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GENOME SEQUENCES

Identification of the Coding-Complete Genome of *Cycas Necrotic Stunt Virus* in Transcriptomic Data Sets of Alfalfa (*Medicago sativa*)

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ABSTRACT We present evidence here that alfalfa (*Medicago sativa* L.) can be a natural host species for a new strain of *Cycas necrotic stunt virus* (CNSV), for which the name CNSV-A (alfalfa) is proposed. Prior to this report, the virus has not been identified in alfalfa.

Cycas necrotic stunt virus (CNSV), genus *Nepovirus*, family *Secoviridae*, was first detected in the gymnosperm *Cycas revoluta* in Japan (1, 2). The severely affected plants deteriorated and subsequently died (1). CNSV isolates have also been detected in other species (1–7). Prior to this report, the virus had not been identified in alfalfa.

Publicly available transcriptomic data sets have become a valuable tool for discovering new pathogens, particularly viruses (8–11). In this study, CNSV sequences were identified in the accessions SRR7751381, SRR7751384, and SRR7751386 of NCBI BioProject PRJNA487676 (12). A total of 14,402 CNSV sequences were found in 157,755,564 raw Illumina reads (0.009%) generated from alfalfa seedlings using TRIzol reagent for RNA extraction, the NEBNext1 Ultra RNA kit for library preparation, and the Illumina HiSeq platform for sequencing, as described by the submitters (12). Sequencing reads from accession number SRR7751381, which were not mapped to the reference genomes of *Medicago sativa* (Cultivated Alfalfa at the Diploid Level [CADL] v.0.95P) and *Medicago truncatula*, were aligned to the NCBI viral genome database. Alignments were performed using BBMap (v.37.66), SeqMan NGen (v.15.2.0), and Bowtie 2 (v.2.3.4) with sensitive settings. Reads mapped to the viral sequences were collected and reassembled *de novo* using SPAdes (v.3.12.0) with *k*-mers 21 to 81. The fold coverage was 57.47× and 80.37× for RNA1 and RNA2 of the assembled viral genome, respectively.

The assembled virus had a bipartite (RNA1 and RNA2 segments) single-stranded positive-sense RNA genome that appeared to be essentially complete. RNA1 was 7,632 nucleotides (nt) long and encoded a single polyprotein (polyprotein 1 [P1]) with conserved motifs for RNA helicase (Pfam identifier [ID] PF00910) and RNA-dependent RNA polymerase (RdRP; Pfam ID PF00680), as predicted by Pfam 32.0 (13). Presence of the RdRp motif was additionally confirmed by a BLASTX search against a database of RdRp sequences, including those experimentally confirmed in our laboratory (11). Other putative P1 domains were characteristic for the genus *Nepovirus* (14). RNA2 was 4,717 nt long and translated into polyprotein 2 (P2), containing predicted domains for nepovirus coat protein (CP; Pfam IDs PF03689, PF03391, and PF03688). The 5′ and 3′ untranslated regions (UTRs) in RNA1 and RNA2 were nearly identical. The 3′ UTRs of both RNAs incorporated an 80-nt-long fragment with no apparent homology to known CNSV strains and a high percent identity to the 3′ UTRs of other secoviruses, suggesting possible recombination events. The P1 consisted of 2,338 amino acids (aa) and showed 94.3% aa identity to the P1 of the reference CNSV sequence (GenBank accession number NP620619), while P2 was 1,356 aa long and had 91.3% aa identity to the P2 of the CNSV (accession number NP620620). The predicted CP region of P2 shared ~97.8%

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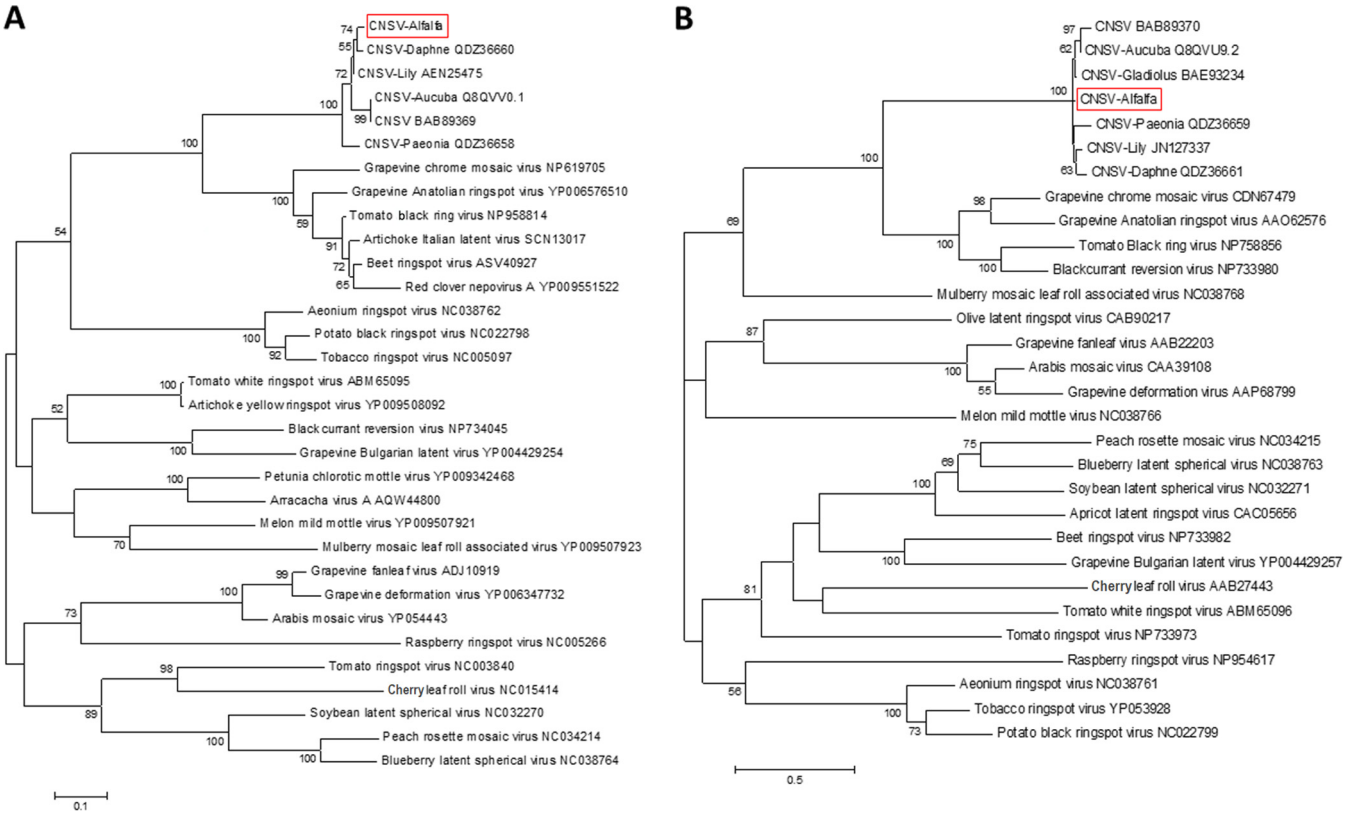

FIG 1 Phylogenetic trees generated based on the alignment of the predicted amino acid sequences of the RdRP (A) and CP (B) of CNSV-A and other nepoviruses. The trees were constructed using the neighbor-joining algorithm of Molecular Evolutionary Genetics Analysis version 7 (MEGA7) (15) with 1,000 bootstrap replicates.

identity with the CP of CNSV (accession number NP620620), and the predicted RdRP region was 94.1% identical to the RdRP of the CNSV (accession number NP620619). Based on these observations, the virus represents a new strain of CNSV that is adapted to alfalfa, for which we propose the name CNSV-A (alfalfa). Phylogenetic analyses based on the predicted RdRP and CP sequences of CNSV-A and other nepoviruses grouped the alfalfa strain together with CNSV isolated from other species, indicating their origin from the same ancestral virus (Fig. 1). Further research is required to confirm the *in silico* identification of the virus and to determine its symptomatology, geographic distribution, and economic importance to the alfalfa industry.

Data availability. Raw data associated with this announcement were retrieved from the publicly available NCBI BioProject number PRJNA487676, accession number SRR7751381. Nucleotide sequence data of the CNSV-A strain reported here were deposited to the Third Party Annotation section of the DDBJ/ENA/GenBank databases under the TPA accession numbers BK010916 and BK010917.

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