Arch Virol (2017) 162:2473–2476
DOI 10.1007/s00705-017-3333-1

ANNOTATED SEQUENCE RECORD

Molecular characterization of a novel bipartite begomovirus isolated from *Lycianthes biflora* in China

Y. F. Tang[1,2] · Z. F. He[1,2] · J. K. Brown[3] · X. M. She[1] · G. B. Lan[1]

Received: 6 November 2016 / Accepted: 21 February 2017 / Published online: 25 April 2017

Abstract A bipartite begomovirus isolate GD was isolated from *Lycianthes biflora* plants showing yellow mosaic symptoms in Nanxiong, Guangdong Province, China. The apparently full-length DNA-A and DNA-B viral components were cloned after enrichment of circular DNA by rolling circle amplification, restriction digestion, cloning, and DNA sequencing. The DNA-A component (2752nt, KT582302) shares highest (80.2%) nucleotide (nt) sequence identity with tomato leaf curl Sulawesi virus [Indonesia-Sulawesi-Langowan F101-2006] (ToLCSuV-[ID-Sul -LanF09-06], FJ237618), reported in Indonesia as causing yellow leaf curl disease of chilli pepper. The DNA-B component (2704nt, KT582303) shares highest (76.3%) nt sequence identity with pepper yellow leaf curl Indonesia virus-[Indonesia-tomato2-2005] (PepYLCIV-[ID-Tom2-05 AB213599) reported in Indonesia, and associated with yellow leaf curl disease in tomato. Based on the ICTV guidelines for begomoviral species demarcation, the virus is a new, previously undescribed bipartite begomovirus species for which the name "*Lycianthes yellow mosaic virus*" is proposed.

Begomoviruses (genus, *Begomovirus*; family, *Geminiviridae*) are important viral pathogens that cause diseases of cotton, vegetables, and other dicotyledonous crop plants, worldwide [1]. They represent the largest genus within the *Geminiviridae*, comprising 322 recognized species (http://www.ictvonline.org). Begomoviruses are transmitted in a persistent manner by the whitefly *Bemisia tabaci* (Gennadius) sibling species group. They have a bipartite genome consisting of two single-stranded circular DNA components, referred to as DNA-A and DNA-B, or a monopartite genome comprised of a single DNA-A-like component of approximately 2.7 kb in size. All begomoviruses endemic to the New World, except for the recently discovered monopartite tomato leaf deformation virus (ToLDeV), reported in Peru and Ecuador [2–4], have bipartite genomes. Some bipartite begomoviruses are endemic to the Old World as well, and co-exist, sometimes in a mixed infection, with those having a monopartite genome, consisting of a single DNA-A component. Many monopartite begomoviruses have been found associated with different types of non-viral molecules, referred to as 'satellites', which are approximately 1.3 kb in size [5–10].

During May, 2015, leaf samples were collected from five *Lycianthes biflora* (Lour. Bitter) (*Solanaceae*) plants exhibiting yellow mosaic symptoms, planted along the Meiling ancient road in Nanxiong, Guangdong Province of China (Fig. 1). Total DNA was extracted from five symptomatic leaves of each *L. biflora* plant, using the CTAB method [11]. The results of polymerase chain reaction (PCR) amplification with degenerate primers AV494/CoPR [12, 13], cloning, and DNA sequencing, indicated the presence of a novel begomovirus.

The suspect begomoviral genome(s) were cloned from purified, total nucleic acid preparations by first enriching for circular DNA using rolling-circle amplification (RCA) (TempliPhi^{TM}kit; GE Healthcare, Buckinghamshire, UK), followed by digestion with *Bam*H I and *Pst* I

✉ Z. F. He
hezf@gdppri.com

[1] Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

[2] Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Guangzhou 510640, China

[3] School of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA

Fig. 1 Symptomatic *Lycianthes biflora* plants exhibiting foliar yellowing and mosaic symptoms

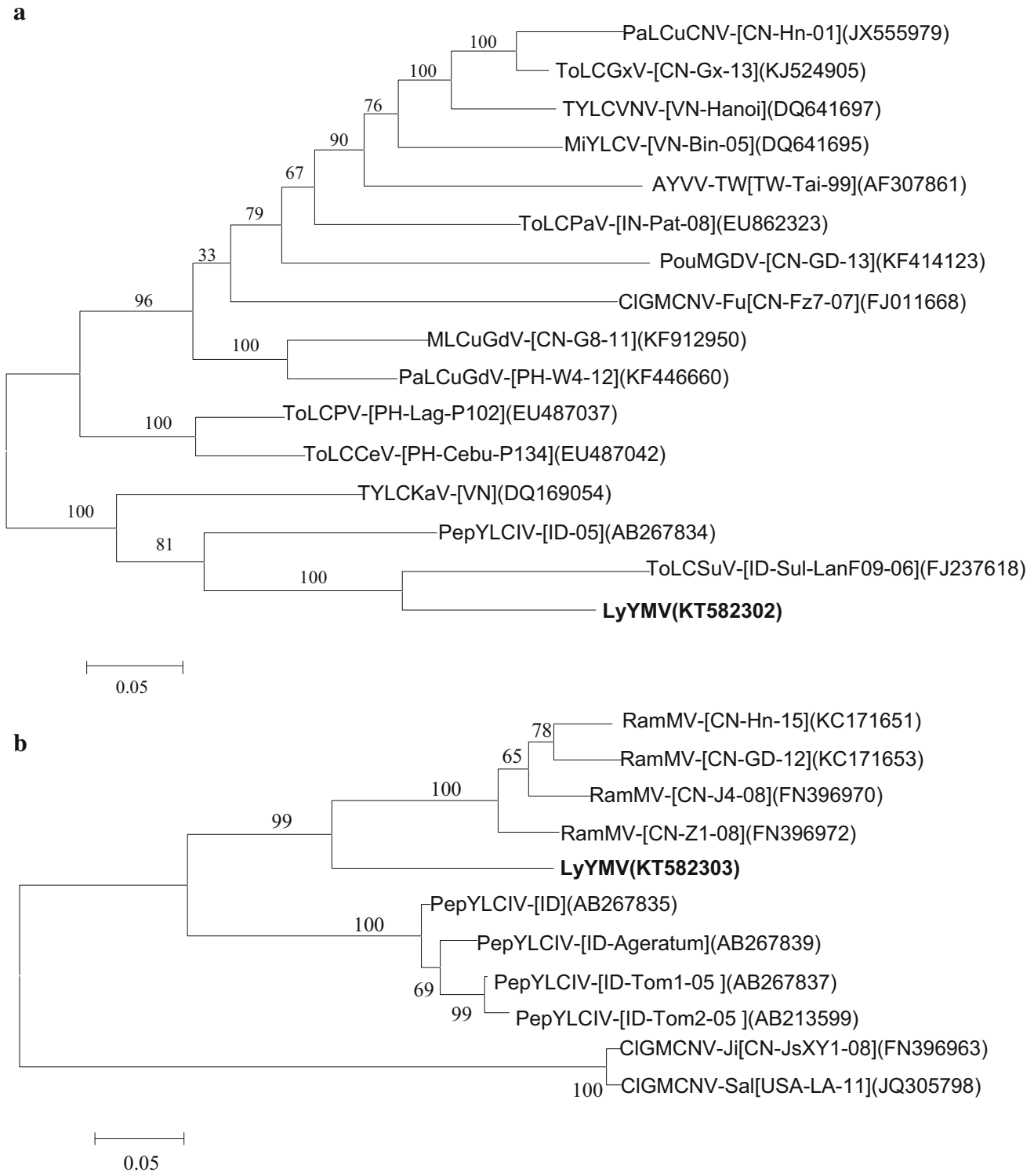
endonucleases (Fermentas, GlenBurnie, Maryland, USA) respectively. The resultant expected-size fragments corresponding to full-length begomoviral genomic components were separated by agarose gel (1.0%) electrophoresis in 1×TAE buffer, pH 8.0, gel-purified, and ligated into the plasmid vector pGEM-3Z (Promega Co.Madison, WI, USA), previously digested with the respective restriction enzymes. The recombinant plasmids were transformed into the bacterial host, *Escherichia coli* DH5α. Three clones from each sample were selected to sequence (Invitrogen Co., Shanghai, China). The DNA sequences were assembled, edited, and analyzed using DNAStar software version 5.0 (DNAStar Inc. Madison,WI, USA). A similarity search for each sequence was carried out using Blastn [14] to identify the most closely related isolates among the sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov). Pairwise nucleotide (nt) sequence analysis of the complete genome sequences, and of the predicted viral coding regions were determined using the Sequence Demarcation Tool (SDT1.2) [15]. Phylogenetic analysis was conducted in MEGA 5.2 using the Maximum Likelihood (ML) method [16].

Among the five samples (fifteen clones), apparently full-length, begomoviral genome sequences of either 2,752 or 2,704 nt were obtained from the RCA products digested by *Bam*H I and *Pst* I, respectively. The Blastn (GenBank) results showed that the sequence of the 2,752 nt molecule had highest similarity with other previously reported begomovirus DNA-A components, whereas, the 2,704 nt molecule was most similar to other begomovirus DNA-B components. The cloned DNA-A and DNA-B component sequences, respectively, shared 99-100% nt identity to one another, and one representative sequence for each was deposited in GenBank with the accession number KT582302, and the GenBank accession number KT582303.

The DNA-A component (accession KT582302) encoded seven predicted open reading frames (ORF). The two ORFs (nt coordinates are indicated parenthetically), AV1 (286-1086) and AV2 (57-467) were located on the virion sense DNA strand, whereas, the five ORFs AC1 (1511-2599), AC2 (1204-1611), AC3 (1059-1469), AC4 (2185-2422), and AC5 (559-957), were located on the complementary sense strand. The common region (CR) of 173 nt for bipartite begomoviruses, within a non-coding intergenic region between the AC1 and AV2 ORF, was present on the DNA-A component, and contained the 'hallmark' geminivirus-conserved nonanucleotide sequence, TAA-TATTAC, required for the initiation of rolling circle replication of geminiviruses [17].The DNA-B component (accession KT582303) encoded two predicted ORFs, with the BV1 ORF (182-1012) located on the virion sense strand and BC1 (1026-1862) on the complementary sense strand. The CR associated with the DNA-B component was 191 nt, which was 18 nt longer than the DNA-A component CR. Thus, they shared only 86.7% identity, but nonetheless, harbored an identical inverted repeated sequence, or 'iteron', GCGGCCCTCG, involved in viral *rep*-protein binding.

The pairwise distance analysis conducted using the Sequence Demarcation Tool (SDT) showed that the complete sequence of the DNA-A and DNA-B components shared less than 81 and 77% nt identity, with the most closely related, previously reported begomoviral components, respectively. The DNA-A component shared highest nt identity, at 80.2%, with tomato leaf curl Sulawesi virus-[Indonesia-Sulawesi-LangowanF101-2006] (ToLCSuV-[ID-Sul-LanF09-06], FJ237618) reported in Indonesia, and associated with yellow leaf curl disease of chilli pepper [18].Whereas, the DNA-B component shared highest similarity, at 76.3%, with the DNA-B component of pepper yellow leaf curl Indonesia virus-[Indonesia-tomato2-2005] (PepYLCIV-[ID-Tom2-05], AB213599) from Indonesia, associated with yellow leaf curl disease of tomato [19].

Phylogenetic analysis (ML at >70% bootstrap and 1000 iterations) of the DNA-A component and the 99 sequence hits (Blastn) indicated that it clustered with ToLCSuV-[ID-Sul-LanF09-06] to form a unique clade, in relation to the 15 other begomoviruses included in the analysis (Figure 2a). Phylogenetic analysis of the DNA-B component and the 10 closest relatives (Blastn) indicated that it grouped with Ramie mosaic virus (RaMV)-[China-Hunan-2015], RaMV-[China-Guangdong-2012], RaMV-[China-Jiangsu-J4-2008], and RaMV-[China-Zhejiang-Z1-2008], forming a unique clade, with respect to all other DNA-B components analyzed here (Figure 2b).



No beta- or alpha-like satellite molecules were obtained, based on an expected size fragment of 1.3 kbp or smaller, following digestion of RCA products. Further no band(s) of the expected size were obtained by PCR amplification of the RCA product using the beta-satellite-specific primer pair β01/β02[20], suggesting the absence of an associated beta- or alpha-satellite(s) in the symptomatic *L. Biflora* plants.

◀**Fig. 2** Phylogenetic trees showing the relationships of the apparent full-length nucleotide sequences of the DNA-A component (a) and DNA-B component (b) of lycianthes yellow mosaic virus, with the most closely related begomovirus components, respectively. The tree was reconstructed using the Maximum Likelihood method implemented in MEGA 5.2 (on-line). The bootstrap (>70%) consensus tree was inferred from 1000 iterations. Legend: AYVV: Ageratum yellow vein virus, MiYLCV: Mimosa yellow leaf curl virus, TYLCVNV: Tomato yellow leaf curl Vietnam virus, PaLCuCNV: Papaya leaf curl China virus, ToLCGxV: Tomato leaf curl Guangxi virus, ToLCPaV: Tomato leaf curl Patna virus, MLCuGdV: Malvastrum leaf curl Guangdong virus, PaLCuGdV: Papaya leaf curl Guangdong virus, PouMGDV: Pouzolzia mosaic Guangdong virus. CIGMCNV: Clerodendrum golden mosaic China virus, ToLCPV: Tomato leaf curl Philippine virus, ToLCCeV: Tomato leaf curl Cebu virus, TYLCKaV: Tomato yellow leaf curl Kanchanaburi virus, ToLCSuV: Tomato leaf curl Sulawesi virus, PepYLCIV: Pepper yellow leaf curl Indonesia virus, RamMV: Ramie mosaic virus

Based on the highest shared nt identity, at 80.2%, for the DNA-A component with its closest relative, ToLCSuV-[ID-Sul-LanF09-06], and at 76.3% for DNA-B with its closest relative, PepYLCIV-[ID-Tom2-05], and in accordance with the threshold of 91% (DNA-A component) for the begomovirus species demarcation [21], the begomovirus isolate associated with symptomatic *L. biflora*is is a previously undescribed, bipartite begomovirus species, for which the name "*Lycianthes yellow mosaic virus"* is herein proposed. Isolates of this virus should be referred to as lycianthes yellow mosaic virus, abbreviated to LyYMV.

Acknowledgments This work was funded by the National Natural Science Foundation of China (No. 31501606), Guangdong Provincial Natural Science Foundation of China (No. 2014A030313571), the Science and Technology Program of Guangdong, China (No. 2014B070706017) and the President funds of Guangdong Academy of Agricultural Sciences of China (201424).

Compliance with ethical standards

Conflict of interest All of the authors declare that they have no conflict of interest.

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

References

1. Varma A, Mandal B, Singh MK (2011) Global emergence and spread of whitefly (*Bemisia tabaci*) transmitted Geminiviruses. In: Thompson WMO (ed) The whitefly, *Bemisia tabaci* (Homoptera: *Aleyrodidae*) interaction with geminivirus-infected host plants. Springer, The Netherlands, pp 205–292
2. Melgarejo TA, Kon T, Rojas MR, Paz-Carrasco L, Zerbini FM, Gilbertson RL (2013) Characterization of a New World monopartite begomovirus causing leaf curl disease of tomato in Ecuador and Peru reveals a new direction in geminivirus evolution. J Virol 87:5397–5413
3. Márquez-Martín B, Aragón-Caballero L, Fiallo-Olivé E, Navas-Castillo J, Moriones E (2011) Tomato leaf deformation virus, a novel begomovirus associated with a severe disease of tomato in Peru. Eur J Plant Pathol 129:1–7
4. Sánchez-Campos S, Martínez-Ayala A, Márquez-Martín B, Aragón-Caballero L, Navas-Castillo J, Moriones E (2013) Fulfilling Koch's postulates confirms the monopartite nature of tomato leaf deformation virus, a begomovirus native to the New World. Virus Res 173:286–293
5. Saunders K, Bedford ID, Briddon RW, Markham PG, Wong SM, Stanley J (2000) A unique virus complex causes Ageratum yellow vein disease. Proc Natl Acad Sci 97:6890–6895
6. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, Dhawan P, Rishi N, Siwatch SS, Abdel-Salam AM, Brown JK, Zafar Y, Markham PG (2003) Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses. Virology 312:106–121
7. Briddon RW, Bull SE, Amin I, Mansoor S, Bedford ID, Rishi N, Siwatch SS, Zafar Y, Abdel-Salam AM, Markham PG (2004) Diversity of DNA 1: a satellite-like molecule associated with monopartite begomovirus-DNA β complexes. Virology 324:462–474
8. Zhou XP, Xie Y, Tao X, Zhang Z, Li Z, Fauquet CM (2003) Characterization of DNAβ associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA-A. J Gen Virol 84:237–247
9. Xie Y, Wu PJ, Liu P, Gong HR, Zhou XP (2010) Characterization of alphasatellites associated with monopartite begomovirus/betasatellite complexes in Yunnan, China. Virol J 7:178–187
10. Briddon RW, Stanley J (2006) Subviral agents associated with plant single-stranded DNA viruses. Virology 344:198–210
11. Doyle JJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
12. Wyatt SD, Brown JK (1996) Detection of subgroup III geminivirus isolates inleaf extracts by degenerate primers and polymerase chain reaction. Phytopathology 86:1288–1293
13. He ZF, Yu H, Luo FF (2004) Detection of whitefly-transmitted geminivirusesfrom tomato by PCR. Virol Sinca 19:67–69
14. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
15. Muhire B, Varsani A, Martin DP (2014) SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:e108277
16. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
17. Stenger DC, Revington GN, Stevenson MC, Bisaro DM (1991) Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. Proc Natl Acad Sci 88:8029–8803
18. Tsai WS, Shih SL, Green SK, Lee LM, Luther GC, Ratulangi MM, Sembel DT, Jan FJ (2009) Identification of a new begomovirus associated with yellow leaf curl diseases of tomato and pepper in Sulawesi, Indonesia. Plant Dis 93:321
19. Kon T, Hidayat SH, Ito K, Hase S, Takahashi H, Ikegami M (2005) Begomoviruses associated with leaf curl disease of tomato in Java, Indonesia. J Phytopathol 153:562–566
20. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG (2002) Universal primers for the PCR-mediated amplification of DNA β. Mol Biotechnol 20:315–318
21. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Briddon RW, Fiallo-Olivé E, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593–1619