Diseases Caused by Viruses

First Report of a Secovirus Associated with Mountain Celery Chlorotic Spot Disease in Heilongjiang, China

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Funding: This study is financially supported by the Program for the Scientific Activities of Selected Returned Overseas Professionals in Heilongjiang Province (2018QD0002) and the China National Funds for Excellent Young Scientists (32022071). Plant Dis. 106:1311, 2022; published online as <https://doi.org/10.1094/PDIS-07-21-1418-PDN>. Accepted for publication 15 October 2021.

Mountain celery (*Heracleum moellendorffii* Hance), an edible perennial herb of Northeast Asia, is sporadically cultivated as a vegetable crop or for medicinal purposes in Northeast China and Korea (Son 2020). In July 2019, a small field of mountain celery showing chlorotic spots was found in Wangkui, Heilongjiang, China. A small-RNA (sRNA) library was constructed with equal amounts of leaf tissues of a diseased mountain celery and a tomato sample showing mottling symptoms from a nearby field using the TruSeq small RNA library preparation kit (Illumina, San Diego, CA). The library was sequenced by the HiSeq 4000 sequencer at Lianchuan Biotechnology Co., Ltd (Hangzhou, China). After trimming adaptor sequences and discarding low-quality reads by Cutadapt (Martin 2011), the remaining 6,949,946 reads of 17 to 27 nucleotides (nt) were de novo assembled as described (Che et al. 2020). The resulting 395 contigs were searched against the GenBank viral sequence database using the BLASTn and BLASTx algorithms. Twenty-three contigs showed high nt sequence similarities (89 to 100%) to the genomic sequence of tomato mosaic virus (ToMV). The deduced amino acid (aa) sequences of 30 contigs had 22 to 96% aa sequence identities to viruses in the family *Secoviridae*, for example, surrounding nonlegume associated secovirus (snLaSV) and lettuce secovirus 1 (LSV-1). No contig homologous to the genomic sequences of other plant viruses was identified. Total

RNAs were extracted from the mountain celery and tomato separately and reverse transcribed into cDNAs by random hexamer plus Oligo-dT₍₁₈₎ using the Super IV Reverse transcription (Invitrogen, Shanghai, China). Polymerase chain reactions (PCR) showed that the secovirus was derived from the mountain celery, whereas the tomato was infected by ToMV. The genome of this secovirus was determined by reverse transcription (RT)-PCR and rapid amplification of cDNA ends (RACE). Amplicons were cloned and Sanger sequenced with at least three independent clones per amplicon. Sequences were assembled by SeqMan Pro 7.1.0 in the Lasergene (DNASTAR, Madison, WI). The genome of this virus is composed of two RNAs of 6,616 and 5,356 nt (excluding the polyadenylic acid tails) (GenBank accession nos. MW143070 and MW143071, respectively). The 30 contigs assembled from sRNAs could be mapped to the genome. Pairwise sequence analyses showed that RNA1 and RNA2 and their encoded polypeptides shared the highest nt (82.7 and 82.2%) and aa (93.4 and 91.8%) sequence identities with the respective RNAs (GenBank accession nos. MN412739 and MN412740) and their encoded polypeptides of snLaSV (Gaafar et al. 2020). In the phylogenetic trees, this virus sequence clustered with snLaSV and LSV-1 in a separate branch neighboring viruses of the subgenus *Stramovirus* or *Satsumavirus* in the genus *Sadwavirus*. These results suggest that this virus is an isolate of the unclassified snLaSV and was referred to as snLaSV-CHN. RT-PCR with primers SecoR1-3700F and SecoR1-5100R confirmed the presence of snLaSV-CHN in other mountain celeries (11 of 23 tested) showing chlorotic spot symptoms but not in healthy ones from the same field. To the best of our knowledge, this is the first report of snLaSV infecting mountain celery in China, and a more orthodox name, mountain celery chlorotic spot virus (MCCSV), is tentatively proposed. Our findings provide better insight into the distribution and host range of this virus, and further surveys are necessary to determine its incidence and damage in mountain celery.

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The author(s) declare no conflict of interest.

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Keywords: Secovirus, mountain celery, deep sequencing, chlorotic spots

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