Nanopore sequencing of tomato mottle leaf distortion virus, a new bipartite begomovirus infecting tomato in Brazil

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Research Article

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Abstract

During a survey in a tomato field in Luziânia (Goiás State, Brazil), a plant showing mottling, chlorotic spots, and leaf distortion was found. A new bipartite begomovirus was found by Nanopore sequencing, and the full DNA-A sequence was confirmed by Sanger sequencing. The highest nucleotide identity match of this DNA-A genome (2596 bases) was 81.65% with tomato golden leaf deformation virus (HM357456). Due to the current species demarcation criterion of 91% of nucleotide identity (DNA-A), we propose it as a new member of the genus *Begomovirus*, named Tomato mottle leaf distortion virus.

Main Text

Viruses of the genus *Begomovirus* (family *Geminiviridae*) have emerged as important plant pathogens in tropical and subtropical regions [1]. Begomoviruses have small, circular, single-stranded DNA genomes and are vectored by whiteflies of the *Bemisia tabaci* cryptic species complex. The genome is arranged either in two components, referred to as DNA-A and DNA-B, or in a single component [2]. Begomoviruses have a wide diversity due to high mutation [3] and recombination frequencies [4]. Notably, tomato plants are hosts to many begomoviruses in Brazil, and the emergence of new species has been continuously observed [5]. Here, we describe a new bipartite tomato begomovirus sequence discovered in Brazil.

During a survey in a processing tomato field in Luziânia, Goiás state, Brazil (16°18’50.8” S, 47°42’35.2” W) in April 2020, a tomato plant (cv. AP533) with strong symptoms of mottling, leaf distortion, chlorotic spots, vein clearing, and mosaic (Fig. 1a) was observed. Total DNA was extracted, and the viral circular DNA was amplified by rolling-circle amplification (RCA, New England Biolabs – NEB, Ipswich, USA). Then, the RCA product was partially digested with PstI, BglII, NcoI, and EcoRI (NEB), pooled and sequenced using the Ligation Sequencing Kit (SQK-LSK109) (Oxford Nanopore Technologies - ONT, Oxford, UK). Briefly, the digested DNA was treated with NEBNext Ultra™ II End Repair/dA-Tailing Module (NEB), ligated to AMX sequencing adapter with NEB Blunt/TA ligation Master Mix (NEB), and loaded onto the Flongle flow cell (FLO-FLG106) on a MinION Mk1B (ONT). Raw FAST5 files were base-called under high accuracy mode (Guppy 4.4.2). Draft genomes were generated with TideHunter [6] and polished with Medaka v1.2.3 (https://github.com/nanoporetech/medaka). The contigs were transferred to Geneious 8.1.9 software (Biomatters Ltd. Auckland, New Zealand) and analyzed using the BLAST algorithm. Contigs presenting high identity with DNA-A and -B of begomoviruses were detected, and the full genome sequence of a potential new bipartite begomovirus was identified. To confirm it, the DNA-A was Sanger sequenced from the RCA product, and a dimeric genome was cloned into pCAMBIA0380 (Cambia Laboratory, Canberra, Australia) after partial digestion by NcoI (Thermo Fisher Scientific, Waltham, USA).

The assembled DNA-A genomes from the RCA product, 2 clones, and the Nanopore sequence contig were highly similar with 0-2 nucleotides mismatch. Clone 16 (GenBank MW561191) was 2596 nt-long, while the DNA-B consensus sequence from Nanopore was 2578 nt-long (GenBank MW650837). The genome organization was typical of bipartite New World begomoviruses (Fig. 1b; DNA-A: CP, Rep, TrAP, REn, AC4; DNA-B: NSP, MP). The conserved geminiviral nonanucleotide sequence (TAATATTAC) was detected in

both components, and they shared a common region of 180 nt with 98.33% identity. Furthermore, upstream the TATA box, the putative Rep binding sites TGTAA and TGTAC were identified in both segments, strongly suggesting that they are cognate DNA segments. These putative core iteron sequences were observed in two tomato mottle leaf curl virus (ToMoLCV) sequences, whereas other ToMoLCV isolates and the closest begomoviruses contained other iteron-like sequences (Supplementary Fig. S1).

An SDT analysis [7] of the DNA-A showed the highest genome-wide identity of 81.65% with tomato golden leaf distortion virus (ToGLDV, HM357456), while the DNA-B shared 82.57% identity with tomato interveinal chlorosis virus-2 (ToICV2, MK087039) (Supplementary Fig. S2). Since the current species demarcation criterion of *Begomovirus* is 91% nucleotide identity of the DNA-A, we conclude that this isolate is a new member of the genus, and the name Tomato mottle leaf distortion virus is proposed. Although tomato mottle leaf distortion virus (ToMoLDiV) DNA-A sequence is closer to ToGLDV, it clustered in a phylogenetic tree with tomato mosaic severe dwarf virus (ToMSDV) (Fig. 2a). The DNA-B clustered with ToICV2 (Figure 2b) in the same branch with ToMSDV. ToMoLDiV is thus closely related to ToGLDV, ToMSDV, and ToICV2.

Declarations

Conflict of interest

The authors declare that they have no known competing interests or personal relationships that could have influenced the work reported in this article.

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Author contributions

All authors contributed to the study conception, design, material preparation, data collection and analysis. The first draft of the manuscript was written by Thais Pereira Martins, Erich Yukio Tempel Nakasu and Alice Kazuko Inoue Nagata, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Rojas MR, Macedo MA, Maliano MR et al (2018) World Management of Geminiviruses. Annu Rev Phytopathol 56:637–677. https://doi.org/10.1146/annurev-phyto-080615-100327
2. Zerbini FM, Briddon RW, Idris A et al (2017). ICTV virus taxonomy profile: *Geminiviridae*. J Gen Virol 98:131-133. https://doi.org/10.1099/jgv.0.000738
3. Lima A, Silva JC, Silva FN et al (2017) The diversification of begomovirus populations is predominantly driven by mutational dynamics. Virus Evol 3:1-14. https://doi.org/1093/ve/vex005
4. Padidam M, Sawyer S, Fauquet CM (1999) Possible emergence of new geminiviruses by frequent recombination. Virol 265: 218–225. https://doi.org/10.1006/viro.1999.0056
5. Inoue-Nagata AK, Lima MF, Gilbertson RL (2016) A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. Horticult Bras 34:8–18. https://doi.org/10.1590/S0102-053620160000100002
6. Gao, Y, Liu, B, Wang, Y et al (2019) TideHunter: efficient and sensitive tandem repeat detection from noisy long-reads using seed-and-chain. Bioinformatics 35: i200-i207. https://doi.org/10.1093/bioinformatics/btz376
7. Muhire BM, Varsani A, Martin DP (2014) SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:e108277. https://doi.org/10.1371/journal.pone.0108277
8. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol and Evol 33:1870-1874. https://doi.org/10.1093/molbev/msw054
9. Edgar, RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797. https://doi.org/10.1093/nar/gkh340

Figures

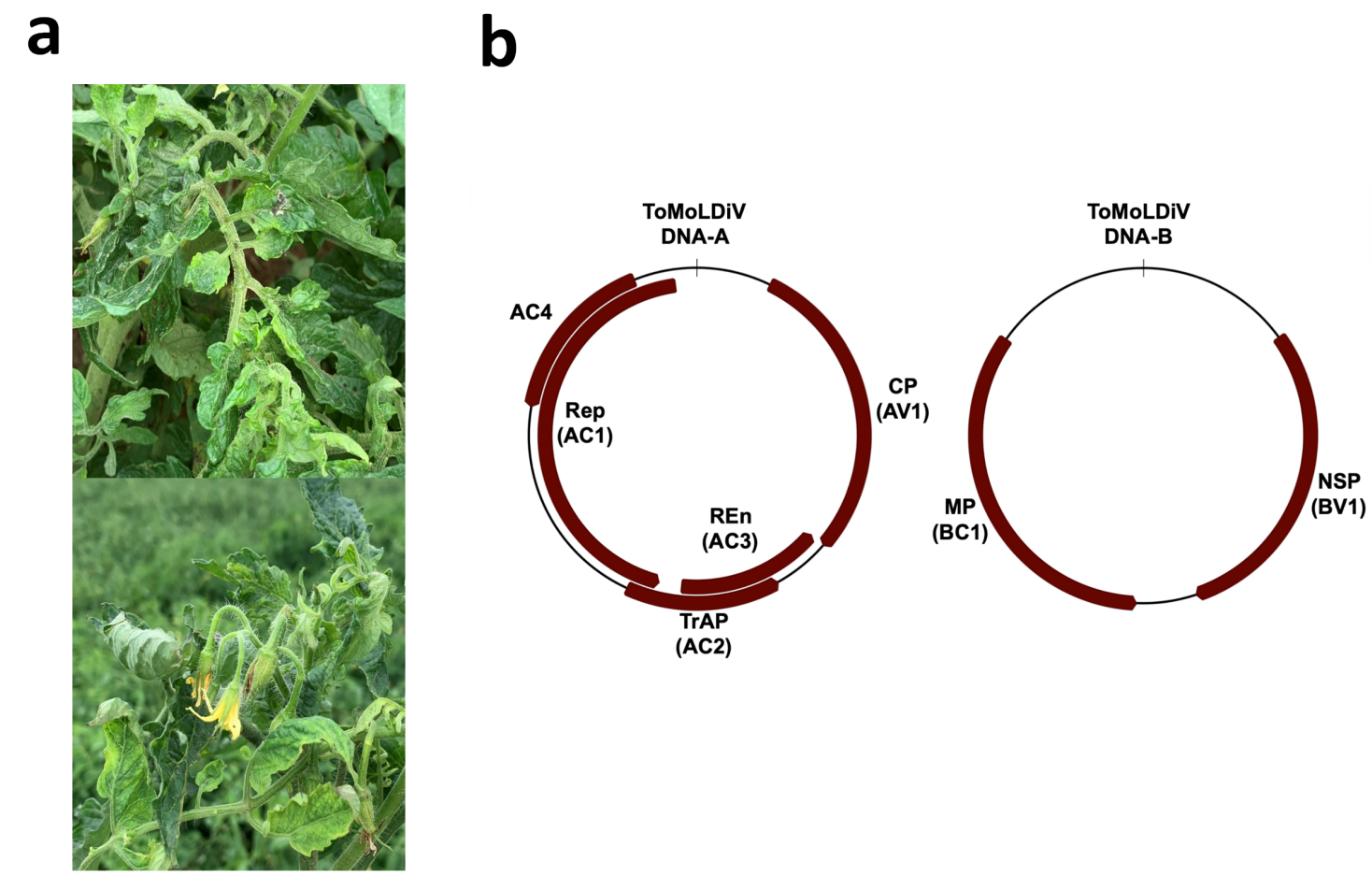

Figure 1

a. Tomato plant (cv. AP533) showing mottling, leaf distortion, chlorotic spots, vein clearing, and mosaic symptoms collected in Luziânia, Goiás state, Brazil. b. Genome of ToMoLDiV containing two segments, and conding 5 ORFs in DNA-A and 2 ORFs in DNA-B

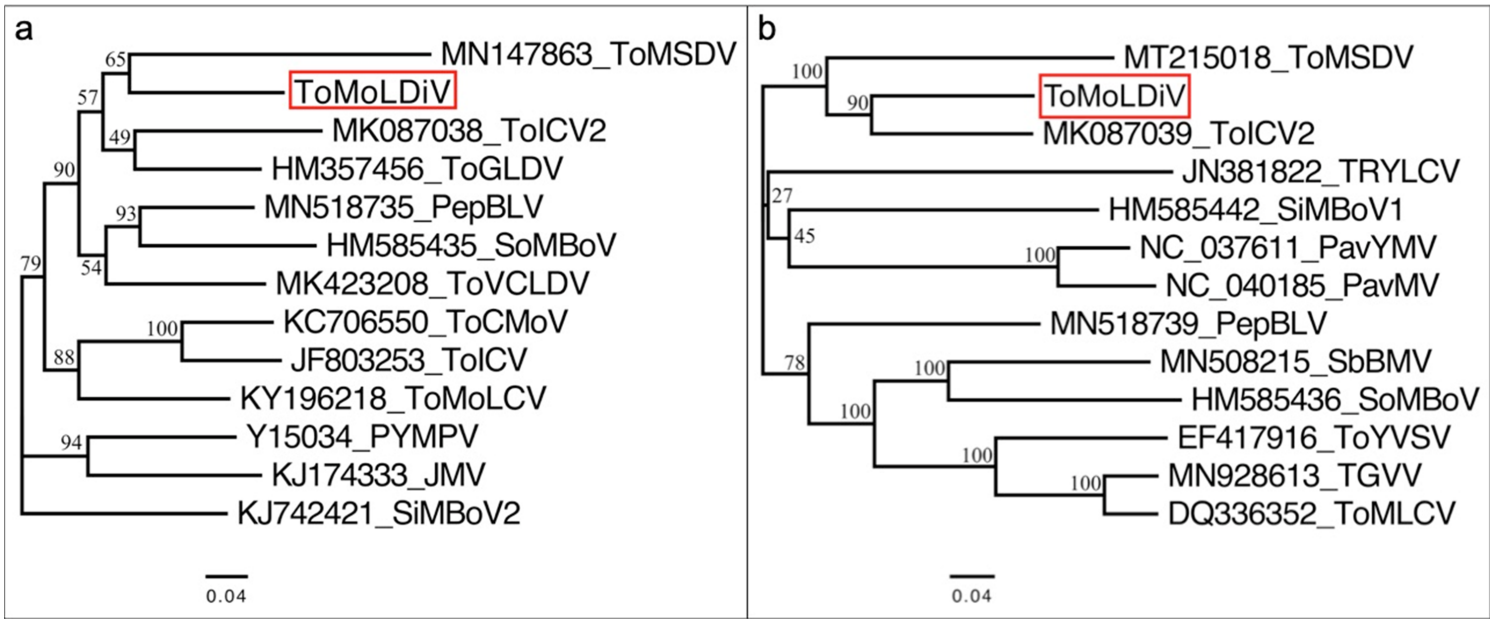

Figure 2

Phylogenetic unrooted trees of ToMoLDiV and the closest begomoviruses generated with the maximum likelihood method (3000 replications), performed in Mega X [8] using the general time-reversible (a, DNA-A), and Hasegawa-Kishino-Yano (b, DNA-B) models, after aligning with MUSCLE [9]. The bootstrap values are shown at the nodes. The complete virus names are found in: https://talk.ictvonline.org/ictv-reports/ictv_online_report/ssdna-viruses/w/geminiviridae/479/member-species-begomovirus. Bar = substitutions per site

Supplementary Files

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