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Complete Genome Sequence of a *Tomato Brown Rugose Fruit Virus* Isolated in the United States

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ABSTRACT The complete genome sequence of a U.S. isolate of a *Tomato brown rugose fruit virus* (ToBRFV) (CA18-01) was obtained through Illumina and MinION sequencing. The U.S. ToBRFV isolate shared a high nucleic acid sequence identity (>99%) with known ToBRFV isolates. Phylogenetic analysis revealed a tight cluster for ToBRFV isolates throughout the world, suggesting a short evolutionary history.

Tomato (*Solanum lycopersicum* L.), an important vegetable crop throughout the world, is susceptible to many viral diseases (1). *Tomato brown rugose fruit virus* (ToBRFV), an emerging tobamovirus, was capable of breaking the *Tm-2²* gene in tomato that is resistant to other tobamoviruses (2, 3). ToBRFV, in genus *Tobamovirus* and family *Virgaviridae*, was first identified in the Middle East (2, 4). ToBRFV has since been reported in a number of countries in Asia (5, 6), Europe (7–11), and North America (12–14) and is likely to spread to other countries. To curb virus spread, the U.S. Department of Agriculture issued a federal order in 2019 for ToBRFV (15). Here, we report the complete genome sequence of a U.S. isolate of ToBRFV using Illumina and MinION sequencing technologies. The U.S. isolate of ToBRFV, designated CA18-01, was originally collected in the fall of 2018 in southern California (14). For Illumina sequencing, libraries were prepared using the TruSeq stranded total RNA (tomato leaves) with a Ribo-Zero plant kit and sequenced in a MiSeq instrument (150 cycles, paired-end reads). A total of 1,982,141 read pairs were generated. Adapter trimming and quality filtering of reads were performed with Trimmomatic v. 0.39 (16). Clean reads were *de novo* assembled using the SPAdes v. 3.10.0 assembler (17) with default parameters. The MinION sequencing library was prepared using the SQK-PCS108 cDNA PCR kit (Oxford Nanopore Technologies) and sequenced in a MinION Mk1B device (MIN-101B). A total of 3,001,250 reads were generated. Adapters were removed and chimeric reads were discarded using Porechop v. 0.2.3 (https://github.com/rrwick/Porechop). Clean reads were *de novo* assembled using Canu v. 1.8 (18) with default Nanopore assembly parameters with readSamplingCoverage = 1000 based on a genome size of 10 kb. The resulting contigs were searched against an in-house database generated from GenBank virus and viroid sequences through BLASTn. The depth of coverage was estimated for all positively identified viruses by mapping reads to contigs using BBMap (19). A single contig with high-depth coverage (mean depth, 7,576) was mapped to the reference ToBRFV-Jordan (GenBank accession no. KT383474). To determine the exact 5′-terminal sequence of ToBRFV CA18-01, rapid amplification of cDNA ends (RACE) was used with a First Choice RLM-RACE kit (Invitrogen), and PCR products were sequenced using Sanger sequencing.

The completed genome of ToBRFV CA18-01 (GenBank accession no. MT002973) had 6,389 nucleotides (GC content, 41.51%) comprising four open reading frames (ORFs), with ORF1 encoding a 126-kDa replicase protein, ORF 2 encoding an RdRP protein of 183 kDa, ORF3 encoding a movement protein of 30 kDa, and ORF4 encoding a coat
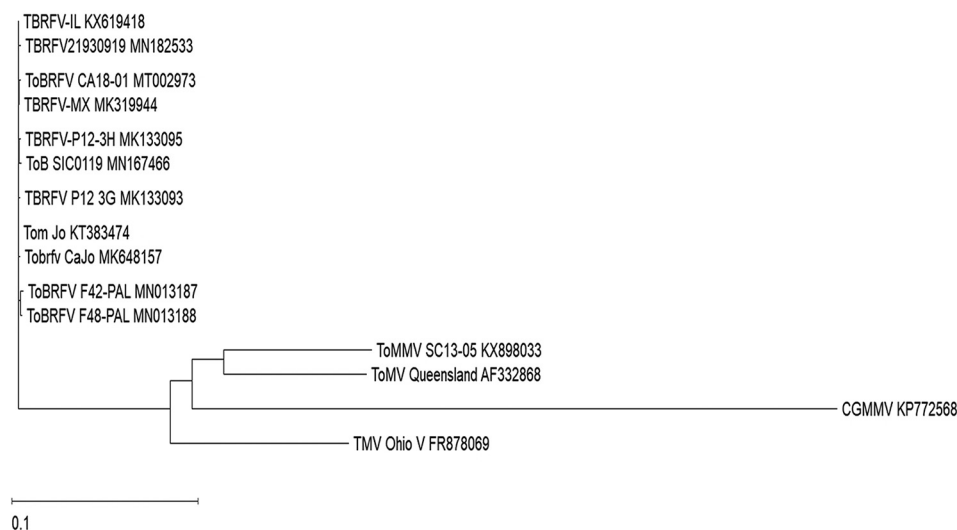
FIG 1 Phylogenetic relationship of the complete genome sequence of *Tomato brown rugose fruit virus* (ToBRFV) isolate CA18-01 (GenBank accession no. MT002973) with those of 10 other ToBRFV isolates and four related tobamoviruses, including *Tomato mosaic virus* (ToMV), *Tomato mottle mosaic virus* (ToMMV), *Tobacco mosaic virus* (TMV), and *Cucumber green mottle mosaic virus* (CGMMV). A multiple sequence alignment was created using ClustalOmega in the MegAlign Pro program of Lasergene 16 (DNASTar, USA). The phylogenetic tree was generated using BIONJ with default parameters.

protein of 17.5 kDa. The 5′ and 3′ untranslated regions spanned 72 and 199 nucleotides, respectively. A multiple sequence alignment (Lasergene 16) using 10 ToBRFV isolates revealed 99.6 to 99.9% nucleotide sequence identities among each other and less than 82.2% identities to other tobamoviruses. Such high sequence identities among ToBRFV isolates suggest a short evolutionary history of this novel virus. Phylogenetic analysis showed a tight cluster of ToBRFV isolates separating from other tobamoviruses (Fig. 1). The complete genome sequence of the ToBRFV isolate from the United States will enhance our understanding of the genetic diversity of this emerging virus as it spreads further across the world.

Data availability. The high-throughput sequencing data sets were submitted to the SRA database with the accession no. SRR11794481 (Illumina reads) and SRR11794480 (MinION reads) and the BioProject no. PRJNA632574. The genome sequence of ToBRFV CA18-01 was deposited in GenBank with accession no. MT002973.

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