First Report of Alfalfa Mosaic Virus on Soybean in Heilongjiang, China

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Funding: Funding was provided by Department of Education of Heilongjiang Province (2018QD0002) and Natural Science Foundation of Heilongjiang Province (LH2019C027 and ZD20018002). Plant Dis. 104: 3085, 2020; published online as <https://doi.org/10.1094/PDIS-04-20-0850-PDN>. Accepted for publication 12 June 2020.

Alfalfa mosaic virus (AMV), the only species of genus *Alfavirus*, infects a wide range of plants (Hill and Whitham 2014). In July 2018, soybean (*Glycine max*) plants with yellow mottling patterns or vein yellowing were found at the university trial field in Harbin, Heilongjiang, China. To determine the causal agent of this disease, two small RNA sequencing libraries (three leaves per library) were constructed using TruSeq Small RNA Library Preparation Kits (Illumina, Shanghai, China) and sequenced with an Illumina HiSeq 2000 platform in LC-Bio Technologies (Hangzhou, China). After adapter trimming and quality control, the remaining 22,840,947 and 40,344,895 reads of 17 to 27 nucleotides (nt) in length were assembled separately using Velvet 1.1 and Oases 0.2.09 with k-mers of 17 (Schulz et al. 2012). The contigs were searched against the NCBI nucleotide and amino acid databases using BLASTn and BLASTx programs (Johnson et al. 2008). A total of 41 contigs of 133 to 2,088 nt showed high homologies to the genomic sequences of AMV. None of these contigs were homologous to the genomic sequences of other plant viruses. Reverse transcription polymerase chain reaction (RT-PCR) using primers AMVcp-F (5'-ATGAGTCTTCACAAAAGAAAGCTGGT-3') and AMVcp-R (5'-ATGACGATCAAGATCGTCAGCTTCGTC-3'), which target coat protein gene, confirmed AMV in all six samples. Primers were designed to

amplify the genome of this AMV isolate (named AMV-soybean) from one of the six plants by RT-PCR and rapid amplification of cDNA ends. Amplicons were cloned in pEASY-Blunt vector (TransGen Biotech, Beijing, China) and Sanger sequenced with at least four clones per amplicon. RNA1, RNA2, and RNA3 of AMV-soybean are 3,643, 2,594, and 2,008 nt, respectively (GenBank accession nos. MT362607 to MT362609), which share the highest nt sequence identities with RNA1 and RNA2 of a Canadian AMV isolate (Ca175-1) from potato (98.4%; MF990284) and RNA3 of an Argentinian AMV isolate (Manfredi) from Medicago (98.21%; KC881010), respectively. A total of 5,180,064 AMV-derived siRNAs were found and were mapped to all three genomic fragments at both orientations. AMV-soybean was mechanically inoculated to 22 soybean cultivars including Kennong-28, Dongnong-253, Mengdou-28, Dongnong-50, Hefeng-25, Huajiang-1, Heinong-52, Shengdou-58, Heidou, Huangposhanzibai, Dongnong-48, Bayuezha, Heihe-43, Wanhuangdou, Wuyuezha, Jinyuan-1, Suinong-14, Xiaolidou, Baimaodou, Suinong-11, Beidou-40, and Wujiang-1 with at least five plants per cultivar. Wuyuezha and Baimaodou remained symptomless, whereas others developed symptoms such as leaf yellowing, yellow mottling, and/or leaf distortion symptoms at 14 days postinoculation. Total RNA was extracted from noninoculated leaves using the FastPure Plant Total RNA Isolation Kit (Vazyme, Nanjing, China) and analyzed by RT-PCR using primers AMVcp-F and AMVcp-R. Results suggest that Wuyuezha and Baimaodou may be tolerant, whereas the other 20 soybean cultivars are susceptible to AMV-soybean. Subsequent RT-PCR screening confirmed the presence of AMV on five soybean and six common bean samples with yellowing symptoms on two other fields in Harbin. To the best of our knowledge, this is the first report of AMV on soybean in China. AMV on soybean can be transmitted by seeds, by mechanical transmission, and by more than 10 aphid species (He et al. 2010). Special attention should be paid to the damage that it may cause to soybean in northern China.

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The author(s) declare no conflict of interest.

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Keywords: Alfalfa mosaic virus, field crops, soybean, epidemiology, infectivity, pathogen detection

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