Complete Genome Sequence of an American Isolate of Pepino Mosaic Virus

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ABSTRACT

Pepino mosaic virus (PepMV) is a widely distributed tomato virus. The complete genome sequence of the PepMV isolate US3 from infected tomato fruit was determined. The genome is 6,410 nucleotides long and has a poly(A) tail. US3 shares the highest similarity with strains belonging to the European genotype.

Pepino mosaic virus (PepMV) is a devastating tomato disease worldwide. PepMV belongs to the genus Potexvirus in the family Alphaflexiviridae and order Tymovirales. PepMV was initially isolated from pepino (Solanum muricatum), a tomato plant relative, in Peru during the 1970s. In the past 2 decades, PepMV has been recovered from all five continents. Tomato yield losses can reach up to 38%.

Symptoms of PepMV range from mild mottling to interveinal yellowing and more severe symptoms, such as necrosis on leaves, whereas infected tomato plants produce marbled and discolored fruits. Symptoms largely depend on the infecting strain. Currently, PepMV can be classified into five genotypes, namely, American (US1), Chilean 2 (CH2), European (EU), Peruvian (LP), and southern Peruvian (PES).

PepMV isolate US3 was previously collected and partially sequenced from fresh tomato fruit in Maryland. In this study, we report the full-genome sequence of isolate US3. PepMV US3 purified virus particles stored at –80°C were rubbed onto healthy young Nicotiana benthamiana leaves. Plants were tested positive with PepMV immunostrips (Agdia, Inc., Elkhart, IN). Infected leaves were homogenized in 50 mM phosphate buffer (pH 7.0), and PepMV was serially passaged onto a new set of healthy N. benthamiana leaves by rub inoculation. Systemically infected leaf tissues from the initial and serially passaged plants were homogenized in RLT buffer with 2-mercaptoethanol. Total RNA was extracted using the RNeasy kit (Qiagen, Hilden, Germany). The full-length cDNA was generated using an oligo(dT) primer with restriction sites at the 5’ end of the primer using the AffinityScript cDNA synthesis kit (Santa Clara, CA). Primers were designed based on conserved regions of the full-length sequences of a PepMV multiple-sequence alignment. cDNA was diluted and subsequently used for PCR with the long-range PCR kit (Qiagen). Primers 4585-F (5′-GGTTCAACCTAGGGCTTGGCTA-3′) and oligo(dT) were used to amplify a 1,850-bp amplicon of the 3′-end genome region. Primers PA1f2 (5′-GAAAACAAAATAA-3′) and 4585-R (5′-TAGCAAGGGCGGTGAAACC-3′) were used to amplify a 4,604-bp segment of the 5′ end of the virus genome. Primers 4625F (5′-CCAAATGGGTGATGAGCTGC-3′) and 5237R (5′-TGCTCCAGCAACAGTTGG-3′) were used to amplify a 973-bp segment that overlaps the 4.6-kb and 1.8-kb fragments. Internal sequences were determined by primer walking using the following primers: 714F (5′-GGTGGTGGGGCATATTTCCA-3′), 1473F (5′-GAGCCTGAAACTAGGCCCC-3′), 1860F (5′-GCTCAGAGCTCCGGAAGACG-3′), 2855R (5′-TGAGCAAGGGCGGTGAAACC-3′), 2754F (5′-ATGCAAGACATAGGGCACAAA-3′), 3546F (5′-GTCAAGGTTGGGAAAGGGTGATGC-3′), and 5950R (5′-TGCAGGGTTGGGAAAGGGTGATGC-3′). The 5′ and 3′ terminal ends of the virus were amplified using a 5′ rapid amplification of cDNA ends.
(RACE) PCR kit (Ambion, Inc., Austin, TX) and an oligo(dT) primer, respectively, following the manufacturer’s instructions. The PCR amplicons were gel purified and cloned into the pCR4-TOPO vector (Thermo Fisher Scientific, Waltham, MA). Three or four clones from each PCR product were sequenced by GenScript (Piscataway, NJ) on an Applied Biosystems 3730xl DNA analyzer.

Nucleotide sequences were trimmed, assembled, and annotated using default parameters in Geneious Prime 2019.2.1 (Biomatters Ltd., Auckland, New Zealand) into a single contig representing the full-length sequence of PepMV. The mean coverage by position was 9.5 ×. The complete genome sequence of US3 was 6,410 nucleotides (nt) long without the poly(A) tail and has a G+C content of 40.9%. The open reading frames (ORFs) were annotated in Geneious based on a manually curated PepMV database retrieved from GenBank. The virus has five predicted ORFs, the replicase, triple gene block 1 (TGB1), TGB2, TGB3, and coat protein (CP). The 5’ untranslated region (UTR) and

![FIG 1 Maximum likelihood tree of pepino mosaic virus (PepMV) complete genome sequences built using the generalized time-reversible substitution model and gamma rate of heterogeneity created in RAxML v8.2.11 (6). The tree was rooted with the ancestral LP clade. GenBank accession numbers for the five genotypes EU, CH2, PES, LP, and US1 are indicated in brackets next to the isolate names. The PepMV US3 isolate sequenced in this study is highlighted in blue. Bootstrap values with support above 70% are indicated on branches. The country of origin is indicated next to the name of the corresponding isolates.](image-url)
3’ UTR are 86 and 64 nt, respectively. The virus genome has a 3’-poly(A) tail. PepMV US3 has the canonical octanucleotide (GTAAAGTT) sequence in the core subgenomic promoter regions upstream of the TGB1 and CP genes and shared between 98.8% to 99.5% pairwise nucleotide identity with isolates within the EU phylogroup, which was determined using the MUSCLE sequence alignment tool. The highest nucleotide identity was shared with the French (Fr) isolate at 99.5% and the lowest with an American isolate (Euro-6) at 98.8% nucleotide identity. The CP nucleotide sequence shared 99.8% pairwise nucleotide identity with EU_CAHN8, EU-tomato, and Fr isolates. The TGB1, TGB2, and TGB3 genes shared the highest nucleotide similarity with European isolates Fr, EU, and EU-tomato. Isolates belonging to the EU genotype are considered mild strains and do not incite severe symptoms in tomato plants. A maximum likelihood phylogenetic tree of isolate US3 with all of the complete genomes of PepMV isolates in the GenBank database is depicted in Fig. 1. Isolate US3 is more closely related to the European isolates than to the American isolates of the EU genotype.

Data availability. The complete genome sequence of PepMV isolate US3 was deposited in GenBank under accession number MN395046.

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