas (*Cycas revoluta*) in Japan (Kusunoki et al. 1986). CNSV has been detected in other hosts and geographical areas (Jiang et al. 2019). To our knowledge, this is the first report of CNSV infecting *C. officinale* in South Korea. Further research is needed to understand the transmission, epidemiology, and pathological properties of the virus.

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The author(s) declare no conflict of interest.

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Keywords: *Cnidium officinale*, virus, CNSV, HTS

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