Arch Virol (2015) 160:581–585
DOI 10.1007/s00705-014-2216-y

ANNOTATED SEQUENCE RECORD

Tomato mottle wrinkle virus, a recombinant begomovirus infecting tomato in Argentina

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Received: 26 March 2014 / Accepted: 26 August 2014 / Published online: 25 September 2014

Abstract Begomoviruses seriously threaten tomato production in South America. Here, we present the molecular characterization of a novel tomato-infecting begomovirus isolated in Argentina and demonstrate its infectivity. After cloning and sequencing the complete genome of this new virus, pairwise genetic distance and phylogenetic analyses revealed that it is a novel virus that is closely related to other begomoviruses found in Argentina, Brazil and Bolivia. We have proposed naming the virus tomato mottle wrinkle virus (ToMoWrV), based on symptoms produced upon its biolistic inoculation into tomato plants. Recombination analysis revealed that ToMoWrV is a recombinant, with parental sequences likely belonging to the South American begomoviruses soybean blistering mosaic virus (SoBlMV) and tomato yellow vein streak virus (ToYVSV).

The family *Geminiviridae* includes viruses with single-stranded DNA genomes that have been classified into seven genera [1]. The genus *Begomovirus* includes the majority of the described geminiviruses, most of which infect dicotyledonous plants and are transmitted by the whitefly *Bemisia tabaci* (Gennadius). Although most begomoviruses originating from the New World (NW) have bipartite genomes consisting of two circular ~2.6-kb components, named DNA-A and DNA-B, many of the begomoviruses found in the Old World have monopartite genomes consisting exclusively of a DNA-A like molecule [2].

The DNA-A components of all known NW begomoviruses encode five proteins (Rep, TrAP, REn, AC4 and CP) that redirect the host metabolic processes and cellular components necessary to establish a productive infection [3]. On the other hand, the DNA-B components of bipartite begomoviruses encode two proteins involved in intra- and intercellular movement (MP and NSP) [4].

The genomic components display a very low degree of sequence identity to each other, except for an approximately 200-nt-long common region (CR) in the intergenic region at the origin of replication. The DNA-A and DNA-B CRs generally share >90 % identity and contain conserved repeated sequences (iterons) of about 4-7 nt that are generally specific to each species [5].

Tomato crops have been severely affected by emergent begomoviruses across South America [6–11]. The main contributors to this situation are the capacity of whiteflies to transmit begomoviruses to multiple host species and the great diversity of begomoviruses with broadly overlapping host ranges, including cultivated and uncultivated solanaceous, malvaceous and legume species. Besides having high mutation rates (as high as many RNA viruses), begomoviruses, within the context of mixed infections, also have the capacity to evolve very rapidly through recombination and genome component reassortment (also called pseudo-recombination) [12, 13].

Argentina produces about 900,000 tons of fresh tomatoes per year, and begomoviruses seriously constrain tomato yields [14]. A great diversity of begomoviruses has

Electronic supplementary material The online version of this article (doi:10.1007/s00705-014-2216-y) contains supplementary material, which is available to authorized users.

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been reported in Argentina, and many of these are still in the process of being molecularly and biologically characterized [15–17]. Here, we present the molecular characterization of one of these tomato-infecting begomoviruses and demonstrate its recombinant origin and infectivity.

Tomato plants with curling, yellowing, leaf wrinkling, and chlorotic mottling symptoms were collected from greenhouses in Pichanal (2008) and Oran (2013), in the Argentinian province of Salta. Total DNA was extracted using a Nucleospin Plant II kit (Macherey-Nagel). Plants infected with begomoviruses were identified by PCR using degenerate primers [18]. Begomovirus genomes were amplified by rolling-circle amplification (RCA) using a Templi-phi^{TM} amplification kit (GE, Healthcare). DNA-A and DNA-B components were cloned with *Apa*I into the pBluescriptSK II (+) vector. Genome components were sequenced by primer walking (Macrogen, Korea). The BLAST algorithm [19] was used to calculate the percent identity to begomovirus sequences available in GenBank.

We constructed two alignments, one with 69 full-length DNA-A sequences and another with 50 DNA-B sequences, using MUSCLE [20] as implemented in Geneious 5 (Biomatters). Besides the novel begomovirus DNA-A and DNA-B sequences (denoted AR:Pichanal:400:08), the alignments contained previously published DNA-A and DNA-B from neighboring South American countries (Online resource 1).

Best-fit nucleotide substitution models were selected for each dataset using jModeltest 2.1.2 [21]. A maximum-likelihood phylogenetic tree was constructed using PhyML [22]. Non-parametric bootstrapping with 1000 replicates was used to test the statistical support for inferred phylogenetic tree branches.

Evidence of recombination within the newly sequenced genome components was tested using the RDP, Maxchi, GENECONV, Bootscan, Chimaera, Siscan, and 3Seq methods implemented in the RDP 4.22 package [23].

The infectivity of a representative cloned genome component of the AR:Pichanal:400:08 isolate was evaluated by biolistic inoculation using a PDS-1000/He particle delivery system (Bio-Rad). The RCA products derived from re-circularized DNA-A and DNA-B genomic components were mixed with tungsten particles and then inoculated into 12 *Solanum lycopersicum* plants, each of them at the 4- to 5-leaf stage [24]. Begomovirus infection was assayed by RCA-RFLP [25].

PCR results confirmed begomovirus infection of three tomato plants sampled in Pichanal (2008) and one in Oran (2013). We cloned and sequenced two complete genomes (DNA-A and DNA-B) from isolates AR:Pichanal:398:08 (KM243019 and KM243017) and AR:Pichanal:400:08 (JQ714137 and JQ714138), and two full-length DNA-A components belonging to isolates AR:Pichanal:397:08 (KM243018) and AR:Oran:611:13 (KM243020). The AR:Pichanal:397:08 isolate appeared to be a mixed infection with another begomovirus with the proposed name tomato dwarf leaf virus (ToDLV) [17].

All of the cloned begomovirus genome components have the characteristic begomovirus DNA-A open reading frames (ORFs) corresponding to the *AV1, AC1*, *AC2*, *AC3*, *AC4* and *AC5* genes and the DNA-B ORFs corresponding to the *BV1* and *BC1* genes.

Pairwise analysis indicated that the four analyzed DNA-A sequences share 98-99 % nucleotide sequence identity with each other, and the two DNA-B sequences share 95 % identity.

The highest pairwise sequence identity between the DNA-A of AR:Pichanal:400:08 (JQ714137) and another previously characterized begomovirus DNA-A component sequence was 86 %, with ToYVSV Ba-3 (EF417915). The DNA-B of AR:Pichanal:400:08 (JQ714138) was most similar to that of ToYVSV Ba-3 (EF417916), with which it shared 83 % identity. According to the recommended ICTV species demarcation recommendations for begomoviruses, the AR:Pichanal:400:08 isolate appears to be a tomato-infecting member of a new begomovirus species [26].

The percent pairwise identity in the common region (CR) that is shared by cognate begomovirus genome components was >97 % for the AR:Pichanal:400:08 DNA-A and DNA-B. Two repeats of a potential replication-associated iterated sequence (GGGGTA) and one inverted version of this repeat (TACCCC) were identified. The CR of the AR:Pichanal:400:08 isolate and soybean blistering mosaic virus (EF016486) have 90 % identity and the same iterated sequences (Online resource 2a).

DNA-A and DNA-B maximum-likelihood phylogenetic trees were constructed using the GTR + I+G nucleotide substitution model. The DNA-A of AR:Pichanal:400:08 is phylogenetically most closely related to tomato dwarf leaf virus (ToDLV), solanum mosaic Bolivia virus (SoMBoV), soybean blistering mosaic virus (SoBlMV) and tomato yellow vein streak virus (ToYVSV), all of which have been reported previously to infect soybean and tomato in Argentina, Brazil and Bolivia (Fig. 1) [5, 8, 15, 16, 27]. The DNA-B of AR:Pichanal:400:08 is most closely related to ToYVSV (Online resource 3).

Using the seven recombination detection methods implemented in the RDP4 package, a recombination event was identified in the DNA-A component of AR:Pichanal:400:08 (Online resource 2b). These methods indicated that the sequences in the analyzed dataset that most closely resemble the parental sequences of AR:Pichanal:400:08 are SoBlMV (EF016486) and ToYVSV (KJ413253) (Online resource 2b). The beginning and ending breakpoints are at approximately 1847 and 2533 nt, respectively, with the beginning breakpoint falling close to the 3′ end of *AC1* and

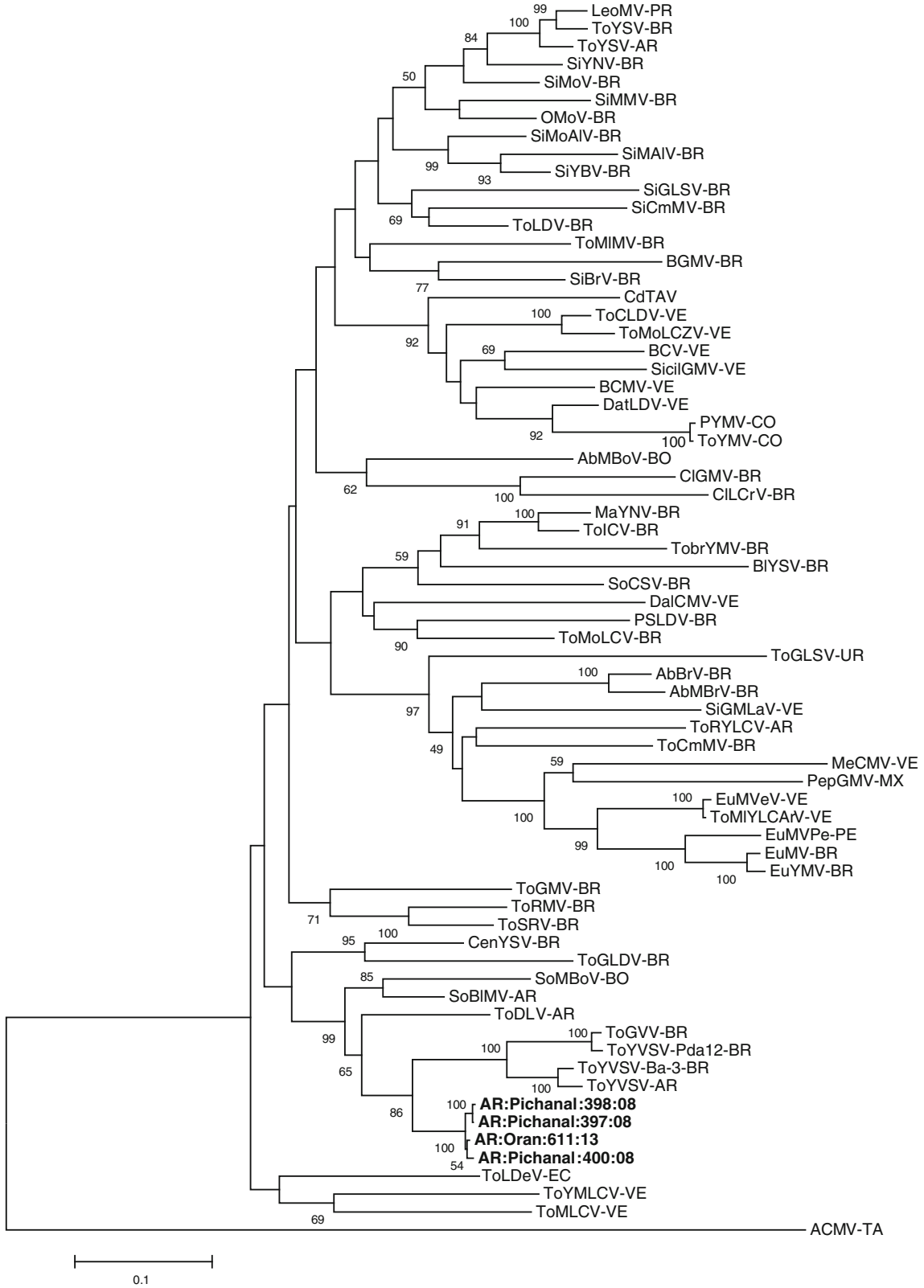

Fig. 1 Maximum-likelihood (ML) phylogenetic tree constructed using a multiple alignment of the AR:Pichanal:400:08, AR:Pichanal:397:08, AR:Pichanal:398:08 and AR:Oran:611:13 DNA-A sequences with the DNA-A sequences of 64 other South American begomoviruses (Online resource 1). Only non-parametric bootstrap values over 50 are shown as node support. African cassava mosaic virus (ACMV-AY795982) was selected as an out-group

the ending breakpoint falling near the origin of virion-strand replication within the CR (Online resource 2b).

These recombination breakpoint positions are close to sites that have been reported to be recombination hotspots in begomovirus genomes [28, 29]. The patterns of sequence exchange that occur between begomoviruses can be complex but are strongly influenced by the degree of sequence similarity shared by the recombining viruses. Selection also favours the preservation of co-evolved longer-range protein-protein and protein-DNA interactions [30, 31]. All of the results presented here indicate that the AR:Pichanal:400:08 DNA-A acquired CR and *AC1* (Rep) fragments from a SoBlMV-like parental virus. The recombinational transfer of genome segments that include the CR and a portion of the *AC1* (Rep) are evolutionarily important because they have the potential to generate entirely new begomoviruses. A recombination event in the DNA-A component that replaces the rep and iteron sequences of one virus with that of another requires either replacement of a cognate DNA-B CR with that of the newly acquired genome fragment or capture of the DNA-B component of the donor virus. This is to ensure that the newly formed recombinant DNA-A can continue to effectively trans-replicate its associated DNA-B. Since there are no SoBlMV DNA-B sequences available in public databases, it was not possible to detect evidence of such compensatory recombination events within the DNA-B of AR:Pichanal:400:08.

It is likely that the frequent occurrence of mixed begomovirus infections in tomato and other cultivated and uncultivated South American plants provides numerous opportunities for inter-species begomovirus recombination events to occur in this part of the world.

The representative DNA-A and DNA-B components of AR:Pichanal:400:08 were shown to be infectious by biolistic inoculation. Six inoculated plants developed mild mottling and wrinkling leaf symptoms (Online resource 4), and the presence of the AR:Pichanal:400:08 isolate in these plants was confirmed by RCA-RFLP (data not shown). The symptoms that developed in the infected plants during the inoculation assay resembled those observed in the tomato plant from which AR:Pichanal:400:08 was isolated. The other sampled tomato plants exhibited more-severe symptoms, possibly due to these having mixed infections with other begomoviruses. Based on the symptoms observed in the infectivity assay, we propose the name tomato mottle wrinkle virus (ToMoWrV) for this new begomovirus.

Acknowledgments This work was supported by AEBIO-242411-INTA-2009 projects. We thank Agr. Eng. Joaquin Fernández De Ullivarri and Agr. Eng. (M.Sc.) Ceferino René Flores for providing us with the infected tomato sample from Salta, and Msc María Lorena Giachero for tomato photography.

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