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Complete genome sequence of two tomato-infecting begomoviruses in Venezuela: evidence of a putative novel species and a novel recombinant strain

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Abstract At least six begomovirus species have been reported infecting tomato in Venezuela. In this study the complete genomes of two tomato-infecting begomovirus isolates (referred to as Trujillo-427 and Zulia-1084) were cloned and sequenced. Both isolates showed the typical genome organization of New World bipartite begomoviruses, with DNA-A genomic components displaying 88.8% and 90.3% similarity with established begomoviruses, for isolates Trujillo-427 and Zulia-1084, respectively. In accordance to the guidelines for begomovirus species demarcation, the Trujillo-427 isolate represents a putative new species and the name "*Tomato wrinkled mosaic virus*" is proposed. Meanwhile, Zulia-1084 represents a putative new strain classifiable within species *Tomato chlorotic leaf distortion virus*, for which a recombinant origin is suggested.

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Tomato (*Solanum lycopersicum* L.), considered one of the most important vegetable crops worldwide, is affected by a large number of begomovirus species [11]. Viruses classified within the genus *Begomovirus* (family *Geminiviridae*) have one or two genomic components (known as DNA-A and DNA-B), circular single-stranded DNA and represent the largest group of plant viruses [3]. Begomoviruses are transmitted by whiteflies within the *Bemisia tabaci* sibling group in a persistent circulative manner. The global spread of this insect has boosted the emergence of begomoviruses in tropical and subtropical regions [8]. In Venezuela, outbreaks of begomoviruses have caused severe yield losses in tomato fields, where they are frequently associated with high populations of the insect vector [18]. In this country, at least 12 begomovirus species have already been described among which six are infecting tomato [7, 19]. In the present study, tomato samples showing begomovirus-like symptoms were collected in two different tomato fields in Venezuela. Sample 427 was collected in the Bolivar County, Trujillo State, in 2006, whereas sample 1084 was collected in the Jesus Enrique Lossada County, Zulia State, in 2008. The main symptoms associated with sample 427 were mosaic and distortion of leaves (Fig. 1A), while those observed with sample 1084 were chlorosis and severe curling of leaves (Fig. 1B). Total DNA [9] from both samples was used to confirm begomovirus infection by PCR using universal primers PAL1v1978 and PAR1c946 [16]. In order to obtain the complete viral genome sequences, rolling-circle amplifications (RCA) were performed using the TempliPhi kit (GE Healthcare). Restriction analysis of the RCA products using endonucleases *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Sac*I and *Sal*I suggested single viral infection in each sample (data not shown). Restriction products with expected full-length sizes for begomovirus components (~ 2.6 kb) were used for cloning into pBluescript

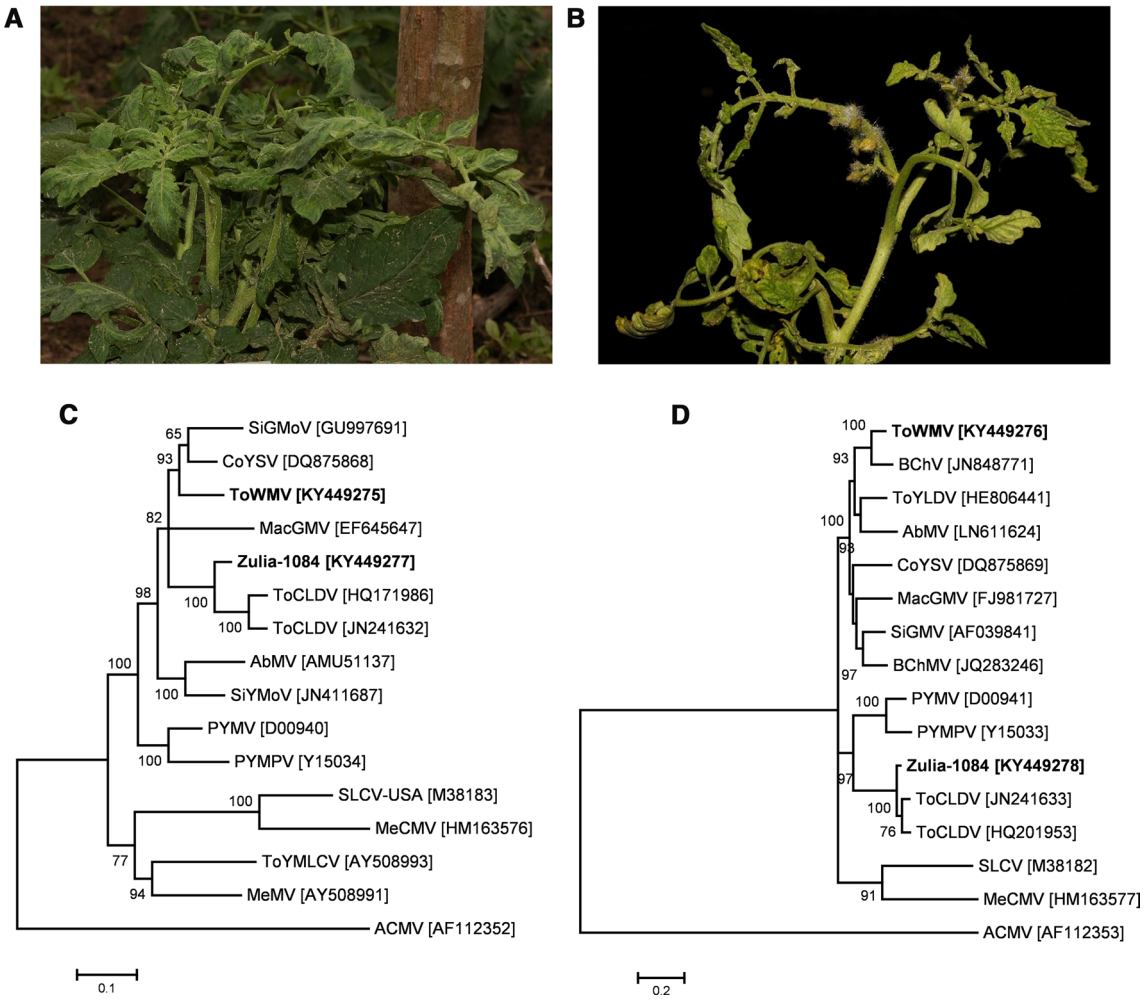

Figure 1 Symptoms and phylogenetic relationships between two novel begomoviruses infecting tomato. A) Symptoms associated with tomato wrinkled mosaic virus (ToWMV). B) Symptoms associated with isolate Zulia-1048, a tomato chlorotic leaf distortion virus (ToCLDV). C) Phylogenetic tree based on the whole DNA-A sequences from ToWMV, Zulia-1048 and related begomoviruses. D) Phylogenetic tree based on the whole DNA-B sequences from ToWMV, Zulia-1048 and related begomoviruses. Phylogenetic trees were constructed based on the maximum likelihood method using GTR + G as the nucleotide substitution model. Bootstrap values (500 iterations) above 60% are indicated for each node. ToWMV and Zulia-1048 isolates are highlighted in bold. African cassava mosaic virus (ACMV) sequences were used as outgroups in both trees. Nucleotide sequences are identified with their GenBank accession number in brackets. Abutilon mosaic virus (AbMV), bean chlorosis virus (BChV), Corchorus yellow spot virus (CoYSV), Macroptilium golden mosaic virus (MacGMV), melon chlorotic mosaic virus (MeCMV), Merremia mosaic virus (MeMV), potato yellow mosaic virus (PYMV), potato yellow mosaic Panama virus (PYMPV), Sida golden mottle virus (SiGMoV), Sida yellow mottle virus (SiYMoV), squash leaf curl virus (SLCV), tomato yellow margin leaf curl virus (ToYMLCV)

II SK+ vector (Stratagene, La Jolla) and transformed into *Escherichia coli* DH10B cells using the heat-shock method. DNA-A and DNA-B components from sample 427 were obtained with *Bam*HI and *Eco*RI, respectively, while DNA-A and DNA-B from sample 1084 were obtained with *Bam*HI. Begomovirus components were fully sequenced at Macrogen Inc. (Amsterdam, The Netherlands). Contigs for each DNA component were assembled using BioEdit [10].

The nucleotide sequences for each component were compared with those of begomoviruses available in GenBank using BLASTn. Multiple alignments of sequences from New World begomoviruses were generated with MUSCLE [5]. The software SDT v.1.2 [15] was used for pairwise comparisons and nucleotide identity calculations. Phylogenetic analyses were carried out using the Maximum likelihood method (500 bootstrap replicates) implemented in MEGA 6 [21]. Recombination analyses were performed using RDP4 [14] and Simplot [13].

The DNA-A (GenBank No. KY449275) and DNA-B (GenBank No. KY449276) components from sample 427 were 2637 and 2584 nucleotides (nt) in length, respectively. These components shared a common region (CR) of 199

Table 1 Recombinant events detected in isolate Zulia-1084 of tomato chlorotic leaf distortion virus (ToCLDV) by at least six methods included in the RDP4 software

Recombinant DNA	Recombination breakpoints	Potential major parents[a]	Potential minor parents[a]	P Value	Detection methods[b]
DNA-A	1994-26	ToCLDV (JN241632)	MacGMV (EU158096)	8.77×10^{25}	R, G, B, M, C, S, 3S
DNA-B	251-6	ToCLDV (JN241633)	MacGMV (EU158097)	1.93×10^{15}	R, G, B, M, C

[a] ToCLDV, tomato chlorotic leaf distortion virus; MacGMV, Macroptilium golden mosaic virus. GenBank No. are indicated in parentheses

[b] Recombination method abbreviations: R, RDP; B, BoostScan; C, Chimera; G, GENCOV; M, MaxChi; S, SiScan; 3S, 3Seq. Methods showing the lowest *p*-value are underlined

nt with 94% nt sequence identity, suggesting both components form a cognate pair in the same viral isolate, referred to in this work as isolate Trujillo-427. The full-length DNA-A (GenBank No. KY449277) and DNA-B (GenBank No. KY449278) isolated from sample 1084 were 2650 and 2631 nt, respectively. The CR of both components was 174 nt with 97% nt sequence identity, suggesting they are a cognate pair from the same viral isolate (hereafter named Zulia-1084). Based on pairwise nucleotide sequence comparisons, DNA-A of isolate Trujillo-427 displayed the highest nt sequence identity (88.8%) with Corchorus yellow spot virus (CoYSV, GenBank No. DQ875868) while DNA-B showed the highest nt sequence identity (86.5%) with bean chlorosis virus (BChV, GenBank No. JN848771). CoYSV is a begomovirus first reported in Yucatan, Mexico, infecting the malvaceous weed *Corchorus siliquosus* [12], while BChV is a begomovirus reported in Táchira, Venezuela, infecting common bean (*Phaseolus vulgaris* L.) [6]. Táchira State belongs to the Venezuelan Andean region like Trujillo State, where sample 427 was collected. The DNA-A and DNA-B of isolate Zulia-1084 showed the highest nt sequence identity (90.3 and 90.6%, respectively) with tomato chlorotic leaf distortion virus (ToCLDV, GenBank Nos. HQ201952 and JN241633). In a previous survey, ToCLDV was reported infecting tomato and *Capsicum chinense* in the Zulia State, Venezuela [22], where sample 1084 was also collected. Furthermore, DNA-A and DNA-B of isolates Trujillo-427 and Zulia-1084 shared 86.2 and 73.9% nt sequence identity, respectively.

In accordance to the species demarcation criteria established by the International Committee on Taxonomy of Viruses (ICTV), begomoviruses sharing DNA-A nt sequence identity < 91% are recognised as different species [3]. Since DNA-A from Trujillo-427 exhibited nt sequence identity < 91% with previously described begomoviruses, this isolate represent a putative new species and the name "*Tomato wrinkled mosaic virus*" is proposed, with isolates being referred to as tomato wrinkled mosaic viruses (ToWMV). Although the isolate Zulia-1084 also displayed nt sequence identity < 91% with previously described begomoviruses, it was closely related to ToCLDV (90.3%). Following the ICTV criteria for begomovirus species demarcation [3], isolate Zulia-1084 is tentatively suggested as an outlier, classifiable within the species *Tomato chlorotic leaf distortion virus*. Sequencing of additional isolates will be required to clarify its taxonomical status.

According to phylogenetic analysis, ToWMV and Zulia-1084 isolates fall in the *Abutilon mosaic virus* clade of the New World begomovirus group (Fig. 1C, Supplementary Fig. S1), which classifies begomoviruses from the Northern regions of South America, Central America, Mexico and Southern United States [17]. Accordingly, DNA-A and DNA-B components from Zulia-1084 clustered with ToCLDV (Fig. 1C-1D, Supplementary Fig. S1). The DNA-A and DNA-B components of ToWMV showed distinct phylogenetic relationships, clustering with CoYSV and BChV isolates, respectively. In begomoviruses, both DNA components may have different molecular evolutionary histories which seem to be driven by varying evolutionary forces, such as recombination [2]. Recombination was not detected in either DNA-A or DNA-B of ToWMV using the RDP4 software. In contrast, recombination events were detected in both DNA components from Zulia-1084 in at least five out of the seven methods included in RDP4 (Table 1). In both DNA-A and DNA-B, the recombination events involved ToCLDV and Macroptilium golden mosaic virus (MacGMV) as the putative major and minor parents, respectively. MacGMV is a begomovirus that was reported infecting the weed *Macroptilium lathyroides* in Jamaica; it is also able to infect bean, tomato and pepper [4]. Interestingly, ToCLDV has only been found in Venezuela in the same geographic region (Zulia State) where Zulia-1084 was found, despite the fact that other tomato-infecting begomoviruses, such as potato yellow mosaic virus (PYMV) and tomato yellow leaf curl virus (TYLCV) are widespread throughout the country [7].

Interestingly, the recombinant-like sequence of Zulia-1084 DNA-A involves the 5′ end of the gene *AC1* that encodes the replication associated protein (Rep) and the CR (Supplementary Fig. S2A). Indeed, when using this region (682 nt) for pairwise sequence comparisons, Zulia-1084 shared 89% nt sequence identity with MacGMV and

79% with ToCLDV (data not shown). Despite this fact, the Zulia-1084 DNA-A has 90.3% nt sequence identity with ToCLDV. Furthermore, a recombination event in DNA-B was also detected involving the CR (Supplementary Table 1 and Supplementary Fig. S2B), in which the break points were identified between nts 2514 and 6. The CR contains essential motifs, referred to as iterons, which are specific binding sites for the Rep to initiate virus replication [1]. Zulia-1084 and MacGMV exhibited three identical iterons that are different from those reported for ToCLDV [22] (Supplementary Fig. S3). Since South America contains major species diversity within the family *Solanaceae* [20], our results raise questions about the role of wild solanaceous plants in begomovirus diversity along with the widespread distribution of the vector *B. tabaci* throughout the region [8, 19]. Overall, this study uncovered a putative new begomovirus species (“*Tomato wrinkled mosaic virus”*) and a new recombinant strain of ToLDV (Zulia-1084), which infect tomato and are genetically related to weed begomoviruses. These findings strengthen the argument for increased begomovirus surveys including crops and weeds in Venezuela.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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

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