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BRIEF REPORT

Tomato dwarf leaf virus, a New World begomovirus infecting tomato in Argentina

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Abstract Begomovirus infection is becoming a threat in fresh-market tomato in Argentina, where mixed infections with begomoviruses are common. The complete sequence of a begomovirus isolate infecting tomato sampled in Salta was molecularly characterized. Phylogenetic analysis showed that this virus isolate is closely related to previously reported Brazilian, Bolivian and Argentinean begomoviruses. The associated symptoms in *Nicotiana benthamiana* and *Solanum lycopersicum* were determined by biolistic delivery of infectious DNA-A and DNA-B clones. This begomovirus isolate induced leaf mottling, rugosity and dwarfing, and growth retardation in tomato. Based on these symptoms, we propose the name of tomato dwarf leaf virus (ToDLV) for this new begomovirus.

The *Geminiviridae* are a family of circular, single-stranded DNA plant viruses. Members of this family have twinned icosahedral particles. This family comprises four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus* [1]. Members of the genus *Begomovirus* can have either a mono- or bipartite genome, can infect dicotyledonous plants, and are transmitted by the whitefly *Bemisia tabaci* (Gennadious). Begomoviruses are widespread all over the world and cause economically important diseases to horticultural and fiber crops [2–4]. Most of the New World begomoviruses have bipartite genomes (DNA-A and DNA-B) of about 2.6 kb each. These components do not share nucleotide identity, except for the common region (CR), which is about 200 bases long [5]. The CR contains sequences that are essential for replication and transcription of the bipartite genome [6]. DNA-A codes for proteins involved in particle encapsidation (CP), viral replication (Rep and REn), regulation of gene expression (TrAP) and AC4 protein [7–9]. The latter protein performs different functions, such as pathogenicity or RNA silencing suppression, depending on the begomovius species [10, 11]. Some begomoviruses encode an additional AC5 protein [12]. On the other hand, DNA-B encodes two proteins (MP and NSP) involved in virus movement [13–16].

The annual tomato production in Argentina is about 900,000 tons, with a total cultivated area of 14,000 ha [17]. At present, Salta and Jujuy are important fresh-market-tomato-producing areas, and begomovirus infection is becoming a major threat. Tomato begomoviruses became economically important due to the migration of the B biotype of *Bemisia tabaci* to Brazil in the mid-1990s [18]. The B biotype has also been detected in Argentina [19]. At least 80 begomovirus species whose members infect tomato have been recognized and proposed, 16 of which are from Brazil [20–22]. Tomato yellow vein streak virus (ToYVSV) and soybean blistering mosaic virus (SbBMV) are the only tomato begomoviruses reported in Argentina [23, 24], where mixed infections with begomoviruses are common [25]. This scenario allows recombination and the emergence of novel viruses [26].

Here, we report the molecular characterization, phylogenetic analysis, and measurement of infectivity of a new begomovirus infecting tomato. We sampled tomato collected from Pichanal, province of Salta, Argentina, that showed mottling, dwarfing and leaf deformation. Total DNA was purified with a Nucleospin® Plant II total DNA

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purification kit (Macherey-Nagel). Infected plants were identified by polymerase chain reaction (PCR) with universal primers for DNA-A [27]. The entire genome of positive samples was amplified by rolling-circle amplification (RCA) using *phi*29 polymerase (Templiphi™, GE, Healthcare) [28]. The DNA-A and DNA-B monomers were obtained by digestion with the restriction enzyme *Apa*I, and then ligated to a digested and dephosphorylated pBluescript SK+ vector (Stratagene). Transformed *E. coli* JM109 competent cell clones were evaluated by PCR. The clones carrying the full genome were sequenced by primer walking (MACROGEN, Korea). Contigs of DNA-A and DNA-B components were assembled using Geneious 5.4.4 (Biomatters Development), and pairwise comparison was done using the MegAlign DNAStar Lasergene 8 package.

We determined the complete bipartite genome sequence of the isolate AR:Pichanal:397:08. Both components have the typical genome organization of bipartite New World begomoviruses. DNA-A (JN564749) is 2540 nt long, and five ORFs (Rep, REn, TrAP, CP, AC4) were identified. By contrast, DNA-B (JN564750) contains two ORFs (MP and NSP) and is 2494 nt long.

The CR of DNA-A and DNA-B of AR:Pichanal:397:08 is about 160 nt long, and pairwise comparison showed 96.1 % identity. A more detailed analysis of the CR sequence showed the elements involved in begomovirus replication: the TATA-box, the iterons (Rep-binding site), and the stem-loop containing the nonanucleotide TAATATTAC, which is conserved in the geminivirus family [29]. We indentified two iteron motifs TGGTGACA, and one inverted TGTCACCA located upstream of the TATA box (Figure 1).

The comparison of the complete nucleotide sequence of DNA-A of the isolate studied with those of the previously reported New World begomoviruses showed highest sequence identity to SoBMV (83 %), ToYVSV (81 %) and solanum mosaic Bolivia virus (SoMBoV) (80 %) (Table 1). For the DNA-B nucleotide sequence, the highest identity percentage was 71 % with ToYVSV and with tomato mild leaf curl virus (ToMiLCV) (Table 1). However, only the DNA-B sequence has been reported for ToMiLCV [30, 31], and DNA-A sequences for SoBMV [24]. It was concluded that this sample contained a member of a new begomovirus species.

Full-length DNA-A and DNA-B sequences were aligned with the selected New World begomoviruses using the

Table 1 Pairwise comparison of the complete sequences of DNA-A and DNA-B of tomato dwarf leaf virus (ToDLV) with those of the most closely related New World begomoviruses

Pairwise identity percentage		
Virus name*	DNA-A	DNA-B
ToYVSV	81	71
SoMBoV	80	68
SiMBoV1	76	62
ToYSV	77	61
ToGMV-YV	79	62
ToMiLCV	–	71
SoBMV	83	–

Nucleotide (nt) percentages were determined

*ToYVSV (EF417915-EF417916): tomato yellow vein streak virus isolate Ba-3; SoMoBoV (HM585435-HM585436): solanum mosaic Bolivia virus; SiMBoV1 (HM585441-HM585442): sida mosaic Bolivia virus 1; ToYSV (DQ336350-DQ336351): tomato yellow spot virus; ToGMV-YV (K02029-K02030): tomato golden mosaic virus-yellow vein; ToMiLCV (DQ336352): tomato mild leaf curl virus; SoBMV (EF016486): soybean blistering mosaic virus

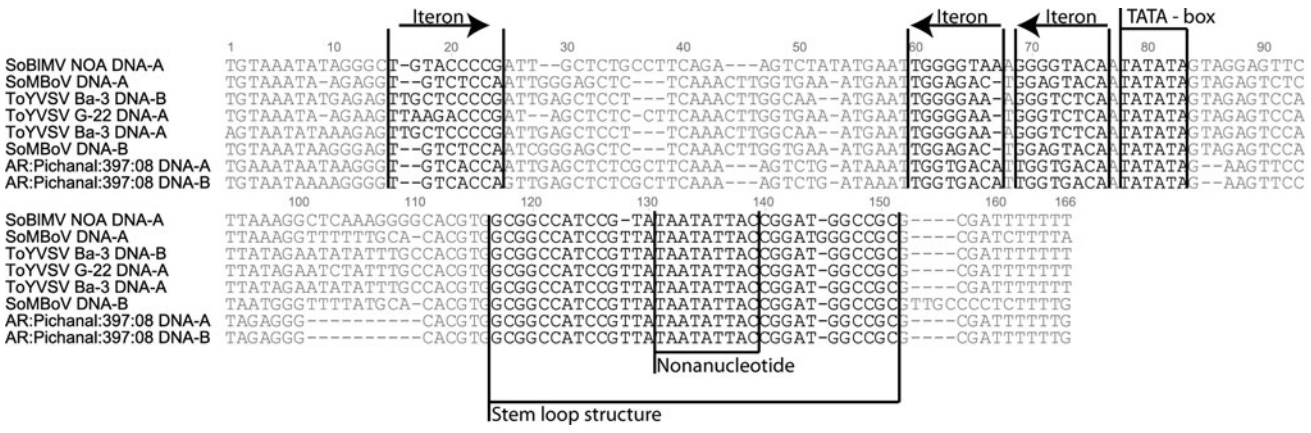

Fig. 1 Comparison of the common-region sequences of DNA-A and DNA-B between tomato dwarf leaf virus (ToDLV), tomato yellow vein streak virus (ToYVSV), solanum mosaic Bolivia virus (SoMBoV) and soybean blistering mosaic virus (SoBMV). Vertical lines demarcate significant replication-related regions: the TATA-box, the iterons, the stem-loop structure, and the conserved nonanucleotide TAATATTAC

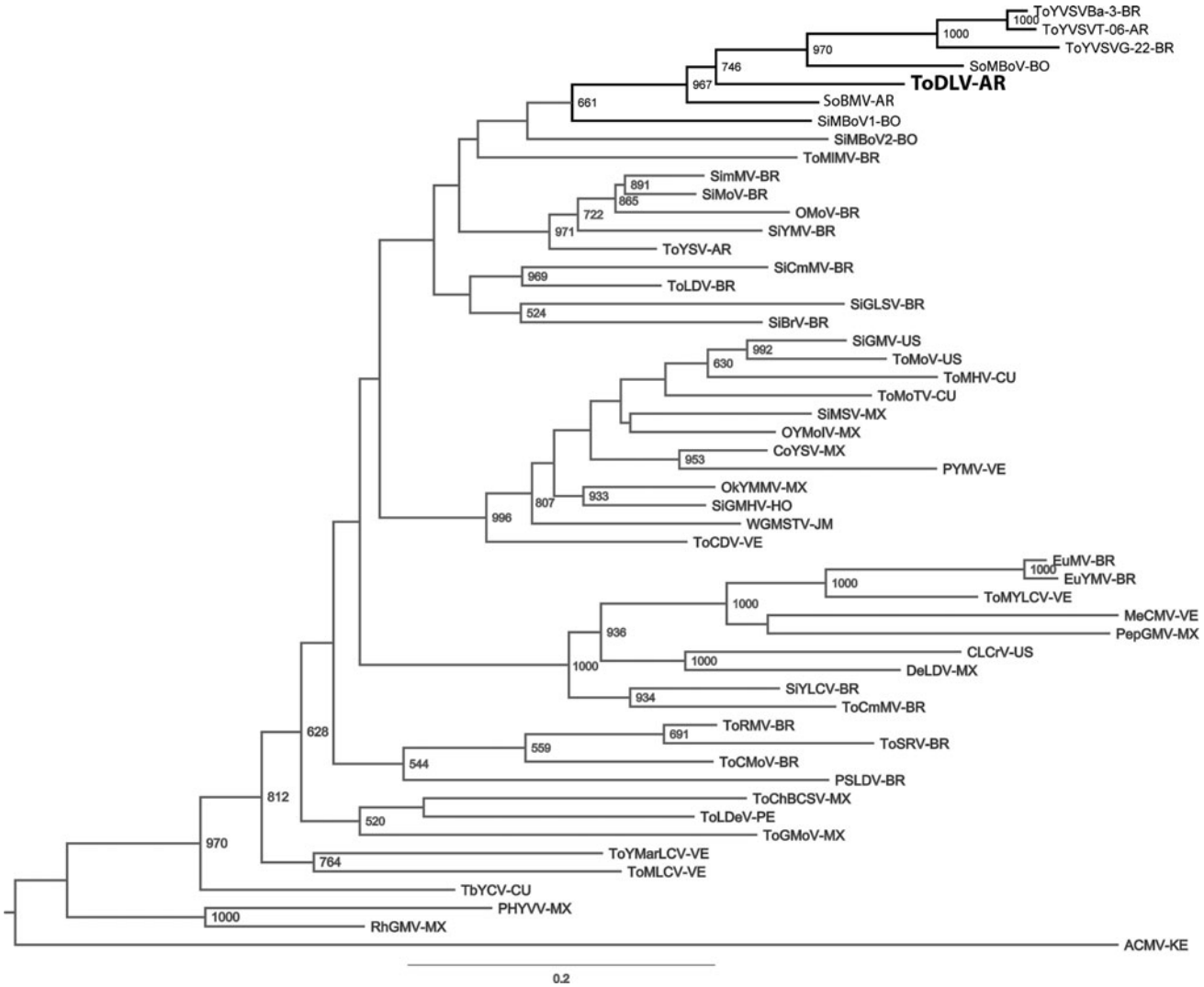

Fig. 2 Maximum-likelihood (ML) phylogenetic trees based on multiple alignments of complete DNA-A sequences of tomato dwarf leaf virus (ToDLV) plus other representative New World begomoviruses (summarized in Supplementary Table S1). Non-parametric bootstrap values for nodes supported in more than 500 replicates are shown. African cassava mosaic virus was selected as an outgroup

MAFFT algorithm with the iterative E-INS-i method [32, 33]. Phylogenetic analysis of multiple sequence alignments of the complete genomes of New World begomoviruses was done by likelihood mapping analysis with Tree-Puzzle [34, 35] (data not shown).

The phylogenetic relationships between the complete genome sequences of the AR:Pichanal:397:08 isolate obtained in Salta and previously reported New World begomoviruses were inferred by the maximum-likelihood criterion using PhyML 3.0 [36] under the GTR+I+G substitution model. Phylogenetic trees with 1000 bootstrap replicates were obtained. Trees constructed from DNA-A sequences showed that AR:Pichanal:397:08 sequences are grouped into a monophyletic cluster with SoBMV, SoMBoV and ToYVSV (Figure 2). This close relationship is consistent with the pairwise identity analysis (Table 1) and with previously reported data [37]. This cluster includes all ToYVSV isolates from Brazil and Argentina, as well as SoBMV from Argentina and SoMBoV from Bolivia, suggesting a geographical relationship. Interestingly, the sister group of this cluster contains sida-infecting begomoviruses, showing evidence of a common ancestor that adapts to different hosts.

The DNA-B-based phylogenetic tree confirmed the phylogenetic relationship obtained with DNA-A. The DNA-B sequences of AR:Pichanal:397:08 are most closely

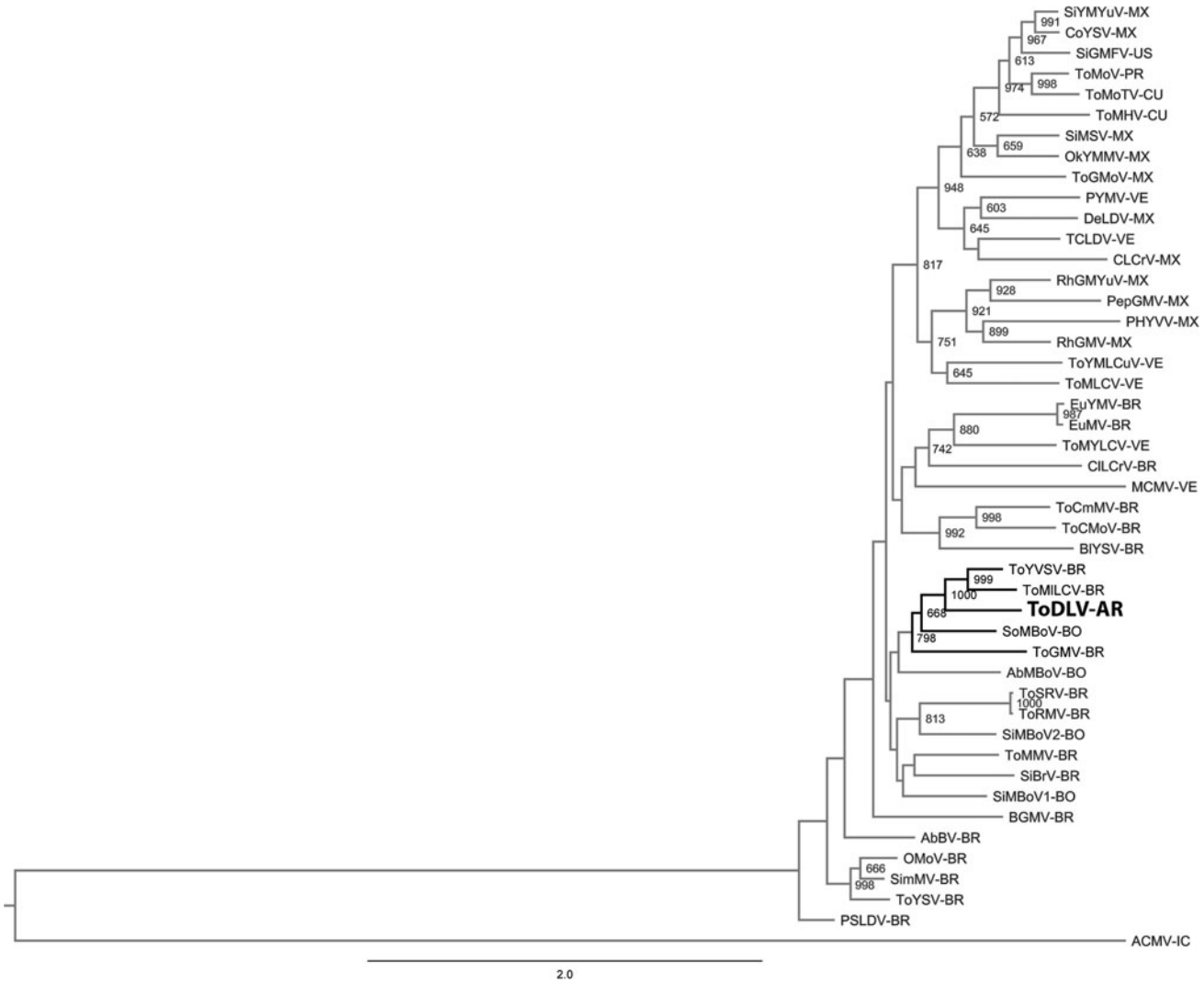

Fig. 3 Maximum-likelihood (ML) phylogenetic trees based on multiple alignments of DNA-B complete sequences of tomato dwarf leaf virus (ToDLV) plus other representative New World begomoviruses (summarized in Supplementary Table S1). Non-parametric bootstrap values for nodes supported in more than 500 replicates are shown. African cassava mosaic virus was selected as an outgroup

related to those of ToYVSV and SoMBoV. The DNA-B tree displays a rapid and recent burst of begomovirus diversification from the most recent common ancestor, in contrast to the gradual accumulation of diversity of DNA-A (Figure 3).

To evaluate the infectivity of the viral DNAs that were cloned, we performed biolistic inoculation of *Nicotiana benthamiana* and *Solanum lycopersicum* plants using a PDS-1000/He particle delivery system (Bio-Rad). Plasmids containing DNA-A and DNA-B of the AR:Pichanal:397:08 isolate were digested with *Apa*I to purify the monomeric genomes and then re-ligated. The RCA amplification product (5 µl) of each component was mixed with tungsten particles and then inoculated into six *N. bentamiana* and 11 *S. lycopersicum* plants with 4-5 leaves, and into the respective control plant [38]. All *N. bentamiana* plants showed chlorosis, wrinkling and leaf deformation. Eight days after inoculation, symptoms developing in eight *S. lycopersicum* plants included leaf mottling, rugosity and dwarfing, and growth retardation (Figure 4). The infection was confirmed by PCR analysis.

We have molecularly characterized a novel member of the genus *Begomovirus* from Pichanal, Salta, Argentina, and established its phylogenetic relationship to other begomoviruses. We have also infected *Nicotiana benthamiana* and *Solanum lycopersicum* plants and determined the symptoms that this novel virus produced on tomato, the original host. Based on these results, we suggest that the

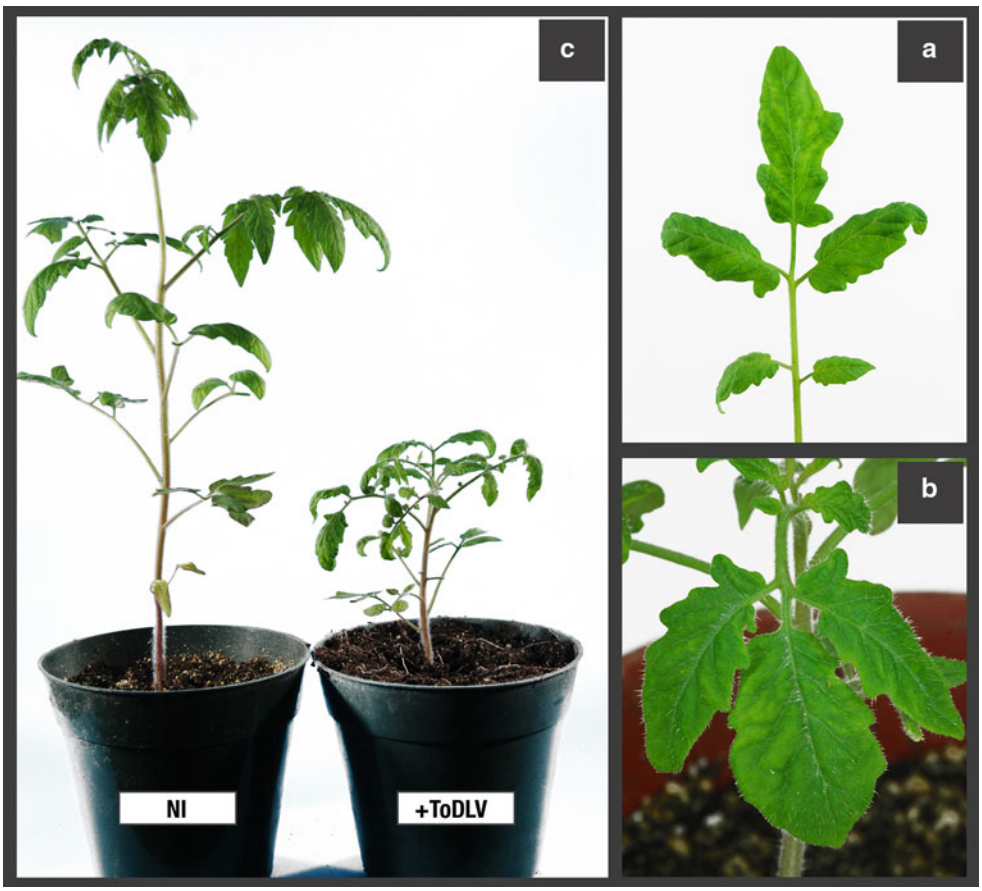

Fig. 4 Detail of leaf symptoms of *Solanum lycopersicum* plants inoculated with DNA-A and DNA-B infectious clones of tomato dwarf leaf virus (ToDLV) by particle bombardment. Dwarfing, rugosity (**a**) and mottling (**b**) are shown. Comparison between a non-inoculated (NI) and a ToDLV-inoculated tomato plant. Leaf dwarfing and growth retardation are shown (**c**)

AR:Pichanal:397:08 isolate belongs to a new species and propose the name "Tomato dwarf leaf virus" (ToDLV) for its identification.

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