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Yambean mosaic virus and lettuce chlorosis virus in Australia

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Abstract

This is the first report of yambean mosaic virus and lettuce chlorosis virus in Australia. Both viruses were initially identified co-infecting the Fabaceae weed, *Calopogonium mucunoides* collected from Umagico in far north Queensland. Full coding sequences of both viruses were obtained with high throughput sequencing and confirmed with RT-PCR and Sanger sequencing. Lettuce chlorosis virus has subsequently been confirmed in several other species collected from far north Queensland and may become a new emerging threat to agronomical crops in Australia.

Keywords Crinivirus · Potyvirus · Fabaceae · Legume

Yambean mosaic virus (YBMV) belongs to the family *Potyviridae*, has a monopartite, positive-sense, single-stranded RNA genome and is transmitted non-persistently by aphids (Fuentes et al. 2012; Damayanti et al. 2008). YBMV was first described in 2012 when the complete genome of a new potyvirus was isolated from yambean in Peru (Fuentes et al. 2012). YBMV has also been isolated from yambean in Indonesia and black bean and yard long bean from Vietnam (Damayanti et al. 2008; Ha et al. 2008).

Lettuce chlorosis virus (LCV), a bi-partite crinivirus, was first identified in the USA in 1991 and has since been identified in Spain, China, Brazil, and Israel (Duffus et al. 1996; Hadad et al. 2019; Ruiz et al. 2014; Zhang et al. 2017; Favara et al. 2020). Criniviruses are positive-sense, single-stranded, mostly bi-partite RNA viruses belonging to the *Closteroviridae* family. They are transmitted semi-persistently by whiteflies, *Bemisia* and *Trialeurodes* species, and primarily infect herbaceous hosts.

During a 2021 disease survey of the Northern Peninsula Area (NPA) of Cape York region, Queensland, Australia, a sample of *Calopogonium mucunoides* Desv. was collected displaying wrinkled leaves with a mild mosaic. Total nucleic acid was extracted using the BioSprint 15 workstation with a BioSprint Plant DNA kit (QIAGEN, catalogue no. 941,514) as per manufacturer's instructions but without the use of RNase A. To identify a suspected potyvirus, cDNA was synthesised using Superscript IV (Thermo Fisher) as per instructions using the poty1 primer (Gibbs and Mackenzie 1997) and Mango Taq DNA polymerase (Bioline, USA) with the universal potyvirus primers U341 and D341 as per (Langeveld et al. 1991). A band of the expected size was obtained and sent for direct sequencing (Macrogen, Korea). The 300 nucleotide (nt) fragment obtained shared 85% nucleotide sequence identity to yambean mosaic virus (YBMV) isolate JN190431. To obtain the complete sequence using high throughput sequencing, total RNA was extracted using Trizol® reagent (Invitrogen) as per Reinhart et al. (2002). Library preparation (Truseq stranded total RNA with Ribo Zero plant library kit) and 150 bp paired-end sequencing with Novaseq6000 was done by Macrogen. Trimming for quality and adaptor and primer sequence removal was carried out using the Trim Galore tool (Krueger 2021) available on Galaxy Australia (https://usegalaxy.org.au/). The total number of reads obtained was

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Table 1 YBMV and LCV isolates identified in Queensland Australia

	Host	family	location	symptoms
YBMV Isolate name				
5693	*Calopogonium mucunoides*	Fabaceae	Umagico	Mild mosaic and wrinkling
LCV Isolate name				
5693	*Calopogonium mucunoides*	Fabaceae	Umagico	Mild mosaic and wrinkling
5886	*Chamaecrista rotundifolia*	Fabaceae	New Mapoon	Mottling
5887	*Calopogonium mucunoides*	Fabaceae	New Mapoon	Mottling
5888	*Chamaecrista rotundifolia*	Fabaceae	New Mapoon	Mottling/yellowing
5889	*Crotalaria goreensis*	Fabaceae	New Mapoon	reddening
5890	*Macroptilium atropurpureum*	Fabaceae	Injinoo	N/R
5891	*Macroptilium atropurpureum*	Fabaceae	Seisia	N/R

Isolate in bold subjected to HTS

N/R, not recorded

46,334,156 which was reduced to 46,276,632 after trimming. Reads were paired and the *de novo* assembly was done using CLC genomic workbench 12.0 (CLCGW) (CLC bio) as per Filardo et al. (2019). The number of contigs produced was 102,710 which were used in a BLAST search using the NCBI plant viral database. Contigs and BLAST results were analysed further in Geneious 10 (Biomatters).

One single contig of 9,630 nt (12,456,114 reads, average coverage of 186,226), which contained the 300 nt RT-PCR fragment (99% nt identity) was obtained and shared 76% nt similarity across the whole genome and 85% amino acid (aa) identity in the polyprotein with YBMV isolate JN190431. According to ICTV, different potyvirus species share less than 76% nt identity across the whole genome and

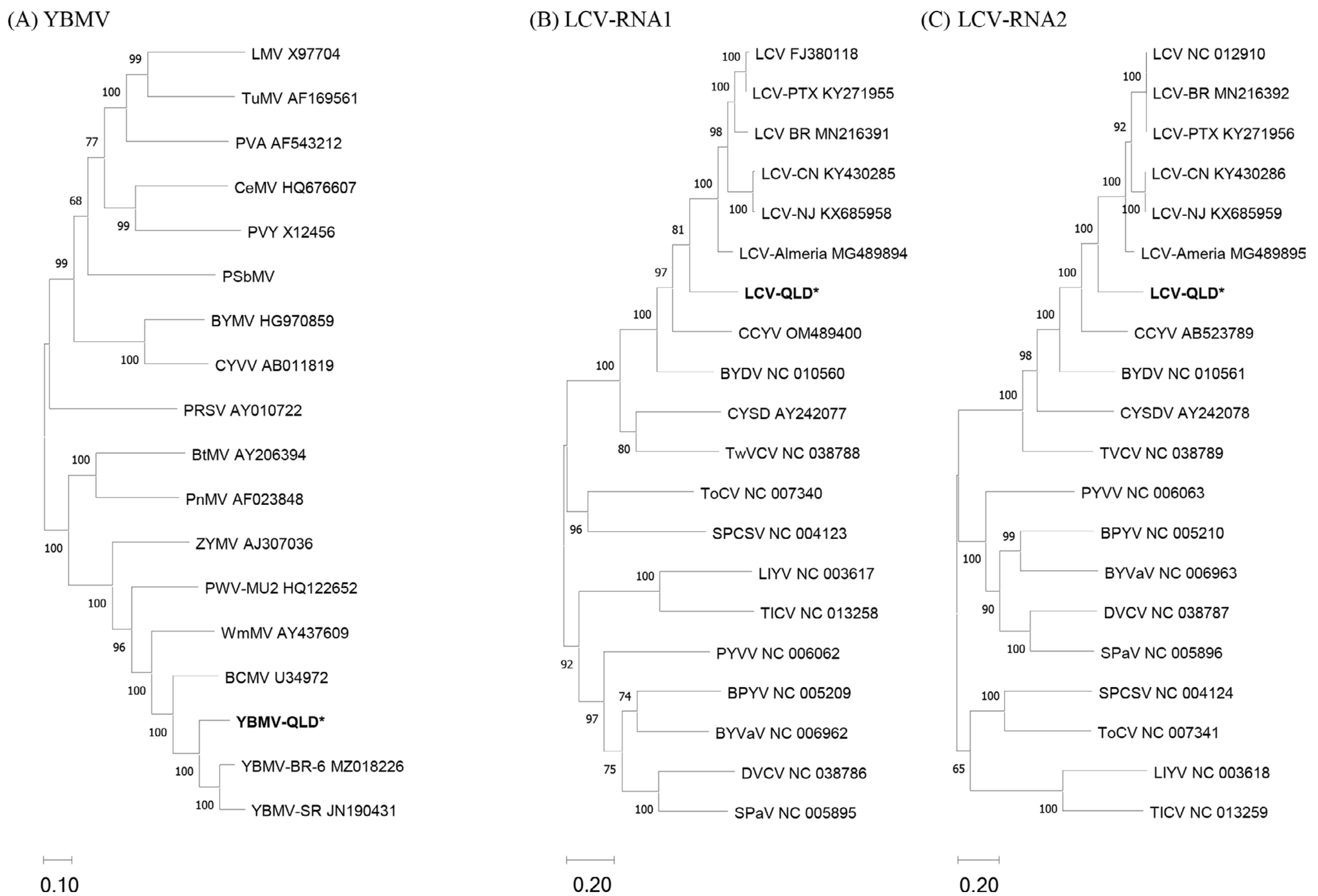

Fig. 1 Maximum-likelihood phylogenetic trees based on nucleotide sequence alignments of (A) YBMV and other members of the *Potyvirus* genus, (B) LCV RNA1 and (C) LCV RNA2 and other members of the *Crinivirus* genus. Sequences were aligned using ClustalW (Geneious 10.2.6) and Maximum-likelihood phylogenetic trees were created in MEGA 11, Tamura-Nei model and 500 bootstrap replicates

80% aa sequence identity in the polyprotein. This is therefore the first identification of YBMV in Australia and in the legume weed *Calopogonium mucunoides* (Table 1; Fig. 1). Freeze-dried leaves of the YBMV-QLD isolate were lodged in the Queensland Department of Agriculture and Fisheries (QDAF) Plant Virus Collection as number 5693. The YBMV genome sequence was lodged in GenBank as accession number OQ377538.

In addition to YBMV, two other contigs, 8589 nt (608,090 reads, average coverage 9,641) and 8672 nt (1,275,002 reads, average coverage 23,147) were obtained from isolate 5693, with the closest match by BLAST being lettuce chlorosis virus (LCV) RNA1 and RNA2 respectively. Across the whole genome, the RNA1 contig shares 72% nt identity to LCV RNA1 (FJ380118) and the RNA2 contig shares 70% nt identity to LCV RNA2 (FJ380119) (Fig. 1). Species demarcation for criniviruses is an amino acid sequence identity less than 75% in the RNA-dependent RNA polymerase (RdRp), coat protein (CP) and Heat shock protein 70 homolog (HSP70h) gene products. This isolate shares 81%, 93, % and 93% aa identity to the LCV reference species for the RdRP, coat protein and HSP70h regions respectively.

The presence of both LCV RNA1 and RNA2 in isolate 5693, was confirmed by RT-PCR, as described above but using primers LCV_6648F1 GATCTAAGTATGGTAAACTTAAGAG and LCV_7261R1 AGAATCATCTCCACTCACAAGG for RNA1 and LCV_2133F2 AACATTATCAGTCTCAATGTTTCCG and LCV_2914R2 GCCATAGGTTTCTTACAAGAACC for RNA 2, with the reverse primer being used for cDNA synthesis in both cases. Expected bands of 613 nt and 781 nt for RNA1 and RNA2 respectively were obtained and confirmed to be LCV with Sanger sequencing. The LCV-QLD genome sequences were lodged in GenBank as accession numbers OQ377539 and OQ377540 for RNA1 and RNA2 respectively.

RT-PCRs using the LCV primers on additional plant samples collected from the survey, showed that LCV was wide spread throughout the NPA area surveyed in several common weedy hosts from the Fabaceae family (Table 1). In contrast, YBMV primers used to amplify the newly found Australian isolate (YBMV_9067F TCAGAGGCAGCTGAAGCCTATATTG or YBMV_9112F CCATACATGCCTA-GGTATGGTCTTCT) with the Poty 1 reverse primer revealed that YBMV is not as widespread, and no other isolates were found in the region from the collected hosts.

Overseas isolates of LCV have been shown to infect a wide range of plant species from several families such as Fabaceae, Asteraceae, Amaranthaceae, Solanaceae, Passifloraceae, Cannabaceae, Convolvulaceae and Malvaceae to name a few (Duffus et al. 1996; Hadad et al. 2019; Vidal et al. 2021). Since LCV has a wide host range and the whitefly vector, *Bemisia argentifolii* (or B. *tabaci* MEAM1) is widespread throughout Australia, including the NPA, there is potential for this virus to spread throughout the agricultural production areas in Australia. Recorded symptoms to look for are interveinal yellowing, yellowing and/or reddening, mosaic, blistering, leaf deformation and brittleness of leaves. However, as there may be differences in host specificity and the disease severity caused by different strains of both LCV and YBMV, pathogenicity testing of key hosts with these genetically distinct Australian isolates would be required to determine if the severity of disease and host range is similar to that recorded for exotic isolates.

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Declarations

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest The authors declare there is no conflict of interest.

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