



Complete Genome Sequence of a Pepper Yellow Leaf Curl Indonesia Virus Isolated from Tomato in Bali, Indonesia

[®]Yutaro Neriya,^a Runa Izumi,^b Fariha Wilisiani,^c Sedyo Hartono,^d G. N. Alit Susanta Wirya,^e Hisashi Nishigawa,^a Tomohide Natsuaki^a

^aSchool of Agriculture, Utsunomiya University, Utsunomiya, Tochigi, Japan
^bUtsunomiya Junior College Attached High School, Utsunomiya, Tochigi, Japan
^cDepartment of Agrotechnology, Faculty of Agriculture, STIPER Institute of Agriculture, Yogyakarta, Indonesia
^dDepartment of Crop Protection, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia
^eDepartment of Agroecotechnology, Faculty of Agriculture, Udayana University, Bali, Indonesia

ABSTRACT We report a complete genome sequence of a pepper yellow leaf curl Indonesia virus (PepYLCIV) isolated in Bali, Indonesia. This virus shares around 90% identity with other PepYLCIV DNA-As and 86% identity with DNA-Bs, suggesting that it is a novel isolate of PepYLCIV.

Most begomoviruses (family *Geminiviridae*, genus *Begomovirus*) possess bipartite circular, single-stranded DNA genomes. Begomoviruses are transmitted by white-fly and cause typical mosaic, yellowing, curling symptoms on leaves and stunted growth of plants, resulting in serious damage to crop production in tropical and subtropical regions.

Plants infected with begomoviruses were reported on Bali Island, Indonesia (1), but no complete genome sequence of a begomovirus has been deposited in the DDBJ/ ENA/GenBank database.

In August 2019, we collected tomato leaves showing yellowing and a mosaic pattern, typical of begomovirus infections in Bali (8°14'8"S, 115°24'19"E). Total DNA was extracted from collected tomato leaves using a DNeasy plant minikit (Qiagen, Germany), followed by PCR amplification (BIOTAQ, Bioline, UK) of a part of the DNA-A components of the begomovirus genome, using universal primers (UPV1 and PAV1c715; product size, 1,622 nucleotides [nt]) (2). Amplified fragments were cloned into the pGEM-T Easy vector (Promega, USA) or pCRZeroT (3), and at least three independent plasmids were sequenced using the BigDye Terminator version 3.1 cycle sequencing kit and the Applied Biosystems 3500 genetic analyzer (Thermo Fisher Scientific, USA) with vector-specific primers (5'-GTAAAACGACGGCCAG-3' and 5'-CAGGAAACAGCTATGACC-3' for pGEM-T Easy; 5'-CGCAATTAATGTGAGTTAGC-3' and 5'-GGGCCTCTTCGCTATTAC-3' for pCRZeroT). Sequences were assembled using ATGC version 4.3.5 (Genetyx, Japan). We designed a primer set (5'-GAAGTCTGTAT ATATTATTGGCAAAG-3' and 5'-CCCAAGGTATACAACAATGAC-3'; 1,207 nt) for inverse PCR to clone the remainder of the genome to amplify the circular DNA fragment, covering the complete nucleotide of the genome, and sequenced it in the same manner. DNA-B was amplified using pepper yellow leaf curl Indonesia virus (PepYLCIV)-specific primers (PepYLCIV_BFs, 5'-GTCCGACGTGGAATATGCAAGTG-3'; PepYLCIV_BRs, 5'-GTGCGTTCTTTGACACGTTGCTG-3', 1,831 nt), and inverse PCR was performed with PYLCIV-Bali-invBF (5'-AAAAGAAGTGCGTTGTAAATACC-3') and PYLCIV-BaliinvBR (5'-AAAAAAGAGTGCATTCCAGC-3'; product size, 1,124 nt). These products were used for cloning and sequencing, and PYLCIV-Bali-B-2360F (5'-GTTCAAACATTTTAGCC AACA-3') was used for internal sequencing. Nucleotide sequence identities were calcu-

Citation Neriya Y, Izumi R, Wilisiani F, Hartono S, Wirya GNAS, Nishigawa H, Natsuaki T. 2020. Complete genome sequence of a pepper yellow leaf curl Indonesia virus isolated from tomato in Bali, Indonesia. Microbiol Resour Announc

9:e00486-20. https://doi.org/10.1128/MRA .00486-20.

Editor Kenneth M. Stedman, Portland State University

Copyright © 2020 Neriya et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Yutaro Neriya, neriya@cc.utsunomiya-u.ac.jp.

Received 4 May 2020 Accepted 22 May 2020 Published 18 June 2020

TABLE 1 Simila	arity analysis of	PepYLCIV-[IN:Bali:Tom:19] with other PepYLCIV isolates
----------------	-------------------	--------------------------	--------------------------------

	Accession no. of:		Sequence identity (%) with:	
PepYLCIV isolate	DNA-A	DNA-B	DNA-A	DNA-B
Indonesia:Bogor:2000	DQ083764	NA ^a	90.4	NA
Indonesia:Bogor:Tomato:2000	DQ083765	NA	90.2	NA
Indonesia:2005	AB267834	AB267835	92.4	86.7
Indonesia:Tomato:2005	AB267836	AB267837	90.1	86.9
Indonesia: Ageratum: 2005	AB267838	AB267839	91.3	85.4
Indonesia:Sumatra:Pepper:2012, BA_A6-1	LC051112	LC314792	91.5	86.2
Indonesia:Sumatra:Pepper:2012, BA_C1-1	LC051113	LC314793	89.9	85.9
Indonesia:Sumatra:Pepper:2012, BA_D1-1	LC051114	LC314794	89.4	86.6
Indonesia:Sumatra:Pepper:2012, BA_E2-1	LC051115	LC314795	89.5	87.3

^a NA, not available; no sequence data were found in DDBJ/ENA/GenBank.

lated using GENETYX-MAC version 19 (Genetyx). No alphasatellite or betasatellite DNAs were detected using universal primers (4, 5).

DNA-A (2,743 nt; GC content, 41.6%) was predicted to have six open reading frames (ORFs) and DNA-B (2,721 nt; GC content, 39.5%) two ORFs, typical for bipartite Old World begomoviruses, using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). A BLASTn search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated that this virus shared high similarity with PepYLCIV isolates (Table 1). From a homology search analysis using the full-genome sequences of DNA-A and DNA-B, this isolate showed high sequence identity with PepYLCIV isolates (Table 1).

A total of 23 partial DNA-A sequences of PepYLCIV were reported (GenBank accession numbers LC381258 through LC381280). These sequences and this isolate shared 80.8 to 89.2% sequence identity, suggesting that our isolate is different from those previously reported.

According to the species demarcation criteria of the genus *Begomovirus* (6), this isolate was classified as a PepYLCIV. We propose that this isolate be named PepYLCIV-[Indonesia:Bali:Tomato:2019] (PepYLCIV-[IN:Bali:Tom:19]).

Data availability. The genome sequences of PepYLCIV-[IN:Bali:Tom:19] were deposited in the DDBJ/ENA/GenBank database under accession numbers LC542629 (DNA-A) and LC542630 (DNA-B).

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI (Grant-in-Aid for Scientific Research B) grant number 17H04617 and by the JST-GSC Incubation Program for Innovative Students at Utsunomiya University (iP-U).

REFERENCES

- Wilisiani F, Tomiyama A, Katoh H, Hartono S, Neriya Y, Nishigawa H, Natsuaki T. 2019. Development of a LAMP assay with a portable device for real-time detection of begomoviruses under field conditions. J Virol Methods 265:71–76. https://doi.org/10.1016/j.jviromet.2018.10.005.
- Kon T, Hidayat SH, Ito K, Hase S, Takahashi H, Ikegami M. 2005. Begomoviruses associated with leaf curl disease of tomato in Java, Indonesia. J Phytopathol 153:562–566. https://doi.org/10.1111/j.1439-0434.2005.01020.x.
- Motohashi K. 2019. A novel series of high-efficiency vectors for TA cloning and blunt-end cloning of PCR products. Sci Rep 9:6417. https://doi.org/ 10.1038/s41598-019-42868-6.
- 4. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. 2002. Universal

primers for the PCR-mediated amplification of DNA β . Mol Biotechnol 20:315–318. https://doi.org/10.1385/MB:20:3:315.

- Bull SE, Briddon RW, Markham PG. 2003. Universal primers for the PCRmediated amplification of DNA 1: a satellite-like molecule associated with begomovirus-DNA β complexes. Mol Biotechnol 23:83–86. https://doi .org/10.1385/MB:23:1:83.
- Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JCF, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A. 2015. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593–1619. https://doi.org/10.1007/s00705-015-2398-y.