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GENOME SEQUENCES

Complete Genome Sequences of Tomato Leaf Curl Guam Virus, a Novel Tomato-Infecting Begomovirus from Guam, USA

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ABSTRACT Genome sequences of a novel begomovirus infecting tomato on Guam were obtained using primer-walking and sequencing. The complete genome sequences are 2,750 nucleotides long with a typical monopartite organization and display less than 91% nucleotide sequence identity to other begomoviruses. A provisional name, tomato leaf curl Guam virus (ToLCGuV), is proposed.

Since 2007, symptoms of leaf curl, yellowing, and plant stunting (Fig. 1A and B) have been observed on tomato plants (*Solanum lycopersicum* L.) on Guam, a U.S. territory (total area of 549 km^2; 13°33′N, 144°55′E) situated in the Western Pacific Ocean, approximately 2,600 km east of the Philippines. Disease outbreaks have been restricted to northern Guam, with the yield loss estimated at 10% in 2007 and up to a total loss in some cases in 2010 to 2011 (1). To identify the causal agent, two leaf tissue samples were collected from growers' fields in Yigo, Guam (144°88′E, 13°57′N), in May 2012, one with typical leaf curl and yellowing and the other one healthy. Two additional leaf tissue samples with symptoms were collected from other farms in the same area in July 2013. A begomovirus in the family *Geminiviridae* was suspected, as the vector whiteflies (*Bemisia tabaci*) were also present. Plant DNA preparations from each sample were prepared using the DNeasy plant minikit, following the manufacturer's instructions (Qiagen, USA). Using the degenerate primers (PAR1c496: 5′-GGCTTYCTRTACATRGG-3′; PAL1v1978: 5′-GCCCACATYGTCTTYCCNGT-3′) specific to the begomovirus DNA-A component (2), an expected PCR product (1,307 bp) was amplified from the diseased sample but not from the healthy one. No amplification was observed for the DNA-B component using the specific primers (PBL1V2040: 5′-CARTGRTCKATCTTCATACA-3′; PCRc154: 5′-GGTAATATTATAHCGGATGG-3′) (2). Primer-walking with overlapping amplicons and Sanger sequencing (Functional Biosciences, Madison, WI) yielded complete genome sequences from three distinct samples, one collected in 2012 and two others in 2013 (GenBank accession no. KJ744212, KR094066, and KR094067). The sequence identities of two 2013 samples (Guam 13-60 and Guam 13-86) were 99.42 and 99.49% to the 2012 sample (KJ744212 is designated a type sequence, NC_027040.1). The complete genomes (2,750 nucleotides [nt] long; GC content, 42%) were assembled as a single circular genome using SeqMan Pro in Lasergene 16 (DNASTAR, Madison, WI). All tools were run with default parameters unless otherwise specified. The genome had a typical monopartite begomovirus organization, which encoded two virus-sense open reading frames (V1 and V2) and four others in complementary orientation (C1, C2, C3, and C4), as analyzed using SeqBuilder in DNASTAR Lasergene 16 (Fig. 1C). A conserved nanonucleotide—TAATATTAC—presented in the replication initiation site. BLASTn analysis using the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) showed sequence identities below 91% to other known begomoviruses (Table 1). The phylogenetic relationships with representative begomoviruses were analyzed using complete DNA-A sequences in MegAlign Pro by MUSCLE, and a tree was generated using default parameters (Fig. 1D). The three ToLCGuV isolates clustered together, with a closer relationship to ageratum yellow vein virus (AYVV)

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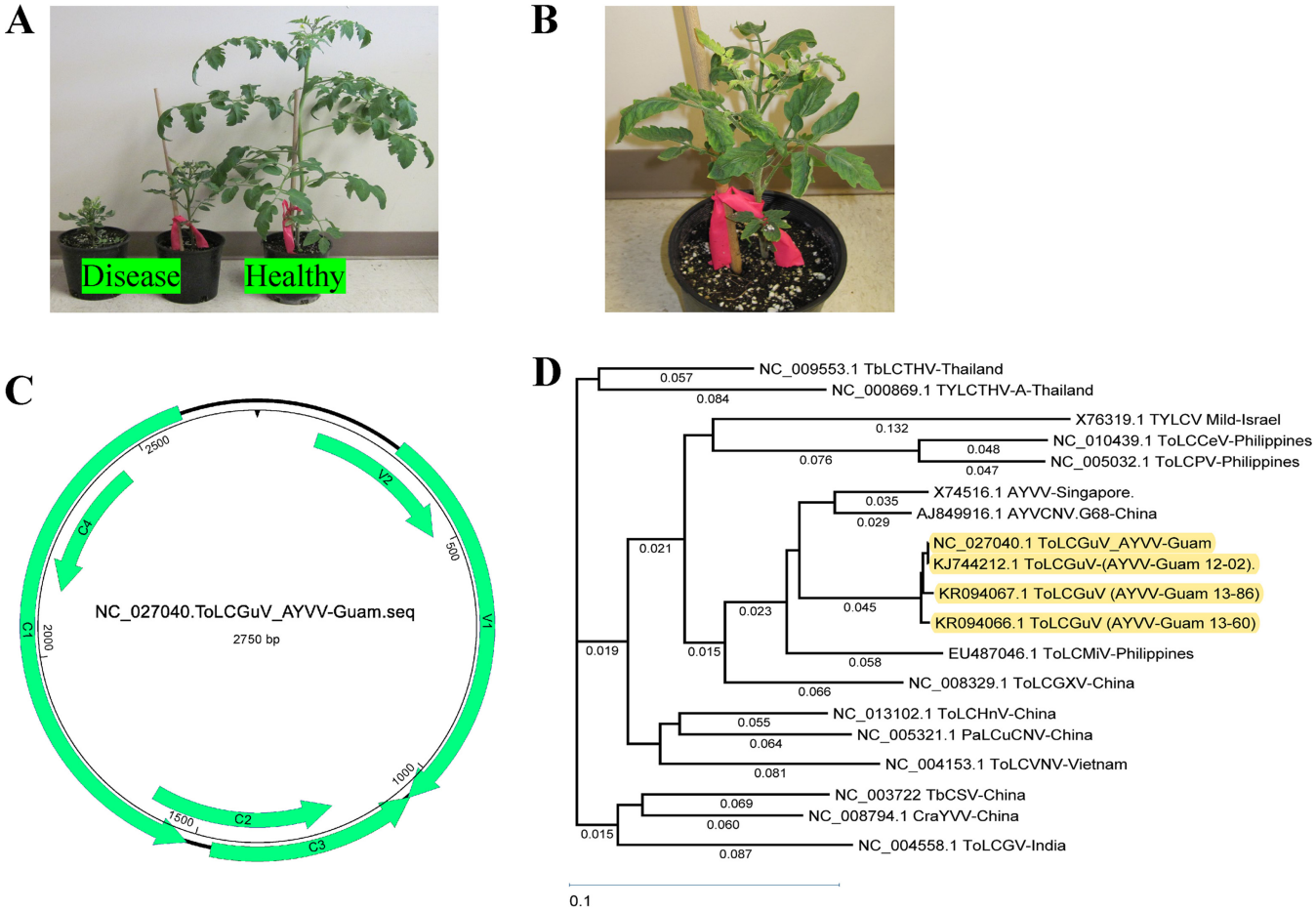

FIG 1 Symptoms on tomato plants infected by tomato leaf curl Guam virus (ToLCGuV) and its genome organization and phylogenetic relationship to other related begomoviruses. (A) Tomato plants (left two) infected by ToLCGuV, with plant stunting, yellowing, and leaf curl symptoms, in comparison to a healthy plant control (right). (B) Closeup look at a diseased tomato plant infected by ToLCGuV. (C) Genome organization of ToLCGuV with two open reading frames (ORFs) in sense orientation (V1 and V2) and four other ORFs in complementary sense orientation (C1, C2, C3, and C4). (D) Phylogenetic relationship of ToLCGuV complete genome sequences (including three samples, Guam 12-02, Guam 13-60, and Guam 13-86, and the type sequence for ToLCGuV that is identical to Guam 12-02) with 15 related begomoviruses, including tobacco curly shoot virus (TbCSV), *Crassocephalum* yellow vein virus (CraYVV), tomato leaf curl Gujarat virus (ToLCGV), tomato yellow leaf curl virus (TYLCV), tomato leaf curl Cebu virus (ToLCCeV), tomato leaf curl Philippines virus (ToLCPV), ageratum yellow vein virus (AYVV), ageratum yellow vein China virus (AYVCNV), tomato leaf curl Mindanao virus (ToLCMiV), tomato leaf curl Guangxi virus (ToLCGXV), tomato leaf curl Hainan virus (ToLCHnV), tomato leaf curl Vietnam virus (ToLCVNV), papaya leaf curl China virus (PaLCuCHV), tobacco leaf curl Thailand virus (TbLCTHV), and tomato yellow leaf curl Thailand virus (TYLCTHV). A multiple sequence alignment was created using MUSCLE in the MegAlign Pro program, and the phylogenetic tree was generated using BIONJ with default parameters in Lasergene 16 (DNASTAR, USA).

(X74516) (3), followed by ageratum yellow vein China virus (AYVCNV) (AJ849916) (4) and then other related begomoviruses (5–17). Initially, these virus isolates were considered to be a unique strain of AYVV, according to the old begomovirus species demarcation standard (18). However, according to the new species demarcation standard of 91% nucleotide sequence identity of the DNA-A component (19), this tomato-infecting begomovirus should be considered a new species, with a provisional name such as tomato leaf curl Guam virus (ToLCGuV). A real-time quantification PCR (qPCR) was developed to detect ToLCGuV and other tomato-infecting begomoviruses, with primers (ToLCGuV_F1: 5′-TAGTTTATAATCATCAAGAGGC-3′; ToLCGuV_R1: 5′-ATCATAGAAATAGATCCTGATCTT-3′; and TaqMan probe ToLCGuV_P1: FAM-AACGCCTTGTTATTGTATATGGCCT_TAMRA). A field survey using this qPCR on 35 randomly collected tomato samples from Guam in 2013 showed an 8.6% positivity rate. With the onset of disease, growers began testing commercially available tomato varieties for field resistance (20). A cultivar with ToLCGuV resistance that is suitable for the Guam climate and its production system would be the most desirable for disease management.

Data availability. The genome sequences of 3 ToLCGuV samples (previously considered ageratum yellow vein virus, Guam strain) have been deposited at GenBank under accession

TABLE 1 Percent nucleotide sequence identities of tomato leaf curl Guam virus isolate Guam 12-02[a] with other related begomoviruses (DNA-A)

Virus name[b]	Isolate name[c]	Country or region of identification	GenBank accession no.	Percent nucleotide sequence identity[d]	Reference
ToLCGuV	Guam 13-60	Guam, USA	KR094066.1	99.49	This work
ToLCGuV	Guam 13-86	Guam, USA	KR094067.1	99.42	This work
AYVV	AF_SP6d	Thailand	JN809821.1	91.40	Shih et al., 2013 (13)
AYVV	pHN419	Singapore	X74516.1	90.04	Tan et al., 1995 (3)
AYVCNV	G68	China	AJ849916	90.84	Huang and Zhou, 2006 (4)
ToLCMiV	P162	Philippines	EU487046.1	88.20	Tsai et al., 2011 (14)
ToLCGXV	GX-1	China	NC_008329.1	85.53	Xu et al., 2007 (17)
TYLCV	Mild	Israel	X76319.1	78.47	Antignus and Cohen, 1994 (21)
ToLCPV	NA	Philippines	NC_005032.1	82.35	Kon et al., 2002 (11)
ToLCCeV	P134	Philippines	NC_010439.1	82.19	Tsai et al., 2011 (14)
ToLCHnV	NA	China	NC_013102.1	80.83	Zhang et al., 2010 (16)
ToLCVNV	NA	Vietnam	NC_004153.1	78.53	Green et al., 2001 (8)
PaLCuCNV	G8	China	NC_005321.1	83.41	Wang et al., 2004 (15)
TbLCTHV	NA	Thailand	NC_009553.1	81.88	Knierim and Maiss, 2007 (10)
TYLCTHV	NA	Thailand	NC_000869.1	77.56	Attethom et al., 1994 (5)
TbCSV	Y41	China	NC_003722.1	79.38	Li et al., 2004 (9)
CraYVV	Jinghong	China	NC_008794.1	77.87	Dong et al., 2008 (7)
ToLCGV	Varanasi	India	NC_004558.1	78.29	Chakraborty et al., 2003 (6)

[a] GenBank accession no. KJ744212 or NC_027040.1.

[b] Virus names: ageratum yellow vein virus (AYVV), ageratum yellow vein China virus (AYVCNV), tomato leaf curl Mindanao virus (ToLCMiV), tomato yellow leaf curl virus (TYLCV), tomato leaf curl Philippines virus (ToLCPV), tomato leaf curl Cebu virus (ToLCCeV), tomato leaf curl Hainan virus (ToLCHnV), tomato leaf curl Vietnam virus (ToLCVNV), papaya leaf curl China virus (PaLCuCHV), tobacco leaf curl Thailand virus (TbLCTHV), tomato yellow leaf curl Thailand virus (TYLCTHV), tobacco curly shoot virus (TbCSV), Crassocephalum yellow vein virus (CraYVV), and tomato leaf curl Gujarat virus (ToLCGV).

[c] NA, not applicable.

[d] Percent nucleotide sequence identity was calculated based on BLASTn analysis in the NCBI database using the DNA-A component of the respective virus to the complete genome sequence of tomato leaf curl Guam virus (ToLCGuV) isolate Guam 12-02 (GenBank accession no. KJ744212 or NC_027040).

no. KJ744212 (sample Guam 12-02), KR094066 (sample Guam 13-60), and KR094067 (sample Guam 13-86). In addition, Guam 12-02 is considered the type member of ToLCGuV (NC_027040.1).

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