Occurrence and molecular characterization of Tomato common mosaic virus (ToCmMV) in tomato fields in Espírito Santo state, Brazil

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SHORT COMMUNICATION

Occurrence and molecular characterization of *Tomato common mosaic virus* (ToCmMV) in tomato fields in Espírito Santo state, Brazil

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Abstract Here, we report for the first time the complete molecular characterization of a begomovirus infecting tomato crops in Espírito Santo state, Brazil. Based on the analysis of partial nucleotide sequences of isolates obtained from 20 samples, collected from 2007 to 2011, all isolates were preliminary classified as *Tomato commom mosaic virus* (ToCmMV). Complete nucleotide sequences of the DNA-A and DNA-B components of one isolate were determined. The DNA-A sequence shares 97.5 % identity with that of ToCmMV-[BR:Coi22:07], thus confirming it to be an isolate of ToCmMV. In addition, recombination analysis showed that this ToCmMV isolate probably evolved from an inter-species recombination event and likely contributed to the emergence of the weed-infecting begomovirus, *Sida yellow leaf curl virus* (SiYLCV).

Keywords *Solanum lycopersicum* · Begomovirus · Characterization · Recombination

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Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) have a genome composed of one (monopartite) or two (bipartite) circular, single-stranded DNA molecules of approximately 2.6 kb and are transmitted by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) in a persistent circulative manner to dicotyledonous plants (Brown et al. 2012). They are considered as a major group of plant pathogens in tropical and subtropical regions worldwide, where they cause severe losses in many economically important crops such as beans (*Phaseolus* spp.), cassava (*Manihot esculenta*) and tomato (*Solanum lycopersicum*) (Polston and Anderson 1997; Ribeiro et al. 2003). Losses caused by begomoviruses increased dramatically after the introduction into the Western Hemisphere of *B. tabaci* Middle East-Asia Minor 1 (MEAM1, formerly known as *B. tabaci* biotype B) (Lourencão and Nagai 1994), known to have a wider host range than the formerly widespread *B. tabaci* New World 1 (NW1, formely *B. tabaci* biotype A) (Bedford et al. 1994). In Brazil, especially after the introduction of *B. tabaci* MEAM1 in the mid 1990's, serious epidemics of begomovirus diseases have been reported (Ribeiro et al. 1998). Currently, there are 11 recognized begomovirus species infecting tomato in different states of Brazil: *Tomato golden mosaic virus* (TGMV), *Tomato rugose mosaic virus* (ToRMV), *Tomato chlorotic mottle virus* (ToCMoV), *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV), *Tomato common mosaic virus* (ToCmMV), *Tomato mild mosaic virus* (ToMlMV), *Tomato yellow vein streak virus* (ToYVSV), *Tomato interveinal chlorosis virus* (ToICV), *Tomato mottle leaf curl virus* (ToMoLCV) and *Tomato golden vein virus* (TGVV) (Rocha et al. 2013). However, at present, ToSRV is reported as the prevalent begomovirus species on tomato crops (Fernandes et al. 2008; González-Aguilera et al. 2012).

In Espírito Santo state, an important tomato growing region, epidemics of begomovirus disease causing yield losses of up to 80 % have been reported since 2006 in counties such as Alfredo

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Chaves, Afonso Cláudio, Domingos Martins, Laranja da Terra and Venda Nova do Imigrante (H. Costa, *unpublished*). Infected plants usually show symptoms of severe mosaic and leaf rolling and these symptoms vary in intensity depending upon the variety and the age of the plant at infection time.

Although the first epidemic cases were only reported from 2006 in Espirito Santo state, Ambrozevicius et al. (2002) had already detected a begomovirus in tomato plants collected in Várzea Alegre in 1999, although the species was not identified. Until now, it remains unknown which begomovirus occurs in Espírito Santo state. In this study, we report the occurrence and the complete molecular characterization of a begomovirus isolated from tomato plant collected in Espírito Santo state.

During 2007 to 2011, tomato plants showing symptoms characteristic of those caused by begomovirus infection were sampled in fields around the counties of Afonso Claudio (six fields; February 2007 to October 2010), Domingo Martins (four fields; May 2011) and Venda Nova do Imigrante (five fields; May 2009 to February 2011). One to three samples were collected from each field for a total of 20 samples. Total DNA was extracted from symptomatic leaf tissue of each sample (Dellaporta et al. 1983) and used for PCR amplification with the begomovirus universal primer pair PAL1v1978/PAR1c496 (Rojas et al. 1993), which direct the amplification of a fragment of approximately 1.1 kbp comprising the 5′-region of the *rep* gene, the entire intergenic region, and the 5′-region of the *cp* gene. PCR products were purified with the QIAquick PCR purification kit (Qiagen) and directly sequenced using the primer PAR1c496, directed to the 5′-region of the *cp* gene.

Approximately 500 nucleotides of the 5′ region of the *cp* gene from each sample were compared to the corresponding sequence of other begomovirus isolates available in public databases. Pairwise nucleotide sequence comparisons performed by Mega 6.0 (Tamura et al. 2013) revealed that the sequences of all the isolates shared 99 to 100 % sequence identity among themselves and with the sequence of *Tomato common mosaic virus* (ToCmMV, GenBank accession EU710754).

As the partial nucleotide sequences for all 20 isolates were almost identical, one isolate, named ES-14, from Venda Nova do Imigrante county, was selected for the complete molecular characterization. For this, total DNA extracted from the sample infected by ES-14 was used as a template for rolling circle amplification (RCA) as described by Inoue-Nagata et al. (2004). RCA products were digested with the restriction endonucleases *Eco*RI, *Bam*HI, *Taq*I, *Hin*dIII, *Kpn*I, *Xba*I and *Stt*II in an attempt to clone the monomeric units of DNA-A and DNA-B molecules. All ca. 2.7 kb fragments corresponding to the unit genome-length of begomoviruses were ligated to the pBLUESCRIPT SK+ plasmid vector (Stratagene) and introduced into *Escherichia coli* DH5α by transformation. Viral inserts were commercially sequenced (Macrogen Inc.) by primer walking. For sequence analysis, the BLAST algorithm (http://www.ncbi.nlm.nih.gov/) was used for sequence similarity searches, Sequence Demarcation Tool (SDT; Muhire et al. 2014) was used to calculate pairwise identity scores, MUSCLE (Edgar 2004) implemented in MEGA 6.0 (Tamura et al. 2013) was used for multiple sequence alignments, the maximum-likelihood (ML) method (3,000 bootstrap replications) was used for phylogenetic reconstruction, Simplot 3.5.1 (Lole et al. 1999) was used for generating similarity plots, and RDP3 (Martin et al. 2010) was used for recombination analysis.

Thirty clones had their inserts sequenced and they shared 100 % nucleotide identity among themselves, but all of them were clones of the DNA-B component. In order to isolate the DNA-A component, 31 additional clones were evaluated by restriction fragment length polymorphism (RFLP) analysis using the *Msp*I restriction endonuclease. The digestion pattern of all clones was identical to that of the DNA-B clones. As an alternative for the determination of the DNA-A component sequence, the RCA product of ES-14 isolate was directly sequenced using the primer pair PAL1v1978 and PAR1c496 (Rojas et al. 1993) plus primers TCmR (5′-TAA CGT GCC CGA CGA GAT G-3′) and TCmF (5′-GAG CCC AAG TTG TAT AAT TT-3′), designed based on the available sequence of ToCmMV (EU710754) (Castillo-Urquiza et al. 2008). High quality sequences were assembled using the Staden package (Staden 1996) to obtain the complete DNA-A nucleotide sequence of the ES-14 isolate.

The DNA-A component is 2592 nucleotides long and shares the highest nucleotide identity (97.5 %) with ToCmMV (EU710754). Therefore, in accordance with the current guidelines for begomovirus species demarcation (Brown et al. 2015), the ES-14 isolate was named *Tomato common mosaic virus*-[Brazil-Venda Nova do Imigrante-ES14-Tomato-2007], acronym ToCmMV-[BR-VdIm-ES14-To-07].

The DNA-A component (GenBank accession KT203558) of ToCmMV-[BR-VdIm-ES14-To-07] shares 97.5 % nucleotide sequence identity with ToCmMV (EU710754) and 85.2 % with *Sida yellow leaf curl virus* (SiYLCV, EU710750) (Fig. 1a). The DNA-B component of ToCmMV-[BR-VdIm-ES14-To-07] (KT203559) is 2568 nucleotides long and shares the highest nucleotide identity (98.8 %) with ToCmMV (EU710755), followed by Tomato crinkle leaf yellows virus (ToCrLYV, AY090556) with 79.2 % (Fig. 1b). No sequences are available for either the DNA-A of ToCrLYV or the DNA-B of SiYLCV, and that is the major reason why the DNA-A and DNA-B trees have slightly different topologies (Fig. 1a and b).

As expected, the begomovirus described here exhibits the typical genome organization of New World, bipartite begomoviruses, with five ORFs in the DNA-A and two ORFs in the DNA-B. The DNA-A and DNA-B of the ToCmMV-[BR-VdIm-ES14-To-07] isolate have the same iteron sequences (GGTG/GGTG), suggesting that they

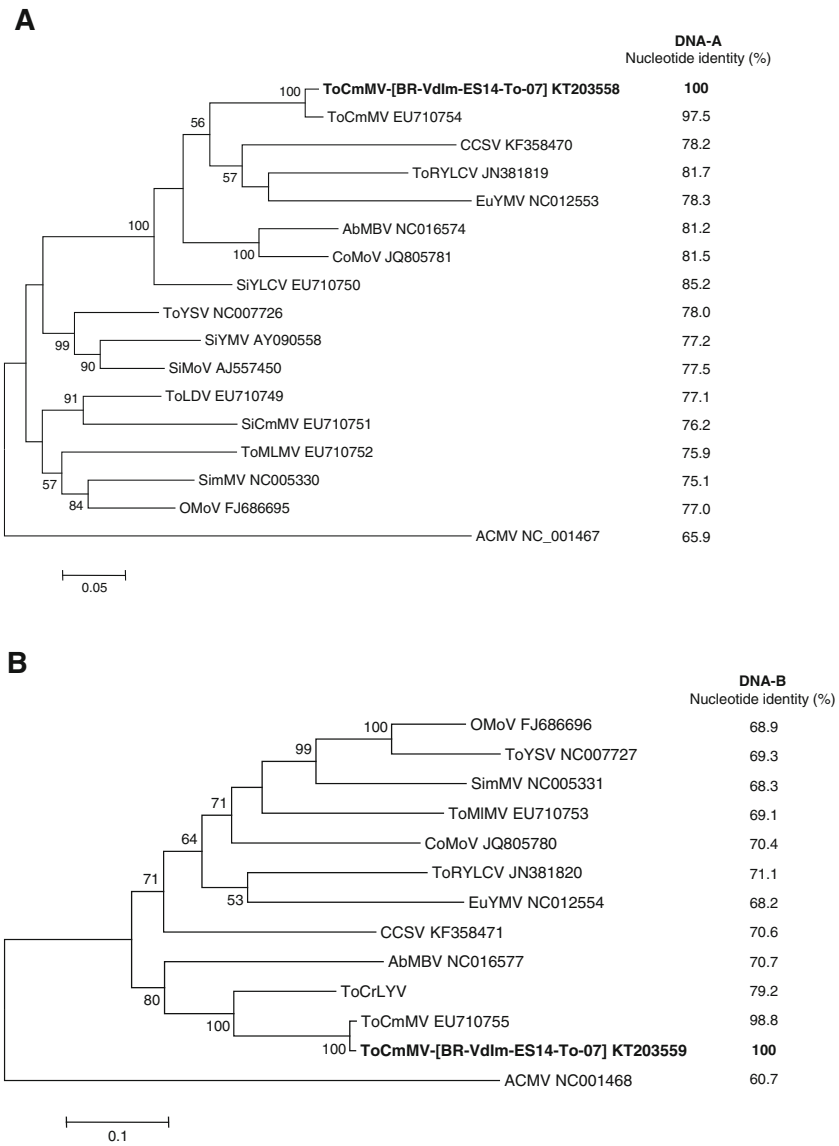


Fig. 1 Phylogenetic trees based upon an alignment of the nucleotide sequences of DNA-A (**a**) and DNA-B (**b**) components of the begomovirus described in this work (*Tomato common mosaic virus*-[Brazil-Venda Nova do Imigrante-ES14-Tomato-2007], acronym ToCmMV-[BR-VdIm-ES14-To-07]) with the sequences of the corresponding components of selected viruses obtained from GenBank. Bootstrap (3,000 replicates) values are shown as percentage values, and only the nodes with values greater than 50 % are labeled. GenBank accession number are show in the tree, and the name of the viruses are as follows: AbMBV, Abutilon mosaic Brazil virus; CCSV, Cotton chlorotic spot virus; CoMoV, Corchorus mottle virus; EuYMV, Euphorbia yellow mosaic virus, OMoV, Okra mottle virus; SiCmMV, Sida commom mosaic virus; SimMV, Sida micrantha mosaic virus; SiYLCV, Sida yellow leaf curl virus; SiYMV, Sida yellow mosaic virus; ToLDV, Tomato leaf distortion virus; ToMlMV, Tomato mild mosaic virus; ToRYLCV, Tomato rugose yellow leaf curl virus; ToYSV, Tomato yellow spot virus. DNA-A and DNA-B components of an isolate of an Old World begomovirus, African cassava mosaic virus (ACMV), were used as outgroups. The *bar* below each tree indicates nucleotide substitutions per site. Nucleotide sequence identities between the DNA-A (KT203558) and DNA-B (KT203559) components of ToCmMV-[BR-VdIm-ES14-To-07] and the viruses in the phylogenetic trees are presented

comprise a cognate pair. However, only inoculation experiments using infectious clones would provide definitive evidence that they are indeed a cognate pair.

Phylogenetic relationships between ToCmMV-[BR-VdIm-ES14-To-07] DNA-A and other closely related begomoviruses was incongruent with nucleotide sequence comparisons (Fig. 1a), which suggested the occurrence of a recombination event. On the other hand, phylogenetic relationships for the DNA-B accurately reflect the nucleotide identities, where ToCmMV-[BR-VdIm-ES14-To-07] clustered with ToCmMV and ToCrYLV (Fig. 1b).

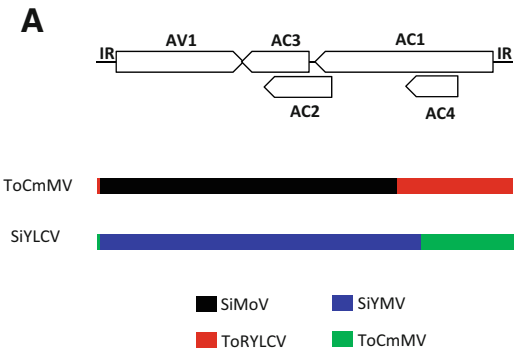
Recombination is an important evolutionary process that has been shown to play a major role in the evolution of begomoviruses and all other geminiviruses. To test the role

of this mechanism of genetic variation on the evolution of ToCmMV, we aligned all full-length South American begomovirus genome sequences obtained from GenBank (March 2015) and submitted to recombination analysis using the methods included in the RDP 3.0 package with default settings. No significant evidence of recombination events involving the DNA-B was detected. For the DNA-A, we detected evidence of individual recombination events and recombination breakpoint positions using RDP 3.0. Putative recombination events were analyzed with Simplot using the putative recombinant sequence as query.

Rocha et al. (2013) identified a recombination event in ToCmMV (EU710754), where *Euphorbia yellow mosaic virus* (EuYMV, FN435995) and *Sida mottle virus* (SiMoV, AJ557450) were the viruses used to infer the minor and major parents, respectively. The same recombination event was also detected here; however, two additional recombination events involving ToCmMV were detected in this study.

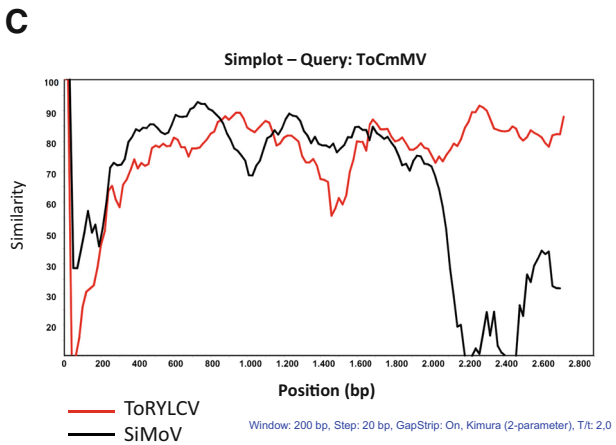
We identified clear evidence of one additional recombination event in the ToCmMV genome. The recombination breakpoints were detected at nucleotide positions 1851 (AC1 ORF) and 9 (intergenic region, IR) with SiMoV (AJ557450) and Tomato rugose yellow leaf curl virus (ToRYLCV, JN381813) as the viruses used to infer the major and minor parents, respectively (Fig. 2a–c). Interesting, ToRYLCV was described recently as a begomovirus infecting tomato crops in northern Uruguay (Marquez-Martin et al. 2012). In the second recombination event, our analyses showed that ToCmMV probably contributed with genetic material (~579 nucleotides) to the emergence of SiYLCV (Fig. 2a, b and d).

This study describes the characteristics of a ToCmMV isolate found infecting tomato plants in Venda Nova do Imigrante, Espírito Santo state, Brazil. ToCmMV was the only begomovirus species detected during surveys performed along a period of 5 years in this region. ToCmMV was first described in tomato plants collected in 2007 in the city of Coimbra, Minas Gerais, as being a distinct lineage of Brazilian begomoviruses (Castillo-Urquiza et al. 2008). The report of ToCmMV in Espírito Santo state is an indicative that this virus may be spreading to other tomato producing regions in Brazil.



B

Event	Recombinant	Major parent	Minor parent	Breakpoints		Methods	P-value
				Begin	End		
1	ToCmMV	SiMoV	ToRYLCV	1851	9	RGBMCST	2.06×10^{-30}
2	SiYLCV	SiYMV	ToCmMV	2023	10	RGBMCST	3.29×10^{-42}



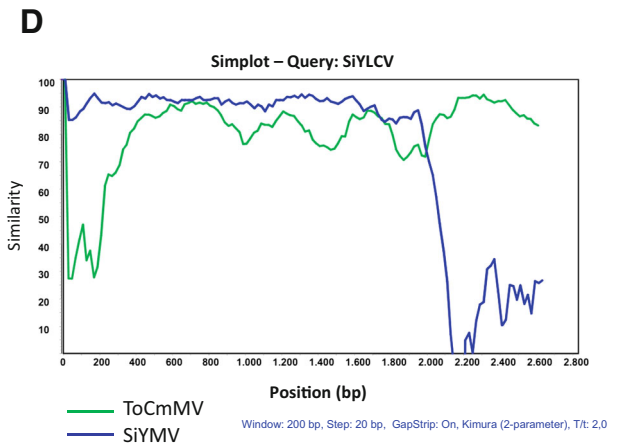


Fig. 2 **a**. Schematic representation of the begomovirus genome (*above*) and recombination events involving the isolates of *Tomato common mosaic virus* (ToCmMV) and *Sida yellow leaf curl virus* (SiYLCV). The colours of blocks correspond to the different begomovirus species. **b**. Details of recombination events in ToCmMV and SiYLCV detected using RDP3. R, G, B, M, C, S and T indicate detection by the RDP, GENCONV, BOOTSCAN, MAXCHI, CHIMERA, SISCAN and 3SEQ methods, respectively, with the present *p*-value being that determined by the method indicated in *bold*. **c**, **d**. Putative recombination events analyzed with Simplot program using ToCmMV (C) and SiYLCV (D) DNA-A sequence as query

This study adds to our understanding on the geographic distribution and the mechanisms that contribute to evolution of this group of viruses in Brazil, which contributes for developing disease management strategies. It is especially important to minimize the risk of the establishment of a damaging ToCmMV epidemic in Brazil.

References

Ambrozevicius LP, Calegario RF, Fontes EPB, Carvalho MG, Zerbini FM (2002) Genetic diversity of begomovirus infecting tomato and associated weeds in Southeastern Brazil. Fitopatol Bras 27:372–377

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG (1994) Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Ann Appl Biol 125:311–325

Brown JK, Fauquet CM, Briddon RW, Zerbini FM, Moriones E, Navas-Castillo J (2012) Family *Geminiviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy. Ninth report of the international committee on taxonomy of viruses. Elsevier Academic Press, London, pp 351–373

Brown J, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JF, Fiallo-Olivé E, Briddon R, Hernández-Zepeda C, Idris A, Malathi VG, Martin D, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593–1619

Castillo-Urquiza GP, Beserra JEJ, Bruckner FP, Lima AT, Varsani A, Alfenas-Zerbini P, Zerbini FM (2008) Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Arch Virol 153:1985–1989

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Report 1:19–21

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Fernandes FR, Albuquerque LC, Giordano LB, Boiteux LS, Avila AC, Inoue-Nagata AK (2008) Diversity and prevalence of Brazilian bipartite begomovirus species associated to tomatoes. Virus Genes 36:251–258

González-Aguilera J, Tavares SS, Sobrinho RR, Xavier CAD, Dueñas-Hurtado F, Lara-Rodrigues RM, Silva DJH, Zerbini FM (2012) Genetic structure of a Brazilian population of the begomovirus *Tomato severe rugose virus* (ToSRV). Trop Plant Pathol 37:346–353

Inoue-Nagata AK, Albuquerque LC, Rocha WB, Nagata T (2004) A simple method for cloning the complete begomovirus genome using the bacteriophage phi29 DNA polymerase. J Virol Methods 116: 209–211

Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, Ingersoll R, Sheppard HW, Ray SC (1999) Full-length *Human immunodeficiency virus* type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol 73:152–160

Lourencão AL, Nagai H (1994) Surtos populacionais de *Bemisia tabaci* no estado de São Paulo. Bragantia 53:53–59

Marquez-Martin B, Maeso D, Martinez-Ayala A, Bernal R, Teresa Federici M, Vincelli P, Navas-Castillo J, Moriones E (2012) Diverse population of a new bipartite begomovirus infecting tomato crops in Uruguay. Arch Virol 157:1137–1142

Martin DP, Lemey P, Lott M, Moulton V, Posada D, Lefeuvre P (2010) RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26:2462–2463

Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9, e108277

Polston JE, Anderson PK (1997) The emergence of whitefly-transmitted geminiviruses in tomato in the Western Hemisphere. Plant Dis 81: 1358–1369

Ribeiro SG, Ávila AC, Bezerra IC, Fernandes JJ, Faria JC, Lima MF, Gilbertson RL, Maciel-Zambolim E, Zerbini FM (1998) Widespread occurrence of tomato geminiviruses in Brazil, associated with the new biotype of the whitefly vector. Plant Dis 82:830

Ribeiro SG, Ambrozevícius LP, Ávila AC, Bezerra IC, Calegario RF, Fernandes JJ, Lima MF, Mello RN, Rocha H, Zerbini FM (2003) Distribution and genetic diversity of tomato-infecting geminiviruses in Brazil. Arch Virol 148:281–295

Rocha CS, Castillo-Urquiza GP, Lima ATM, Silva FN, Xavier CAD, Hora-Junior BT, Beserra-Junior JEA, Malta AWO, Martin DP, Varsani A, Alfenas-Zerbini P, Mizubuti ESG, Zerbini FM (2013) Brazilian begomovirus populations are highly recombinant, rapidly evolving, and segregated based on geographical location. J Virol 87:5784–5799

Rojas MR, Gilbertson RL, Russell DR, Maxwell DP (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis 77:340–347

Staden R (1996) The staden sequence analysis package. Mol Biotechnol 5:233–241

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol