First Report of *Camellia japonica* Associated Emaravirus 1 Associated with *Camellia* Leaf Ringspot and Flower Color-Breaking Disease in China

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Camellia (*Camellia sinensis* L.), one of the most common garden plants, is an evergreen shrub native to China, South Korea, and Japan. In April 2017, we conducted a survey of viral disease of camellia in Jiangshan of Zhejiang, China, and found four plants displaying ringspot symptoms on leaves and color-breaking on flower petals. To determine the associated agent of the disease, a small RNA sequencing library was constructed from total RNA extracted from symptomatic leaf tissues of one plant (Wang et al. 2014) and sequenced using the Illumina HiSeq platform (Lianchuan Bio, Hangzhou, Zhejiang, China). After trimming adapter sequences and removal of low-quality reads, 6,045,676 reads of 17 to 24 nucleotides (nt) in length remained. The reads were assembled into 779 contigs using Velvet 1.1 and Oases 0.2.07 with *k*-mers of 15 and 17 (Schulz et al. 2012). The contigs were searched against the GenBank viral nonredundant coding-sequence database using the BLASTx algorithm (accessed 23 May 2017). Fifteen contigs (length ranging from 101 to 841 nt) had significant identities to genomic sequences of viruses in the genus *Emaravirus* of the *Fimoviridae*, including raspberry leaf blotch virus, High Plains wheat mosaic virus, and fig mosaic virus. None of these contigs were homologous to genome sequences of any other plant viruses. Amplification of this virus using cDNAs transcribed by the degenerate primer PDAP213 that anneals to the conserved 13-nt terminal sequence of emaraviruses (Mielke-Ehret et al. 2012) failed. An rRNA-depleted RNA library was prepared using total

RNA from the same plant and sequenced by an Illumina HiSeq4000 system (Lianchuan Bio). Resultant 103,915,044 paired-end reads (each 50 nt) were edited using Cutadapter and assembled using Bridger assembler (Chang et al. 2015; Martin 2011). A total of 154,144 contigs were obtained and searched against a custom protein database of all RNA viruses (retrieved at 6 April 2019) using NCBI BLAST toolkit (version 2.8.1). Four near full-length and three partial contigs (612 to 7,071 nt) that shared highest homologies to the genome of *Camellia japonica* associated emaravirus 1 (CjEV1) were identified. No contig that is homologous to the genomic sequences of other plant-infecting viruses was identified. Primers were designed to amplify the genome of this virus by reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends. Amplicons were Sanger sequenced and assembled by Seqman Pro 7.1.0 in Lasergene package (DNASTAR, Madison, WI). Pairwise sequence analyses showed that the seven RNAs shared high nt identities (91 to 97%) with RNA1, RNA2, RNA3, RNA4, RNA7, RNA8, and RNA9 of CjEV1 (MN385573 to MN385576 and MN557026 to MN557028). Bioinformatic and RT-PCR analyses using CjEV1-specific primers (designed from MN557024 and MN557025) confirmed no corresponding RNA5 and RNA6 in the sample. All genomic sequences of this CjEV1 isolate (named CjEV1 ZJ-01) have been deposited in GenBank (accession nos. MT263292 to MT263298). RT-PCR using RNA1-derived primers confirmed the presence of CjEV1 ZJ-01 in all four symptomatic plants and its absence in six asymptomatic plants, showing an association of CjEV1 with the observed symptoms of camellia. To the best of our knowledge, this is the first report of CjEV1 in China. The occurrence of CjEV1 in camellia and its associated disease may severely threaten camellia production.

References:

- Chang, Z., et al. 2015. *Genome Biol.* 16:30.
- Martin, M. 2011. *EMBnet. J.* 17:10.
- Mielke-Ehret, N., et al. 2012. *Viruses* 4:1515.
- Schulz, M. H., et al. 2012. *Bioinformatics* 28:1086.
- Wang, Y., et al. 2014. *Virus Res.* 188:103.

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