JVI Journals.ASM.org

GENOME ANNOUNCEMENT

Genome Assembly of Bell Pepper *Endornavirus* from Small RNA

Noa Sela, Neta Luria, and Aviv Dombrovsky

Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

The family *Endornaviridae* infects diverse hosts, including plants, fungi, and oomycetes. Here we report for the first time the assembly of bell pepper endornavirus by next-generation sequencing of viral small RNA. Such a population of small RNA indicates the activation of the viral immunity silencing machinery by this cryptic virus, which probably encodes a novel silencing suppressor.

The family *Endornaviridae* infects diverse hosts, including plants, fungi, and oomycetes (1). Endornaviruses consist of a large double-stranded RNA (dsRNA) element with a nick in the coding strand. Endornaviruses possess unique properties, including no evidence of encapsidation and a single long open reading frame (ORF). The ORF encodes a putative polyprotein with methyltransferase, helicase, glycosyltransferase, and RNA-dependent RNA polymerase domains (5). Recently, the complete genomes of two strains of bell pepper endornavirus (BPEV) of genus *Endornavirus*, family *Endornaviridae*, were sequenced using viral dsRNA as a template for reverse transcription, followed by PCR amplification and 5′ and 3′ rapid amplification of cDNA ends (RACE) (3, 4, 5). Here we report for the first time a complete genome assembly of BPEV from small RNA purified from pepper leaves.

Total RNA was extracted from 5 g of pepper (*Capsicum annuum* L. cv. Yatir) leaves in Israel using TRI reagent, and the pellet was dissolved in nuclease-free water. Small RNA (70 μg) was purified from total RNA using the mirVana microRNA (miRNA) isolation kit (Ambion) and stored at −80°C. Small RNA concentrations were determined by the Agilent 2100 bioanalyzer. Purified small RNA was "deep sequenced" by the SOLiD version 3 instrument (Applied Biosystems Foster, CA) according to the manufacturer's protocols. The bioinformatic analysis used the BFAST (1) alignment tool with the reference genome of BPEV cv. Kyosuzu (BPEV-KY) (GenBank accession number AB597230). Base coverage was 100%, the average coverage depth was 258, and the maximum coverage depth was 2,870 (calculated with Tablet [2]). Alignment against the reference genome of BPEV cv. Yolo Wonder (BPEV-YW) (GenBank accession JN019858) gave 52% coverage, with an average coverage of 57 and a maximum coverage depth of 2,869. The complete genome sequence of the Israeli isolate of BPEV (BPEV-Is) is composed of 14,727 nucleotides, with a typical endornavirus genome organization containing a single open reading frame with a predicted molecular mass of 553 kDa. BPEV-Is has 99% nucleotide identity to BPEV-KY and 88% nucleotide identity to BPEV-YW. Plant viral symptoms are commonly the result of suppressor activity; however, our results indicate that BPEV activates the RNA silencing machinery of the plant immune system without inducing viral symptoms and therefore probably also encodes a novel silencing suppressor.

Nucleotide sequence accession number. This genome sequence was deposited under NCBI GenBank accession number JQ951943.

ACKNOWLEDGMENTS

We thank Mira Korner, Michal Bronstein, and Temima Schnitzer-Perlman for conducting the SOLiD sequencing and the bioinformatic analyses performed at the Center for Genomic Technologies, the Hebrew University of Jerusalem. We thank Victor Gaba for editing the manuscript.

REFERENCES

1. **Homer N, Merriman B, Nelson SF.** 2009. BFAST: an alignment tool for large scale genome resequencing. PLoS One **4**:e7767. doi:10.1371/journal.pone.0007767.
2. **Milne I, et al.** 2010. Tablet—next generation sequence assembly visualization. Bioinformatics **26**:401–402.
3. **Okada R, et al.** 2011. Bell pepper endornavirus: molecular and biological properties, and occurrence in the genus Capsicum. J. Gen. Virol. **92**:2664–2673.
4. **Roossinck MJ, Sabanadzovic S, Okada R, Valverde RA.** 2011. The remarkable evolutionary history of endornaviruses. J. Gen. Virol. **92**:2674–2678.
5. **Valverde RA, Gutierrez DL.** 2007. Transmission of a dsRNA in bell pepper and evidence that it consists of the genome of an endornavirus. Virus Genes **35**:399–403.

Received 23 April 2012 Accepted 24 April 2012

Address correspondence to Noa Sela, noa@volcani.agri.gov.il.

doi:10.1128/JVI.00983-12