Diseases Caused by Viruses

Strawberry, a New Natural Host of Brassica Yellows Virus in China

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Funding: This work was supported by the National Key Research and Development Program of China (2019YFD1001800) and Key Inter-governmental Projects of the National Key Research and Development Program (2017YFE0010900). Plant Dis. 106:1079, 2022; published online as <https://doi.org/10.1094/PDIS-08-21-1617-PDN>. Accepted for publication 17 September 2021.

Brassica yellows virus (BrYV; genus *Polerovirus*, family *Solemoviridae*) has an icosahedral spherical virion with a positive-sense single-stranded RNA genome, and it is distinguished from turnip yellows virus (TuYV) based on differences in ORF0 and ORF5 (Xiang et al. 2011). To investigate the occurrence and distribution of viruses infecting strawberry (*Fragaria ananassa*) in the main production areas in China, a survey of nine greenhouses (667 m² each) was conducted in the cities of Yantai and Beijing, China, in August 2020. About 1% of strawberry plants in each greenhouse showed virus-like symptoms of chlorotic spots; 89 symptomatic leaf samples were randomly collected for virus testing. Total RNA was extracted from a pool of eight samples of four different cultivars (Hokowase, 2; Mibao, 2; Sagahonoka, 2; Monterey, 2) from Yantai using an RNAprep Pure Plant Plus Kit (TianGen, China). A cDNA library was constructed by NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB, U.S.A.) after ribosomal RNA depletion using an Epicentre Ribo-Zero rRNA Removal Kit (Epicentre, U.S.A.). High-throughput sequencing (HTS) was done on an Illumina HiSeq 4000, generating 70,931,850 high-quality 150-bp paired-end reads. Clean reads were de novo assembled by Trinity (version 2.2.0), and the resulting contigs were screened by BLASTn and BLASTx against GenBank database as described previously (Grabherr et al. 2011). A total of 1,432,164 high-

quality reads unmapped to the strawberry genome were obtained and assembled into 93 contigs (ranging from 33 to 8,031 nt). Seven of these contigs (277 to 1,254 nt) shared 98.2 to 100% nt identities with BrYV-A (accession no. HQ388348) and covered 89.5% of the genome of BrYV-A. Subsequent analyses indicated the presence of strawberry pallidosis-associated virus and strawberry mottle virus in the analyzed sample; both have been reported in strawberry in China (Fan et al. 2021; Shi et al. 2018). To confirm BrYV infection, total RNA was isolated from the eight samples used for HTS, and reverse transcription polymerase chain reaction (RT-PCR) was conducted with two pairs of specific primers (CP and rtp) designed based on the assembled contigs. PCR products with expected sizes (587 and 609 bp) were observed in one sample (cv. Mibao). BLASTn analysis indicated that the amplicons (accession nos. MW548437 and MW548438) shared 98.6 and 99.3% nt identity with BrYV-A, respectively. To obtain the complete sequence of the putative BrYV isolate, the gaps were bridged, and the terminal sequences were determined using 5' and 3' RACE kits (Clontech, China) based on the assembled contigs. The complete genome sequence of the putative BrYV isolate has a length of 5,666 nt (accession no. MZ666129) and shares more than 94.3% nt identities with other BrYV isolates. Phylogenetic analysis indicated that the isolate grouped closely with BrYV and further from TuYV. In addition, 11 samples (cv. Benihoppe) of the remaining 81 symptomatic strawberry samples tested positive for BrYV by RT-PCR with the two pairs of primers mentioned above. The sequences (accession nos. MZ407232 and MZ407233) revealed 99.5 and 99.3% nt identities with MW548437 and MW548438. To the best of our knowledge, this is the first report of natural infection of BrYV in strawberry plants. Our findings expand the host range of BrYV, but disease association is difficult to establish due to the presence of mixed infection and nonfulfillment of Koch's postulates.

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The author(s) declare no conflict of interest.

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Keywords: Brassica yellows virus, strawberry, new natural host, first report

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