AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

First Report of Alphapartitiviruses Infecting Alfalfa (*Medicago sativa* L.) in the United States

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ABSTRACT Evidence is presented demonstrating that alfalfa cultivars in the United States could be widely infected with cryptic virus of the genus *Alphapartitivirus*. The nucleotide sequences of several U.S. isolates were obtained. The biological significance or negative effects of the virus on alfalfa are unknown and require further investigation.

The partitiviruses are associated with persistent infections of fungal, protozoan, and plant hosts (1). Plant partitiviruses are transmitted by ovule and by pollen to the seed embryo (2) and may be associated with mutualistic effects (3, 4). Two novel species of the family *Partitiviridae*, *Medicago sativa alphapartitivirus 1* (MsAPV1) and *Medicago sativa deltapartitivirus 1* (MsDPV1), belonging to the genera *Alphapartitivirus* and *Deltapartitivirus*, respectively, were recently reported from South Korea (5). They were computationally deduced from alfalfa transcriptomic data that were published by a group of authors from China (6). In this work, while examining transcriptomic data derived from two U.S. alfalfa (*Medicago sativa* L.) cultivars, Maverick (7) and ZG 9830 (8), we found that all plants used in the experiment ($n = 36$) contained reads that shared high-percentage identity with MsAPV1 (GenBank accession numbers MF443256 and MF443257) (5). Complete viral genomes were obtained from both cultivars by assembly of paired-end reads (2 × 150 bp), generated using an Illumina HiSeq 2500 sequencing platform. The paired-end reads were mapped to the MsAPV1 genome using the aligner Bowtie 2 (9) and assembled using SPAdes 3.11.0 (10) and the Velvet assembler (11). A total of 9,661,606 reads mapped to the MsAPV1 in both cultivars, including 4,329,430 reads in cultivar Maverick and 5,332,176 reads in cultivar ZG 9830. A blast search of the resulting contigs against the NCBI plant virus database was performed using blastn and then manually checked against the nr database. Additionally, 5′ and 3′ rapid amplification of cDNA ends (RACE) was performed for cultivar Maverick using a SMARTer RACE kit (TaKaRa Bio USA, Inc., Madison, WI). The assembled viral genomes were typical for the genus *Alphapartitivirus* and contained two monocistronic segments, double-stranded RNA1 (dsRNA1), encoding RNA-dependent RNA polymerase (RdRP), and dsRNA2, encoding viral coat protein (CP). The sizes of the RdRP segments in virus isolates from cultivars Maverick and ZG 9830 [excluding a poly(A) tail] were 1,865 nucleotides (nt) and 1,868 nt, respectively, while the lengths of the CP segments were 1,774 nt and 1,733 nt (for cultivars Maverick and ZG 9830, respectively). The dsRNA1 and dsRNA2 of both isolates were nearly identical to the respective genome segments of MsAPV1 (>99.6% identity at the nucleotide level and 99.6% to 100% identity at the amino acid level; MegAlign, Clustal W algorithm [DNASTAR, Inc.]). Comparison of the two U.S. isolates revealed 98.1% nucleotide identity for the RdRP and 99.6% for the CP region. At the amino acid level, their RdRPs and CPs were nearly identical (99.8% and 99.6%, respectively).

According to these findings, the U.S. isolates of the virus represent closely related variants of MsAPV1, which may not be a coincidence, because the original transcriptomic study (6) also employed cultivar Maverick that was introduced to China from the

Received 14 September 2018 **Accepted** 7 November 2018 **Published** 29 November 2018

Citation Nemchinov LG, Lee MN, Shao J. 2018. First report of alphapartitiviruses infecting alfalfa (*Medicago sativa* L.) in the United States. Microbiol Resour Announc 7:e01266-18. https://doi.org/10.1128/MRA.01266-18.

Editor Jelle Matthijnssens, KU Leuven

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United States. This is the first evidence that alfalfa cultivars in the United States could be widely infected with seed-transmitted cryptic virus of the genus *Alphapartitivirus*. The biological significance and any negative effects of the virus on alfalfa are currently unknown and require further investigation.

Data availability. The complete genomic sequences of the virus have been deposited in GenBank under the accession numbers MH846124 and MH846123 (cultivar Maverick dsRNA1 and -2, respectively) and MH846126 and MH846125 (cultivar ZG 9830 dsRNA1 and -2, respectively). Raw data were deposited in the NCBI Sequence Read Archive (SRA) under accession number SRR8115067.

ACKNOWLEDGMENT

This research was supported by the United States Department of Agriculture, Agricultural Research Service, CRIS projects 8042-21000-268-00D and 5062-12210-002D.

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