The Occurrence of Strawberry Virus 1 Infecting Strawberry in Shandong Province, China

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Strawberry (*Fragaria × ananassa* Duch.) is one of the most important horticultural plants worldwide with high economic and nutritional value. Strawberry associated virus 1 (SaV1) is a putative Cytorhabdovirus isolated from strawberry in Fujian Province, China (Ding et al. 2019). Strawberry virus 1 (StrV-1) is another putative Cytorhabdovirus characterized from *F. ananassa* (cv. Čačanská raná) and *F. vesca* (cv. Rujana) in Czech Republic (Fránová et al. 2019). The complete genomes of isolates of SaV1 and StrV-1 share 79 to 98% nucleotide (nt) identities. In August 2020, foliar chlorotic spots or streaks were observed in four strawberry cultivars (cvs. Honeoye, Mibao, 8128, and All Star) in Yantai, Shandong Province, China. To identify the associated viruses, symptomatic leaves from two plants of each cultivar (eight samples) were pooled for high-throughput sequencing (HTS). Total RNA was extracted from the composite sample and used for constructing a cDNA library after ribosomal RNA (rRNA) depletion. Sequencing was carried out on an Illumina HiSeq 4000 (Novogene, China). Raw reads were filtered, trimmed, and de novo assembled as described previously (Grabherr et al. 2013; Zhou et al. 2020). The resulting contigs were screened by BLASTn and BLASTx against the GenBank database. Subsequent analyses indicated the presence of strawberry vein banding virus, strawberry pallidosis associated virus, and strawberry mottle virus in the analyzed sample, which had been reported previously in strawberry (Bhagwat et al. 2016;

Martin and Tzanetakis 2013; Shi et al. 2018). Besides, five contigs ranging from 266 to 6,057 nucleotides (nt) were obtained. They shared 87 to 91% nt sequence identity with StrV-1 isolate B (GenBank accession no. MK211271). To confirm StrV-1 infection in the strawberry plants, total RNA was isolated from eight samples (used for HTS) using an RNeasy Pure Plant Plus Kit (Qiagen, China). Reverse transcription polymerase chain reaction (RT-PCR) was conducted with two pairs of specific primers StrVp1 (forward, 5'-CATTACTGAAGCATTCCGTG-3'; reverse, 5'-AGATATCACGCACAGTGAC-3'), and StrVp2 (forward, 5'-TTGCGCGAAGCGGATGTCCG-3'; reverse, 5'-GGTGCCAGAGCGTTGGATG-3'), targeting nt positions 70 to 1,231 and 7,825 to 9,348 of StrV-1 isolate B, respectively. Fragments with expected sizes of 1,162 and 1,524 bp were amplified from two samples of cultivar All Star. The amplicons were cloned, sequenced, and deposited into GenBank database under accession numbers MW419123, MW419124, MW645247, and MW645248. Both protein encoding sequences shared 91 to 92% and 80 to 84% nt identities with the corresponding sequences of StrV-1 isolate B and SaV1, respectively, indicating that the isolates from this study are genetic variants of StrV-1 and distantly related to SaV1. Crude sap was prepared by homogenizing the StrV-1-infected strawberry leaf tissues in 0.02 mol/liter sodium phosphate buffer with 0.45% (w/v) sodium diethyldithiocarbamate trihydrate and then gently rubbed onto five healthy *Nicotiana benthamiana* plants. Neither the inoculated leaves nor the systemically infected leaves showed obvious symptoms 7 days postinoculation. However, StrV-1 was detected by RT-PCR in all five *N. benthamiana* plants using RNA templates prepared from non-inoculated leaves. In addition, a survey of strawberry greenhouses was conducted in August 2020, and approximately 10% of plants in one 667-m² greenhouse in Yantai had StrV-1-like symptoms. To the best of our knowledge, this is the first report of the occurrence of StrV-1 infecting strawberry in Shandong Province, China. Our findings expand the geographic range of StrV-1 and indicate it could be a potential virus threat to strawberry production in China.

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