



First Complete *Squash leaf curl China virus* Genomic Segment DNA-A Sequence from East Timor

Solomon Maina,^{a,b,c} Owain R. Edwards,^{c,d} Luis de Almeida,^e Abel Ximenes,^f Roger A. C. Jones^{b,c,g}

School of Agriculture and Environment, Faculty of Science, the University of Western Australia, Crawley, Western Australia, Australia^a; Institute of Agriculture, Faculty of Science, the University of Western Australia, Crawley, Western Australia, Australia^b; Cooperative Research Centre for Plant Biosecurity, Canberra, Australian Capital Territory, Australia^b; CSIRO Land & Water, Floreat Park, Western Australia, Australia^d; Seeds of Life Project, Ministry Agriculture and Fisheries, Dili, East Timor^e; DNQB-Plant Quarantine International Airport Nicolau Lobato Comoro, Dili, East Timor^f; Department of Agriculture and Food Western Australia, South Perth, Western Australia, Australia^g

ABSTRACT We present here the first complete *Squash leaf curl China virus* (SLCCV) genomic segment DNA-A sequence from East Timor. It was isolated from a pumpkin plant. When compared with 15 complete SLCCV DNA-A genome sequences from other world regions, it most resembled the Malaysian isolate MC1 sequence.

As part of a project to examine genomic connectivity between viruses infecting crops in northern Australia and nearby southeast Asian countries, virus genomes from plant samples from East Timor and Australia were compared (1–10). In 2015, 15 and 22 cucurbit leaf samples with virus-like symptoms were collected in four locations in East Timor and Broome in northwest Australia, respectively, and subjected to next generation sequencing. A complete genomic segment *Squash leaf curl China virus* (SLCCV) DNA-A sequence was obtained from pumpkin sample TM4 from Dili in East Timor. SLCCV belongs to the *Begomovirus* genus, in the family *Geminiviridae*. Members of this genus have two circular single stranded DNA (ssDNA) components (DNA-A and DNA-B) and a genome of approximately 2.7 kb encapsidated within twinned icosahedral particles (11–14). SLCCV is whitefly transmitted and has a restricted host range within the *Cucurbitaceae* family, infecting squash and pumpkin, but not melon or cucumber (15). It occurs in many world regions including parts of southeast Asia (13–15), but has not been found previously in East Timor or Australia. RNA-Seq with rRNA-plant depletion provides reliable metagenomic detection of polyadenylated and nonpolyadenylated RNA viruses and can also detect DNA viruses (16–18). This approach detected SLCCV in sample TM4 (designated isolate SLCCV T4-D).

The 15 East Timorese samples were blotted onto fast technology for analysis of nucleic acids (FTA) cards (19) before dispatch to Australia. The Australian samples studied were recently collected leaves. Total RNA was extracted from both sample types using a ZR Plant RNA MiniPrep kit (Zymo Research). The total RNA extracts were treated with RNase-free DNase (Invitrogen). Quality control was done and RNA subjected to total RNA sample preparation Ribo-Zero plant kit (catalogue no. RS-122-2401, Illumina) as described previously (1–10). Sequencing was by HiSeq 2500 using a Truseq SBS KIT (Illumina) with 151 cycles to generate paired-end reads in a multiplex of 24 samples in one lane. Reads were assembled and genomes annotated using CLC Genomics Workbench 6.5 (CLC bio) and Geneious 8.1.7 (Biomatters) (20, 21).

FTA card sample TM4 yielded 12,867,884 reads and, after trimming, 12,244,994 remained. *De novo* assembly generated 250 contigs and 75,817 reads mapped to the contig of interest with coverage of 4,119. Final coding genome length was 2,737

Received 18 April 2017 Accepted 20 April 2017 Published 15 June 2017

Citation Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2017. First complete *Squash leaf curl China virus* genomic segment DNA-A sequence from East Timor. *Genome Announc* 5:e00483-17. <https://doi.org/10.1128/genomeA.00483-17>.

Copyright © 2017 Maina et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Roger A. C. Jones, roger.jones@uwa.edu.au.

nucleotides (nt). As with other begomoviruses, SLCCV coded for a pre-coat protein, protein C4, and replication-associated protein C1. A BLAST-based search with pairwise sequence comparison (PASC) tool (22), revealed the T4-D genome sequence most resembled Malaysian isolate MC1, GenBank accession number EF197940, with 95.0% nt identity. Since no SLCCV was detected in any Australian samples, further sampling is needed to establish whether SLCCV has spread to Australia from nearby southeast Asian countries. Comparison of any Australian genomic sequences found with ones from neighboring countries would be required.

Accession number(s). This sequence was deposited at DDBJ/EMBL/GenBank under accession number [KY652743](https://doi.org/10.1128/genomeA.00320-17).

ACKNOWLEDGMENTS

Martin J. Barbetti and Mingpei You of the School of Agriculture and Environment, The University of Western Australia (UWA) provided administrative support. The UWA ARC Centre of Excellence in Plant Energy Biology and School of Molecular Sciences, and Laura Boykin also provided initial administrative support at the beginning of this project.

The Cooperative Research Centre for Plant Biosecurity and UWA provided scholarship and operating funds to S.M. The Commonwealth Scientific and Industrial Research Organisation provided additional operating funds.

REFERENCES

- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2016. Complete genome sequences of the *Carlavirus Sweet potato chlorotic fleck virus* from East Timor and Australia. *Genome Announc* 4(3):e00414-16. <https://doi.org/10.1128/genomeA.00414-16>.
- Maina S, Edwards OR, Jones RAC. 2016. First complete genome sequence of *Pepper vein yellows virus* from Australia. *Genome Announc* 4(3):e00450-16. <https://doi.org/10.1128/genomeA.00450-16>.
- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2016. Complete genome sequences of the *Potyvirus Sweet potato virus 2* from East Timor and Australia. *Genome Announc* 4(3):e00504-16. <https://doi.org/10.1128/genomeA.00504-16>.
- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2016. First complete genome sequence of *Suakwa aphid-borne yellows virus* from East Timor. *Genome Announc* 4(4):e00718-16. <https://doi.org/10.1128/genomeA.00718-16>.
- Maina S, Edwards OR, Barbetti MJ, de Almeida L, Ximenes A, Jones RAC. 2016. Deep sequencing reveals complete genome of *Sweet potato virus G* from East Timor. *Genome Announc* 4(5):e00957-16. <https://doi.org/10.1128/genomeA.00957-16>.
- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2016. First complete genome sequence of *Bean common mosaic necrosis virus* from East Timor. *Genome Announc* 4(5):e01049-16. <https://doi.org/10.1128/genomeA.01049-16>.
- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2017. Metagenomic analysis of cucumber RNA from East Timor reveals an *Aphid lethal paralysis virus* genome. *Genome Announc* 5(2):e01445-16. <https://doi.org/10.1128/genomeA.01445-16>.
- Maina S, Coutts BA, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 4 April 2017. *Papaya ringspot virus* populations from East Timorese and northern Australian cucurbit crops: biological and molecular properties, and absence of genetic connectivity. *Plant Disease*. <https://doi.org/10.1094/PDIS-10-16-1499-RE>.
- Maina S, Coutts BA, Edwards OR, de Almeida L, Kehoe MA, Ximenes A, Jones RAC. 2017. *Zucchini yellow mosaic virus* populations from East Timorese and northern Australian cucurbit crops: molecular properties, genetic connectivity and biosecurity implications. *Plant Dis*. <https://doi.org/10.1094/PDIS-11-16-1672-RE>.
- Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RAC. 2017. RNA-seq strand specific library from East Timorese cucumber sample reveals complete *Cucurbit aphid-borne yellows virus* genome. *Genome Announc*. <https://doi.org/10.1128/genomeA.00320-17>.
- Lazarowitz SG, Shepherd RJ. 1992. Geminiviruses: genome structure and gene function. *Crit Rev Plant Sci* 11:327-349.
- Hong Y, Wang X, Tian B, Cai J. 1995. Chinese squash leaf curl virus: a new whitefly-transmitted geminivirus. *Sci China B* 38:179-186.
- Revill PA, Ha CV, Porchun SC, Vu MT, Dale JL. 2003. The complete nucleotide sequence of two distinct geminiviruses infecting cucurbits in Vietnam. *Arch Virol* 148:1523-1541. <https://doi.org/10.1007/s00705-003-0109-6>.
- Kon T, Dolores LM, Bajet NB, Hase S, Takahashi H, Ikegami M. 2003. Molecular characterization of a strain of *squash leaf curl China virus* from the Philippines. *J Phytopathol* 151:535-539. <https://doi.org/10.1046/j.1439-0434.2003.00764.x>.
- Dolores LM, Valdez RB. 1988. Identification of squash viruses and screening for resistance. *Philipp Phytopathol* 24:43-52. <http://agris.fao.org/agris-search/search.do?recordID=PH8912492>.
- Nagano AJ, Honjo MN, Mihara M, Sato M, Kudoh H. 2015. Detection of plant viruses in natural environments by using RNA-Seq. *Methods Mol Biol* 1236:89-98. https://doi.org/10.1007/978-1-4939-1743-3_8.
- Kreuze JF, Savenkov EI, Cuellar W, Li X, Valkonen JP. 2005. Viral Class 1 RNase III involved in suppression of RNA silencing. *J Virol* 79:7227-7238. <https://doi.org/10.1128/JVI.79.11.7227-7238.2005>.
- Kreuze JF, Perez A, Untiveros M, Quispe D, Fuentes S, Barker I, Simon R. 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. *Virology* 388:1-7. <https://doi.org/10.1016/j.virol.2009.03.024>.
- Ndunguru J, Taylor NJ, Yadav J, Aly H, Legg JP, Aveling T, Thompson G, Fauquet CM. 2005. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virol J* 2:45. <https://doi.org/10.1186/1743-422X-2-45>.
- Kehoe MA, Coutts BA, Buirchell BJ, Jones RAC. 2014. Plant virology and next generation sequencing: experiences with a *potyvirus*. *PLoS One* 9(8):e104580. <https://doi.org/10.1371/journal.pone.0104580>.
- Katoh K, Misawa K, Kuma K-i, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059-3066. <https://doi.org/10.1093/nar/gkf436>.
- Bao Y, Chetvermin V, Tatusova T. 2014. Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. *Arch Virol* 159:3293-3304. <https://doi.org/10.1007/s00705-014-2197-x>.