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Complete genome sequence of Zoysia mosaic virus from Japan

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ABSTRACT We report a complete genome sequence of Zoysia mosaic virus (ZoMV) from Japanese lawngrass. This virus shares around 80% nucleotide identity with the previously reported ZoMV-ZM864 isolate from Manila grass.

KEYWORDS *Potyviridae*, *Poacevirus*, Zoysia

Zoysia mosaic virus (ZoMV) is a pathogen responsible for mosaic symptoms in Zoysia grasses. It was first reported in Japan more than 50 years ago (1). Initially, based on virion morphology, it was suspected as a member of the genus *Potyvirus*. However, in 2023, sequencing of the ZoMV-ZM864 isolate (DDBJ/ENA/GenBank accession number OP425115) obtained from Manila grass (*Zoysia matrella*) exported from Japan to the United States suggested that the virus belongs to the family *Potyviridae* and genus *Poacevirus* (2). In this study, we determined the complete genome sequence of a ZoMV (ZoMV-TCG) infecting Japanese lawngrass (*Z. japonicum*) exhibiting mosaic symptoms collected in Nasu-Shiobara City, Tochigi Prefecture in 2022.

Double-stranded RNA (dsRNA) was extracted using a micro-spin column containing cellulose D powder (Advantec, Japan) (3) from symptomatic Japanese lawngrass. A template-switching RT enzyme mix (NEB) was used to synthesize complementary DNA (cDNA) with this dsRNA and TSO (5′- AAGCAGTGGTATCAACGCAGAGTAC ATrGrGrG-3′, rG: guanine ribonucleotide, underline: common sequence according to procedure protocol: https://www.neb.com/ja-jp/protocols/5-race-protocol-using-the-template-switching-rt-enzyme-mix) as template and primer TSO-F1-N6 (5′-AAGCAGTGG TATCAACGCAGAGTTACANNNNNN-3′) as a primer. Since the synthesized cDNA contains a common sequence at its 5′ end and a sequence complementary to the common sequence at its 3′ end, KOD One (TOYOBO, Japan) PCR was performed using AUAP-TSO-F1-in5 primer (5′-GGCCACGCGTCGACTAGTACACAGTGAAGCAGTGGTATCAACGCAGA GT-3′) with the common sequence at its 3′ end. The amplified product was purified using a NucleoSpin Gel and PCR Clean-Up Kit (Takara Bio, Japan), ligated with ligation sequencing DNA V14 (SQK-LSK114, Oxford Nanopore Technologies (ONT), UK), and subjected to whole-genome sequencing with a MinION Mk1B and Flongle Flow Cell (R10.4.1) (ONT), generating 465,063 reads (average read length: 1,241.3). Nanopore sequencing reads containing both expected 5′ and 3′ terminal adapter sequences were extracted using Cutadapt with an allowed error rate of 10%, and reads lacking either adapter were discarded (4). Adapter-filtered reads were further quality- and length-filtered using NanoFilt (minimum Q10 and 200 bp) (5). Filtered reads were directly mapped to ZoMV-ZM864 using minimap2 (6), followed by one round of consensus polishing with Racon (7). Final error correction was performed using Medaka (8). Default parameters were used in these analyses unless otherwise specified.

The complete genome of ZoMV-TCG was determined to be 9,766 nucleotides (nt) in length, excluding the 3′-terminal poly(A) tail, with a guanine or cytosine (GC) content of 40.3%. The average genome coverage of sequences mapped to the ZoMV sequence was 6,666.42-fold. Similar to ZoMV-ZM864, a single large open reading frame (ORF) spanning nt 191–9,565 encoding a polyprotein of 353.9 kDa was predicted using the

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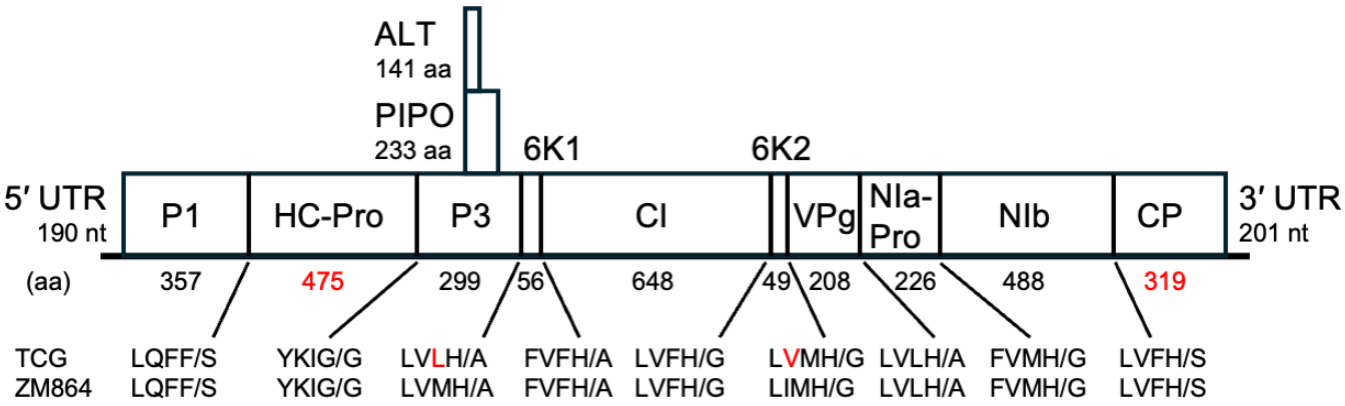

FIG 1 Schematic overview of Zoysia mosaic virus-TCG showing the polyprotein and the mature protein products. The putative cleavage sites in the ZoMV polyproteins are indicated below the genome. Red letters represent differences between ZoMV-TCG and ZoMV ZM864 isolates.

GENETYX-MAC v22.0.5 (Fig. 1). This polyprotein contained nine potential potyvirus cleavage sites, consistent with the previously reported ZoMV-ZM864 isolate. Additionally, small ORFs, called P3N-PIPO (9) and P3N-ALT (10), were also predicted to encode products generated by transcriptional slippage at the GA_8 motif (nt 3,090–3,098). Sequence alignments with ZoMV-ZM864 were performed using the MUSCLE algorithm (11) in GENETYX-MAC under default parameters. The ZoMV-TCG ORF of polyprotein shared 79.6% nucleotide identity and 90.6% amino acid identity. According to the classification criteria of the *Potyviridae* family (12), ZoMV-TCG was confirmed to be an isolate of ZoMV.

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DATA AVAILABILITY

The genome sequences of ZoMV-TCG were deposited in the DDBJ/ENA/GenBank database under accession number LC895292. The MinION data set was deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJDB37752 and SRA run accession number DRR786955.

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