



Genome Sequences of *Beet curly top Iran virus*, *Oat dwarf virus*, *Turnip curly top virus*, and *Wheat dwarf virus* Identified in Leafhoppers

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ABSTRACT Implementation of a vector-enabled metagenomics approach resulted in the identification of various geminiviruses. We identified the genome sequences of *Beet curly top Iran virus*, *Turnip curly top viruses*, *Oat dwarf viruses*, the first from Iran, and *Wheat dwarf virus* from leafhoppers feeding on beet, parsley, pumpkin, and turnip plants.

New molecular-based methods have facilitated the rapid identification of various plant and animal viruses, especially small single-stranded DNA viruses. Furthermore, vector-enabled metagenomic (VEM) approaches using these methods have proven very useful for the identification of plant virus circulation in various agricultural regions (1–4). Here, we used a VEM approach to identify geminiviruses (5) circulating in farms in Zafar-Abad, Iran, in 2013 by sampling of plant leafhoppers from different genera feeding on beet (*Beta vulgaris*; $n = 1$), parsley (*Petroselinum crispum*; $n = 1$), pumpkin (*Cucurbita pepo*; $n = 1$), and turnip (*Brassica rapa*; $n = 3$) plants. The insects were homogenized in SM buffer and processed as described by Dayaram et al. (6–9). The viral DNA was enriched for circular molecules using rolling-circle amplification, and this was sequenced on an Illumina sequencing platform at Novogene (Hong Kong). The paired-end reads were *de novo* assembled using ABySS 1.9 (10), and viral sequences were identified using BLASTx against a local viral protein sequence database. Contigs with BLASTx hits to geminivirus sequences (5, 11) were identified. Abutting primers were designed and used for PCR amplification to recover full viral genomes. These were cloned and sequenced at Macrogen, Inc. (South Korea). Two *Oat dwarf virus* (ODV) genomes that were recovered from leafhoppers feeding on beet and turnip share 99.8% identity and 97% with the only other ODV identified infecting oat (*Avena sativa*) in Germany. To date, ODV has not been reported in Iran, and therefore, these genome sequences (accession numbers KX533458 to KX533459) represent the first genome sequences of ODV from Iran. Six *Wheat dwarf virus* (WDV) genomes recovered from leafhoppers feeding on beet ($n = 2$), turnip ($n = 2$), pumpkin ($n = 1$), and parsley ($n = 1$) display 13.6% diversity (accession numbers KX533460 to KX533465); however, compared with genomes of WDV in public databases, they are most closely related to recently identified sequences from Iran, sharing >97% genome-wide identity. The *Beet curly top Iran virus* (BCTIV; accession number KX533466) and *Turnip curly top virus* (TCTV; accession numbers KX533467 to KX533468) sequences share 94% and 99% identity with previously described isolates, respectively. The BCTIV and TCTV genomes were

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recovered from leafhoppers feeding on turnip, and the two TCTV genomes share 90% identity.

Leafhoppers transmitted several destructive viral and prokaryotic diseases, such as curly top of sugar beet and turnip crops (12) and citrus stubborn (13) in Iran. We analyzed the beet, parsley, pumpkin, and turnip plants being fed on by leafhoppers in this study and did not detect ODV or WDV infection. This is perhaps not surprising, as vectors play a crucial role in viral transmission and movement, and *Psammotettix alienus* has been shown to transmit ODV (14) and WDV (15). It is therefore probable that these leafhoppers acquired ODV and WDV when feeding on infected grasses prior to being sampled.

VEM has proven a useful tool not only in this case where we identified and recovered the genomes of ODVs and WDV's circulating in Fars province of Iran but also in identifying the first mastrevirus in the Caribbean (2), novel begomovirus species (3), and known and novel plant-infecting viruses in an unlikely vector, mosquitoes (16).

Accession number(s). The complete genome sequences for the four viruses have been deposited in GenBank under accession numbers [KX533466](#) (BCTIV), [KX533458](#) to [KX533459](#) (ODV), [KX533467](#) to [KX533468](#) (TCTV), and [KX533460](#) to [KX533465](#) (WDV).

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