



First Complete Genome Sequence of *Suakwa aphid-borne yellows virus* from East Timor

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We present here the first complete genomic RNA sequence of the polerovirus *Suakwa aphid-borne yellows virus* (SABYV), from East Timor. The isolate sequenced came from a virus-infected pumpkin plant. The East Timorese genome had a nucleotide identity of 86.5% with the only other SABYV genome available, which is from Taiwan.

Received 30 May 2016 Accepted 6 June 2016 Published 28 July 2016

Citation 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 doi:10.1128/genomeA.00718-16.

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o examine possible connectivity between viruses infecting crops in northern Australia and nearby Southeast Asian countries, cucurbit samples from East Timor and Australia were studied. In July 2015, 15 leaf samples were collected from cucurbit plants in East Timor and blotted onto Fast Technology for nucleic acids (FTA) cards. In October 2015, 21 leaf samples were collected from cucurbit plants growing in the Broome district of the West Kimberley region of tropical northwest Australia. RNA extracts from 15 East Timorese and 21 Australian samples were subjected to next-generation sequencing. A complete genome of Suakwa aphid-borne yellows virus (SABYV) was obtained from a single pumpkin plant sample (DL76) from the Dili district of East Timor, but no SABYV was detected in any of the Australian samples. SABYV is a single-stranded RNA virus in the genus Polerovirus, family Luteoviridae. It causes leaf interveinal yellowing in cucurbit plants and occurs in the Philippines and Thailand in Southeast Asia, and in China and Taiwan in East Asia (1-3). Currently, there is only one complete SABYV genome in GenBank (accession no. JQ700308 from Taiwan) (4).

Total RNA was extracted from the samples using a ZR Plant RNA MiniPrep kit (Zymo Research), treated with RNase-free DNase (Invitrogen) and measured using Qubit (Invitrogen). RNA integrity was confirmed using RNA screen Tape (TapeStation 2200, Agilent Technologies). Libraries were prepared from total RNA extracts using a TruSeq Stranded Total RNA sample preparation kit with Ribo-Zero Plant (catalogue no. RS-122-2401, Illumina). Final size and concentration of each library was verified using Qubit and D1000 ScreenTape (TapeStation 2200). Sequencing was done with the MiSeq platform reagent kit version 2 (Illumina) using 2×251 paired-end runs with a 1% PhiX spike. Reads were assembled and genomes annotated using CLC Genomics Workbench (CLC Bio) and Geneious version 8.1.7 (Biomatters) (5); further alignment was done by MAFFT (6).

FTA card sample DL76 yielded 788,236 reads and, after trimming, 670,062 remained. *De novo* assembly generated 11 contigs; 13,045 reads were mapped to the contig of interest, giving a coverage of $330 \times$. The assembled full-length genome from DL76 was 5,845 nucleotides in length with the six open reading frames typical of the genus *Polerovirus*. A BLAST-based search through the Pairwise Sequence Comparison (PASC) tool (7) revealed an isolate resembling Taiwanese isolate TW19. Comparison of their genomic sequences found that East Timorese isolate DL76 had a pairwise nucleotide identity of 86.5% to JQ700308. There is a need for further sampling to establish whether SABYV has spread to Australia. Comparison of any Australian SABYV genomic sequences found with ones from neighboring Southeast Asian countries would then be required.

Nucleotide sequence accession number. The new sequence was deposited in GenBank under the accession number KX122023 (DL76).

ACKNOWLEDGMENTS

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

FUNDING INFORMATION

This work, including the efforts of Solomon Maina, was funded by Cooperative Research Centres, Australian Government Department of Industry (CRCs) (PBCRC61056).

The Cooperative Research Centre for Plant Biosecurity and the University of Western Australia provided a scholarship and operating funds to Solomon Maina. The Commonwealth Scientific and Industrial Research Organisation provided additional operating funds. The University of Western Australia and the Department of Agriculture and Food Western Australia provided laboratory facilities for this project.

REFERENCES

1. Shang QX, Xiang HY, Han CG, Li DW, Yu JL. 2009. Distribution and molecular diversity of three cucurbit-infecting poleroviruses in

China. Virus Res 145:341-346. http://dx.doi.org/10.1016/ j.virusres.2009.07.017.

- Knierim D, Deng TC, Tsai WS, Green SK, Kenyon L. 2010. Molecular identification of three distinct polerovirus species and a recombinant *Cucurbit aphid-borne yellows virus* strain infecting cucurbit crops in Taiwan. Plant Pathol 59:991–1002. http://dx.doi.org/10.1111/j.1365-3059.2010.02327.x.
- Knierim D, Tsai WS, Maiss E, Kenyon L. 2014. Molecular diversity of poleroviruses infecting cucurbit crops in four countries reveals the presence of members of six distinct species. Arch Virol 159:1459–1465. http:// dx.doi.org/10.1007/s00705-013-1939-5.
- 4. Knierim D, Tsai WS, Deng TC, Green SK, Kenyon L. 2013. Full-length genome sequences of four polerovirus isolates infecting cucurbits in Taiwan determined from total RNA extracted from field samples.

Plant Pathol 62:633-641. http://dx.doi.org/10.1111/j.1365 -3059.2012.02653.x.

- Kehoe MA, Coutts BA, Buirchell BJ, Jones RAC. 2014. Plant virology and next generation sequencing: experiences with a *Potyvirus*. PLoS One 9:e104580. http://dx.doi.org/10.1371/journal.pone.0104580.
- Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. http://dx.doi.org/10.1093/ nar/gkf436.
- Bao Y, Chetvernin V, Tatusova T. 2014. Improvements to pairwise sequence comparison (PASC): a genome-based web tool for virus classification. Arch Virol 159:3293–3304. http://dx.doi.org/10.1007/s00705-014 -2197-x.