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Clitoria yellow mottle virus: a tobamovirus from Northern Australia

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Abstract A tobamovirus was isolated from feral plants of the African forage legume *Clitoria ternatea* collected in 1995 from two out of nineteen sites in Northern Australia. This virus, which we call Clitoria yellow mottle virus, is most closely related to, although distant from, sunnhemp mosaic virus in all regions of its genome.

Keywords Tobamovirus · Clitoria yellow mottle virus · Genome sequence

There are several species of butterfly peas (*Clitoria* spp.) in tropical Northern Australia. Many are endemic but the most widespread is the African forage species *Clitoria ternatea* (http://www.chah.gov.au/avh/avhServlet). Plants of *C. ternatea* are frequently chlorotic, and may show vein clearing. Leaves from plants showing these symptoms were collected in March/April 1995 from the border of a car park at Larrimah Wayside Inn on the Stuart Highway, Northern Territory (15.7767° S, 128.7386° E), and were dried over silica gel. Sap extracts from these leaves were manually inoculated to a range of test plants grown in a glasshouse in Canberra, and produced a novel combination of virus symptoms in different hosts. Infected *Clitoria ternatea* seedlings showed local chlorotic lesions and a systemic vein clearing followed by mottling and mosaic of the tip leaves (Fig. 1). Local chlorotic and pale necrotic lesions developed in the inoculated leaves of *Chenopodium quinoa, Nicotiana clevelandii, N. glutinosa* and *Pisum sativum*, and chlorotic lesions with dark spreading haloes in the inoculated primary leaves of *Phaseolus vulgaris* (cv Purple King) and with systemic chlorotic flecking. When sap extracts from these plants were tested by inoculating the primary leaves of Purple King bean seedlings, all produced the same chlorotic lesions with spreading dark haloes. No symptoms were shown by inoculated seedlings of *Brassica chinensis, Cucumis sativa, Lactuca sativa, Lycopersicon esculentum, Ocimum basilicum* or *Zinnia elegans*, and when sap extracts from them were inoculated to the leaves of Purple King beans no symptoms appeared.

Sap extracts from leaves of *C. ternatea, N. clevelandii* and *N. glutinosa* contained particles with the shape and size typical of tobamovirus virions. We conclude that the symptoms in the test plants were caused by a single virus, which we call clitoria yellow mottle virus (CYMV).

CYMV virions were extracted by blending infected tissue in 0.05 M sodium phosphate pH 7.5 containing 0.1 % thioglycollate, and the extract clarified by emulsifying with n-butanol and chloroform. The virions were purified by differential centrifugation, and gave diagnostic symptoms when inoculated to Purple King beans.. Standard methods were used to extract nucleic acid from virions and to sequence it (Maniatis et al. 1982; Sambrook et al. 1989). cDNA was synthesized from CYMV RNA using random hexamer nucleotides and avian myeloblastosis reverse transcriptase, and was transcribed into dsDNA by the RNase H method (Gubler and Hoffman 1985). The dsDNA was hydrolysed using several four-base restriction endonucleases, and the resulting fragments were separated to give a series of overlapping clones, and these were sequenced.

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Fig. 1 Tip leaf of a seedling of *Clitoria ternatea* sap inoculated with Clitoria yellow mottle tobamovirus 1 month earlier

The 5′ and 3′ terminal sequences were determined using standard RACE methods (Maniatis et al. 1982). Each nucleotide of the sequence was determined from at least two, usually more, independent overlapping clones in both orientations. Sequences were mostly manipulated using BIOEDIT (Hall 1999).

The complete CYMV sequence (Genbank Accession Code JN566124) is 6,514 nucleotides long. The Genbank sequence database was searched with subsegments of the CYMV sequence using SWeBLAST (Fourment et al. 2008). With the BLASTN facility and a window length of 100 nucleotides and step of 50 nucleotides about half the 129 CYMV subsequences matched various tobamoviruses most closely, and half of those were matches of sequences of sunnhemp mosaic tobamovirus (synonym: cowpea TMV) (SHMV) (Kassanis and Varma 1975; Meshi et al. 1982; Meshi et al. 1981). When the BLASTX facility was used with a window length of 150 nucleotides (50 codons) and step of 50, 119 of the 128 CYMV subsequences matched SHMV sequences, and the other nine had no significant match. This indicates that CYMV is not a genetic recombinant of SHMV and another tobamovirus; any recombination event in the origin of CYMV preceded the divergence of CYMV and SHMV. This pattern of matching confirms that, among all tobamoviruses, CYMV is most closely related to, but distant from, SHMV, as reported in previously published phylogenetic analyses (Gibbs 1999; 2008).

The genome of CYMV has the same structure as most other tobamoviruses (Stobbe et al. 2011). The MolQuest-Softberry viral gene detector (http://www.softberry.com) found four open reading frames (ORFs) in the CYMV sequence. The amino acid sequences encoded by the two ORFs closest to the 5′ end of the sequence included motifs typical of viral RNA replicases, and are linked by a leaky stop codon motif as in other tobamoviruses (Skuzeski et al. 1991). This region has the sequence "AAAUAGCAAUUA CAGAUC", which encodes "K*QLQI". The complete replicase protein is 1,632 amino acids long, and has 70.8 % identity with the SHMV replicase. The third ORF from the 5′ end of the genome encodes a protein which has 60.9 % identity with the movement protein of SHMV. The 3′-terminal ORF encodes a typical tobamovirus coat protein of 163 amino acids with 66.4 % sequence identity to that of SHMV.

On the same 1995 field trip to the Northern Territory and north-west Western Australia, samples of other chlorotic *C. ternatea* samples were collected from eighteen sites and tested in the same way, but only one of them yielded CYMV, as judged by the shape and size of its virions and the symptoms it caused. It came from a courtyard hedge at Hotel Kununurra, Messmate Way, Kununurra, Western Australia (15.5748° S, 133.2164° E); 480 kms from the Larrimah site. Its gene sequences were not determined. The fact that a virus was not isolated from most of the chlorotic *C. ternatea* samples indicates that the presence of a virus and the symptoms shown by the sampled plants were not causally related, and, as the samples were collected from the tropical monsoon region of Australia at the end of the dry season, it is most likely that drought caused the symptoms.

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