

First Complete Genome Sequence of *Bean common mosaic necrosis virus* from East Timor

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We present here the first complete *Bean common mosaic necrosis virus* (BCMNV) genomic sequence isolated from virus-infected common bean (*Phaseolus vulgaris*) in East Timor, and compare it with six complete BCMNV genomes from the Netherlands, and one each from the United States, Tanzania, and an unspecified country. It most resembled the Netherlands strain NL-8 genome.

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To examine possible connections between viruses infecting crops in northern Australia and Southeast Asia, common bean (*Phaseolus vulgaris*) plants with virus-like symptoms were sampled in East Timor and northwest Australia (1–5). *Bean common mosaic necrosis virus* (BCMNV; genus *Potyvirus*, family *Potyviridae*) is spread nonpersistently by several aphid species and transmitted through infected seeds (6). It causes mosaic and curling of leaves and plant dwarfing and is important in common bean production in North America, East Africa, and Europe (6, 7). Before 1992, BCMNV was included within *Bean common mosaic virus* (8). It has not been reported from East Timor, northwest Australia (9), or the rest of Australia (6). Nine complete BCMNV genomes are available in GenBank: six from the Netherlands (including one duplicated genome), and one each from Tanzania, the United States, and an unspecified country (6, 7, 10). BCMNV was detected in only one sample (TM70) collected in May 2015 from the Aileu district of East Timor. It was sequenced, and a complete genome was obtained.

Fifteen East Timorese samples were blotted onto Fast Technology for Analysis of nucleic acids (FTA) cards (11). Total RNA was extracted from these cards using ZR Plant RNA MiniPrep kit (Zymo Research). The total RNA extracts were treated with RNase-free DNase (Invitrogen) and measured using Qubit (Invitrogen). RNA integrity was confirmed using RNA ScreenTape (TapeStation 2200, Agilent Technologies). Libraries were prepared from total RNA using a TruSeq stranded Total RNA sample preparation Ribo-Zero plant kit (catalogue no. RS-122-2401, Illumina). The final size and concentration of each library was verified using Qubit and D1000 ScreenTape (TapeStation 2200). Sequencing was by Macrogen Inc. using HiSeq 2500 with a TruSeq SBS kit version 4 (Illumina) with 151 cycles of paired-end reads. Reads were then assembled and genomes annotated using CLC Genomics Workbench version 6.5 (CLC bio) and Geneious version 8.1.7 (Biomatters) (12). Further alignment was by MAFFT (13).

FTA card sample TM70 yielded 2,248,678 reads and, after trimming, 1,948,678 remained. *De novo* assembly generated 108 contigs, and 858,904 reads were mapped to the contig of interest with coverage of 12,871×. The final complete genome sequence length was 9,640 nucleotides (nt) containing the 5' (157 nt) and 3' (242 nt) untranslated regions. The new sequence coded for 10 proteins, as with other potyviruses (14). A BLAST-based search using a pairwise sequence comparison tool (15) revealed that TM70 most resembled the Netherlands strain NL8 genome, accession number HQ229994 (7). Pairwise nucleotide alignment of these two isolates was 98.0% and was well within the 76% species demarcation limit for *Potyvirus* genomes (16, 17). Since no BCMNV was detected in any Australian samples, further sampling is needed to establish whether BCMNV 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). The sequence was deposited in GenBank under the accession number [KX302007](https://www.ncbi.nlm.nih.gov/nucl/1000000000).

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REFERENCES

1. Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RA. 2016. Complete genome sequences of the *Carlavirus Sweet potato chlorotic fleck virus* from East Timor and Australia. *Genome Announc* 4(3):e00414-16. <http://dx.doi.org/10.1128/genomeA.00414-16>.
2. Maina S, Edwards OR, Jones RA. 2016. First complete genome sequence of *Pepper vein yellows virus* from Australia. *Genome Announc* 4(3):e00450-16. <http://dx.doi.org/10.1128/genomeA.00450-16>.
3. Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RA. 2016. Complete genome sequences of the *Potyvirus Sweet potato virus 2* from East Timor and Australia. *Genome Announc* 4(3):e00504-16. <http://dx.doi.org/10.1128/genomeA.00504-16>.
4. Maina S, Edwards OR, de Almeida L, Ximenes A, Jones RA. 2016. First complete genome sequence of *Suakwa aphid-borne yellows virus* from East Timor. *Genome Announc* 4(4):e00718-16. <http://dx.doi.org/10.1128/genomeA.00718-16>.
5. 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:e00957-16, in press.
6. Worrall EA, Wamonje FO, Mukeshimana G, Harvey JJ, Carr JP, Mitter N. 2015. *Bean common mosaic virus* and *Bean common mosaic necrosis virus*: relationships, biology, and prospects for control. *Adv Virus Res* 93:1–46. <http://dx.doi.org/10.1016/bs.aivir.2015.04.002>.
7. Larsen RC, Druffel KL, Wyatt SD. 2011. The complete nucleotide sequences of bean common mosaic necrosis virus strains NL-5, NL-8 and TN-1. *Arch Virol* 156:729–732. <http://dx.doi.org/10.1007/s00705-011-0945-8>.
8. McKern NM, Mink GI, Barnett OW, Mishra A, Whittaker LA, Silbernagel MJ, Ward CW, Shukla DD. 1992. Isolates of bean common mosaic virus comprising two distinct potyviruses. *Phytopathology* 82:923–929. <http://dx.doi.org/10.1094/Phyto-82-923>.
9. Coutts BA, Kehoe MA, Webster CG, Wylie SJ, Jones RA. 2011. Indigenous and introduced potyviruses of legumes and *Passiflora* spp. from Australia: biological properties and phylogenetic placement. *Arch Virol* 156:1757–1774. <http://dx.doi.org/10.1007/s00705-011-1046-4>.
10. Fang GW, Allison RF, Zambolim EM, Maxwell DP, Gilbertson RL. 1995. The complete nucleotide sequence and genome organization of bean common mosaic virus (NL3 strain). *Virus Res* 39:13–23. [http://dx.doi.org/10.1016/S0168-1702\(95\)00072-0](http://dx.doi.org/10.1016/S0168-1702(95)00072-0).
11. 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. <http://dx.doi.org/10.1186/1743-422X-2-45>.
12. Kehoe MA, Coutts BA, Buirchell BJ, Jones RA. 2014. Plant virology and next generation sequencing: experiences with a *Potyvirus*. *PLoS One* 9:e104580. <http://dx.doi.org/10.1371/journal.pone.0104580>.
13. Katoh K, Misawa K, Kuma K, 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>.
14. Revers F, García JA, Karl M, Thomas CM. 2015. Molecular biology of potyviruses. *Adv Virus Res* 92:101–199. <http://dx.doi.org/10.1016/bs.aivir.2014.11.006>.
15. 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>.
16. Adams MJ, Antoniw JF, Fauquet CM. 2005. Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Arch Virol* 150:459–479. <http://dx.doi.org/10.1007/s00705-004-0440-6>.
17. King AMQ, Adams MJ, Lefkowitz EJ. 2011. *Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses*, vol 9. Elsevier Academic Press, San Diego, CA.