Diseases Caused by Viruses

First Report of Pear Chlorotic Leaf Spot-Associated Virus on Japanese and European Pears in Japan and Its Detection from an Eriophyid Mite

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In May 2018, three leaf samples were collected from Japanese pear trees (cv. Hosui) that exhibited typical chlorotic spot symptoms in a germplasm nursery in Tsukuba, Ibaraki. Total RNA was prepared using the rapid CTAB method (Gambino et al. 2008) for high-throughput sequencing, as described by Kubota et al. (2020). In brief, after removing ribosomal RNA, a library was constructed by fragmenting RNA, synthesizing cDNA, and polymerase chain reaction (PCR) amplification. Sequencing was performed using a NovaSeq 6000 sequencer (Illumina, San Diego, CA) with paired-end 150-nt reads. De novo assembly was performed using CLC Genomics Workbench 11.0 software (Qiagen, Hilden, Germany), with a minimum length of 500 bp. A total of 36,017 contigs derived from 33,565,182 reads were obtained and subjected to BLASTX search against the GenBank sequence database as of January 2019. Viruses commonly found in stone fruits (i.e., apple stem pitting virus, apple green crinkle-associated virus, apricot latent virus [foveaviruses], and apple stem grooving virus [a

capilovirus]) were detected. In addition, five contigs with amino acid sequence homologies to P1 to P4 of known emaraviruses and the P7 of High Plains wheat mosaic virus (Tatineni et al. 2014) were detected and designated as PEV-Jp. The complete nucleotide (nt) sequences of the five segments of PEV-Jp were determined by Sanger sequencing of cloned reverse transcription (RT)-PCR amplification products using the primers shown in the supplementary materials; the 5'- and 3'-terminal sequences were RACE verified (Takara Bio, Shiga, Japan). In pairwise comparisons, the complete RNA1 to RNA5 of PEV-Jp (LC554756 to 760) shared 90.7 to 98.7% nt identities with those of PCLSav-CG1 (MK602177 to 181), indicating that PEV-Jp is an isolate of pear chlorotic leaf spot-associated virus (PCLSav). Using newly designed segment-specific primers, 12 symptomatic Japanese pear trees (cv. Kosui) sampled in 2020 from the same nursery tested positive for PCLSav by RT-PCR, whereas 12 symptomless trees were negative for the virus. Similar chlorotic spots, sometimes accompanied by necrotic spots, were observed on European pear (*Pyrus communis*) cultivar Le Lectier in Niigata in 2019; PCLSav was detected by RT-PCR in leaf tissue samples from symptomatic trees ($n = 3/3$) but not in symptomless trees ($n = 0/2$). No vector for PCLSav has been identified (Liu et al. 2020), but acaricide sprays in the early spring are effective for preventing occurrence of chlorotic spots in pear orchards (Nakai et al. 2018). Because infestations of *Eriophyes chibaensis* Kadono, an eriophyid mite often observed on the Japanese pear (Kadono 1981), have been associated with occurrences of the chlorotic spots (Shimizu et al. 2019), samples of *E. chibaensis* individuals were collected from PCLSav-positive Japanese pear cultivars Akizuki and Kosui and *P. communis* cultivar Le Lectier for total nucleic acid isolations via phenol–chloroform extraction, followed by quantitative RT-PCR. The expected RNA1- and RNA5-specific 150-bp products were detected from mite samples collected from Akizuki ($n = 6/12$), Kosui ($n = 13/18$), and Le Lectier ($n = 6/8$). The results indicate that *E. chibaensis* can ingest PCLSav and may be a potential vector for the virus, although additional experiments are needed to demonstrate its vector competency. To our knowledge, this is the first report of PCLSav in Japan and the first report of its detection in *E. chibaensis*.

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