Universidade de Brasília
Instituto de Ciências Biológicas
Departamento de Fitopatologia
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Tese de Doutorado

Análise metagenômica-biológica da diversidade de vírus da família *Geminiviridae* e de agentes subvirais em cultivares de tomateiro suscetíveis e tolerantes (*Ty*-1/*Ty*-3) em diferentes regiões brasileiras

IZAÍAS ARAÚJO DE OLIVEIRA

Brasília – DF
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Tese apresentada à Universidade de Brasília como parte dos requisitos parciais obtenção do título de Doutor em Fitopatologia pelo Programa de Pós-Graduação em Fitopatologia.

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Per ipsum, et cum ipso, et in ipso, est tibi Deo Patri omnipoténti, in unitáte Spíritus Sancti, omnis honor et glória per ómnia sæcula sæculórum.

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RESUMO GERAL

De Oliveira, Izaías Araújo de. **Análise metagenômica-biológica da diversidade de vírus da família Geminiviridae e de agentes subvirais em cultivares de tomateiro suscetíveis e tolerantes (Ty-1/Ty-3) em diferentes regiões brasileiras** 2024. Número de páginas (196). Tese (Doutorado em Fitopatologia) – Universidade de Brasília, Brasília, DF.

A cultura do tomateiro (*Solanum lycopersicum* L.) possui grande importância para agricultura nacional e internacional. Diversas viroses podem acometer a cultura causando impactos negativos consideráveis. Dentre os vírus que infectam o tomateiro, as espécies classificadas no gênero *Begomovirus* (família *Geminiviridae*) merecem destaque (**Capítulo 1**). Os begomovírus possuem genoma de DNA circular fita simples que pode ser monopartido ou bipartido e são separados em dois grupos: begomovírus do Velho Mundo e do Novo Mundo. A transmissão dos begomovírus ocorre por meio de um complexo de espécies crípticas de *Bemisia tabaci* (Aleyrodidae: Hemiptera) com o predomínio de *B. tabaci Middle East Asia Minor* 1 – MEAM 1 (= biótipo B) e *B. tabaci Mediterranean* – MED (= biótipo Q). A gama de plantas hospedeiras dos begomovírus é ampla, incluindo dicotiledôneas cultivadas e não cultivadas. Há uma grande diversidade de espécies de begomovírus já descritas e diversas novas espécies vêm sendo caracterizadas na última década. A grande diversidade observada nos begomovírus é gerada por três mecanismos distintos: mutação, recombinação e pseudorecombinação. No Brasil, uma das estratégias promissoras para controle de begomoviroses no tomateiro tem sido o uso de cultivares com dois fatores de resistência/tolerância (*Ty*–1 e *Ty*–3). Com a crescente diversidade de espécies, o uso de ferramentas tais como *High-Throughput Sequencing* (HTS) tem permitido estudos mais amplos nesse tópico. Estudos prévios têm indicado que cultivares portadoras do gene *Ty*–1 reduzem o número de espécies de begomovírus que infectam o tomateiro e impactam a frequência e predominância destes vírus, agindo como um 'filtro biológico'. Além disto, espécies novas e/ou recombinantes podem superar fatores de tolerância presentes na planta hospedeira. Dessa maneira, o objetivo deste trabalho foi realizar o monitoramento e caracterização via sequenciamento com tecnologia HTS da diversidade viral da família *Geminiviridae* e de agentes subvirais associados ao gênero *Begomovirus* junto ao cultivo do tomateiro em cinco regiões geográficas do Brasil entre 2003–2017. Para tanto, 154 amostras de tomateiros sintomáticos (com e sem os fatores *Ty*–1 e *Ty*–3) foram coletadas em cinco regiões brasileiras: Norte (13 amostras), Nordeste (36), Sul (24), Sudeste (39) e Centro-Oeste (42). As amostras foram enriquecidas com DNA circulares por meio de *Rolling Circle Amplification* (RCA). As amostras enriquecidas foram agrupadas em dois *pools*: BP1 (amostras das regiões Norte, Nordeste e Sul) e BP2 (Sudeste e Centro-Oeste) e submetidas ao sequenciamento HTS pela plataforma Illumina NovaSeq-6000 (**Capítulo 2**). As sequências foram montadas em *contigs* utilizando o software CLC genomics Workbench 7.5, analisadas no Geneious R11.1, comparadas com o banco de sequências depositadas no GenBank por meio do algoritmo BLASTn. Do sequenciamento do *pool* BP1 recuperou-se 16 sequências correspondentes a 16 vírus, sendo 15 deles classificados no gênero *Begomovirus*, dos quais 11 correspondem a relatos de espécies já conhecidas e quatro corresponderam a potenciais novos begomovírus (espécie nova #1; espécie nova #2; espécie nova #3 e espécie nova #4), além de uma espécie de *Topilevirus*. Recuperou-se do sequenciamento do *pool* BP2 14 sequências correspondentes a 14 vírus, sendo uma provável nova espécie de begomovírus (espécie nova #5) e também sequências de alfassatélites associados aos begomovírus. *Primers* espécie-específicos foram empregados para recuperação dos genomas por PCR em cada amostra individualmente. Os genes *Ty*–1 e *Ty*–3 impactaram a diversidade viral e o número de amostras infectadas, sendo

que as amostras desprovidas desses fatores apresentam maior número de espécies detectadas e infecções. As frequências de infecções mistas também foram maiores em amostras de tomateiros que não possuíam os fatores *Ty*–1/*Ty*–3. No **capítulo 3**, a recuperação por ensaios de PCR com *primers* espécies-específicos permitiu a detecção do begomovírus bipartido tomato chlorotic mottle Guyane virus – ToCMoGV, até então ausente no Brasil, na amostra denominada AM–035, coletada em Iranduva, estado do Amazonas, norte do Brasil. Análises moleculares e reconstruções filogenéticas demonstraram que ToCMoGV detectado no Brasil, consiste em uma estirpe com alta divergência do isolado da Guiana Francesa, com a qual o DNA–A compartilha 92% de identidade nucleotídica. **No capítulo 4**, através da caracterização molecular, reconstrução filogenética e análises de identidade por meio do *Sequence Demarcation Tool* (SDT) demonstrou-se que isolados virais depositados no *GenBank* identificados como tomato yellow spot virus (ToYSV) e Leonurus mosaic virus (LeMV) somados aos isolados de ToYSV recuperados por HTS no presente estudo se mostravam intimamente relacionados. Alguns compartilhavam os mesmos *iterons* e identidades iguais e/ou superiores a 91%, levando a concluir que foram erroneamente nomeados uma vez que se trata de uma única espécie viral. Esse vírus ocorre apenas esporadicamente no tomateiro, mas é o patógeno predominante em *Leonurus*. Neste contexto, foi elaborada uma proposição de que apenas a nomenclatura original/mais antiga (Leonurus mosaic virus) seja empregada para esse patógeno. O **Capítulo 5** relata a detecção de um novo isolado de begomovírus infectando tomateiro na região norte do Brasil. O novo isolado de begomovírus foi detectado na amostra TO–083, originária de Araguaína, estado do Tocantins. O novo isolado é bipartido e clones infectivos foram produzidos e inoculados em tomateiros. A caracterização molecular do novo isolado identificou o *iteron* GGTGT/ACACC e o domínio da Rep relacionado ao iteron (Rep–IRD): MPRQPTTFRL. O isolado TO–083 foi então denomidado tomato golden leaf spot virus (ToGLSV). No **capítulo 6**, duas novas espécies de begomovírus monopartidos foram recuperadas por PCR com *primers* específicos. A nova espécie #1 foi detectada no nordeste brasileiro, nas amostras: CE–001, originária do estado do Ceará, PE–011 e PE–012, ambas de Pernambuco. A nova espécie #2 foi detectada nas amostras PR–173 e PR–174 ambas coletadas no estado do Paraná, sul do Brasil. As análises de recombinação demonstraram que as duas novas espécies são recombinantes envolvendo segmentos genômico de outros begomovírus previamente reportados infectando o tomateiro no Brasil. O begomovírus correspondente a nova espécie #1, nomeado tomato iridescent mottle virus (ToIMoV) está filogeneticamente mais próxima do tomato interveinal chlorosis virus (ToICV) (JF803252), enquanto que o tomato iridescent apical mosaic virus (ToIAMV), correspondente à nova espécie #2 está mais próxima do tomato mottle leaf curl virus (ToMoLCV) (MT215005). O **capítulo 7** relata a detecção e caracterização molecular de um novo begomovírus bipartido (tomato bright apical mosaic virus –ToBAMV) infectando tomateiros, o qual foi detectado na região centro-oeste do Brasil. ToBAMV foi detectado em amostras de tomateiro oriundas do estado de Goiás (GO) e do Distrito Federal (DF), apresenta o segmento DNA–A de 2561 nucleotídeos (nts) e o DNA–B de 2527 nts. Este vírus está filogeneticamente mais relacionado com tomato golden vein virus (TGVV) (MN928612) com o qual compartilha 89% de identidade nucleotídica. As análises detectaram um evento de recombinação com tomato leaf curl purple vein virus (ToLCPVV) e tomato chlorotic mottle virus (ToCMoV). Estudos para a caracterização biológica dessa nova espécie bipartida serão realizados futuramente. O número crescente de espécies de begomovírus associadas com tomateiro que foram descritas aqui representam um grande desafio para o manejo e controle genético desse complexo de patógenos. O **capítulo 8** compila os principais resultado desta tese, trazendo dados que fornece ao melhoramento genético do tomateiro, um

panorama mais preciso sobre espécies de *Geminiviridae* que apresentam relevância epidemiológica no Brasil.

Palavras chaves: *Geminiviridae*, *Solanum lycopersicum*, sequenciamento de alto rendimento, tolerância.

GENERAL ABSTRACT

De Oliveira, Izaías Araújo. **Metagenomic-biological analysis of the diversity of viruses from the Geminiviridae family and subviral agents in susceptible and tolerant tomato cultivars (Ty-1/Ty-3) in different Brazilian regions** 2024. Number of pages (196). Thesis (Doctorate in Phytopathology) – University of Brasília, Brasília, DF.

The tomato (*Solanum lycopersicum* L.) crop is of great national and international importance. Several viruses can affect the tomato crop, causing considerable negative impacts. Among the tomato-infecting viruses, the species classified in the genus *Begomovirus* (family *Geminiviridae*) deserve to be emphasized (**Chapter 1**). Begomoviruses have a single-stranded circular DNA genomes that can be either monopartite or bipartite, being discriminated into two major groups: Old World and New World begomoviruses. Transmission of begomoviruses occurs through a complex of cryptic species of *Bemisia tabaci* (Aleyrodidae: Hemiptera) with a predominance of *B. tabaci Middle East Asia Minor 1* – MEAM 1 (= biotype B) and *B. tabaci Mediterranean* – MED (= biotype Q). The range of host plants for begomoviruses is wide, including cultivated and uncultivated dicotyledonous. There is a great diversity of *Begomovirus* species already described and several new ones have been characterized in the last decade.The great diversity observed in begomoviruses is generated by three distinct mechanisms: mutation, recombination, and pseudorecombination. In Brazil, one of the promising strategies for controlling begomoviruses in tomato has been the use of cultivars with two resistance/tolerance factors (*Ty*–1 and *Ty*–3). With the increasing diversity of species, the use of tools such as High-Throughput Sequencing (HTS) has allowed extensive studies on this topic. Previous studies have indicated that cultivars carrying the *Ty*–1 gene reduce the number of begomovirus species able to infect tomatoes and impact their frequency and predominance, acting as a biological 'filter'. Furthermore, new and/or recombinant species may overcome tolerance factors present in the host plant. Therefore, the objective of this work was to monitor and characterize via sequencing with HTS technology the viral diversity of the *Geminiviridae* family and subviral agents associated with begomoviruses associated with the tomato crop in five geographic regions of Brazil between the years 2003–2017. To achieve this objective, 154 samples from symptomatic tomato plants (with and without *Ty*–1 and *Ty*–3 factors) were collected in five Brazilian regions: North (13 samples), Northeast (36), South (24), Southeast (39) and Midwest (42). Samples were enriched with circular DNA using Rolling Circle Amplification (RCA). The enriched samples were grouped into two pools: BP1 (samples from the North, Northeast, and South regions) and BP2 (Southeast and Central-West) and submitted to HTS sequencing using the Illumina NovaSeq-6000 platform (**Chapter 2**). The sequences were assembled into contigs using the CLC genomics Workbench 7.5 software, analyzed in Geneious R11.1, compared with the sequence bank deposited in GenBank using the BLASTn algorithm. From the sequencing of the BP1 pool, 16 sequences corresponding to 16 viruses were recovered, 15 of which were classified in the genus *Begomovirus*, of which 11 corresponded to reports of already known species and four corresponded to potential new begomoviruses (new species #1; new species #2; new species #3 and new species #4), in addition to one *Topilevirus* species. Fourteen (14)

sequences corresponding to 14 viruses and subviral agents were recovered from the sequencing of the BP2 pool, being a probable new species of begomovirus (new species #5) and also alphasatellite sequences associated with begomoviruses. Species-specific primers were used to recover the genomes by PCR in each sample individually. The *Ty*–1 and *Ty*–3 genes impacted viral diversity and the number of infected samples, with samples lacking these factors presenting a greater number of detected species and infections. The frequencies of mixed infections were also higher in tomato samples that did not have the *Ty*–1/*Ty*–3 factors. In **Chapter 3**, recovery by PCR assays with species-specific primers allowed the detection of the bipartite begomovirus tomato chlorotic mottle Guyane virus – ToCMoGV, previously absent in Brazil, in the sample called AM–035, collected in Iranduva, state of Amazonas, northern Brazil. Molecular analyzes and phylogenetic reconstructions demonstrated that ToCMoGV from Brazil consists of a strain with high divergence from the isolate of French Guiana, sharing 92% nucleotide identity in their DNA–A segment. In **Chapter 4**, it was demonstrated via molecular characterization, phylogenetic construction, and identity analysis (using the Sequence Demarcation Tool – SDT) that viral isolates identified as tomato yellow spot virus (ToYSV) and Leonurus mosaic virus (LeMV) in GenBank (as well as the ToYSV isolates recovered by HTS in the present study) were closely related. Some shared the same iterons and identities equal to and/or greater than 91%, leading to the conclusion that they were wrongly named since they are a single viral species. This virus occurs only sporadically in tomato, but is the predominant pathogen in *Leonurus*. In this context, a proposal was made that only the original/oldest nomenclature (i.e. Leonurus mosaic virus) should be used for this pathogen. The **Chapter 5** reports the detection of a new begomovirus isolate infecting tomatoes in the Northern region of Brazil. The new begomovirus isolate was detected in sample TO–083, originating from Araguaína, state of Tocantins. The new isolate is bipartite and infective clones were produced and inoculated into tomato plants. Molecular characterization of the new isolate identified the GGTGT/ACACC iteron and the iteron-related Rep domain (Rep–IRD): MPRQPTTFRL. Isolate TO–083 was tentatively named as tomato golden leaf spot virus (ToGLSV). In **Chapter 6**, two new species of monopartite begomoviruses were recovered by PCRs with specific primers. The new species #1 was detected in northeastern Brazil, in samples: CE–001, originating from the state of Ceará, PE–011 and PE–012, both from Pernambuco. The new species #2 was detected in samples PR–173 and PR–174, both collected in the state of Paraná, southern Brazil. Recombination analyzes demonstrated that the two new species are recombinants involving genomic segments of other begomoviruses previously reported infecting tomato in Brazil. The begomovirus corresponding to new species #1, named tomato iridescent mottle virus (ToIMoV), is phylogenetically closer to tomato interveinal chlorosis virus (ToICV) (JF803252), while tomato iridescent apical mosaic virus (ToIAMV), corresponding to new species # 2 is closest to tomato mottle leaf curl virus (ToMoLCV) (MT215005). **Chapter 7**, reports the detection and molecular characterization of a new bipartite begomovirus (tomato bright apical mosaic virus –ToBAMV) infecting tomato plants, which was named in the central-western region of Brazil. ToBAMV was detected in tomato samples from the state of Goiás (GO) and the Federal District (DF), has the DNA–A segment of 2561 nucleotides (nts) and the DNA–B of 2527 nts. This virus is phylogenetically most closely related to tomato golden vein virus (TGVV) (MN928612) with which it shares 89% nucleotide identity. Further analyses detected a recombination event with tomato leaf curl purple vein virus (ToLCPVV) and tomato chlorotic mottle virus (ToCMoV). Studies dealing with the biological characterization of this new bipartite species are ongoing. The increasing number of *Begomovirus* species associated with tomato that have been described here represent a major

challenge for the management and genetic control of this complex of pathogens. **Chapter 8** compiles the main results of this thesis, bringing data that provide the genetic improvement of tomato, a more precise overview of Geminiviridae species that have epidemiological relevance in Brazil.

Keywords: *Geminiviridae*, *Solanum lycopersicum*, high-throughput sequencing

INTRODUÇÃO GERAL

O tomateiro (*Solanum lycopersicum* L.), originário das regiões andinas do Peru, Bolívia e Equador, é uma cultura adaptada às condições tropicais e subtropicais (EMBRAPA 1993). A China está em primeiro lugar entre os maiores produtores de tomate do mundo com uma produção de 68.241.810,69 milhões de toneladas (ton). O Brasil encontra-se na oitava posição com 3.809.986,00 de toneladas produzidas (FAOSTAT 2024). No âmbito nacional, as regiões Sudeste e Centro-Oeste possuem as maiores produções, destacando-se o Estado de São Paulo (SP) o maior produtor nacional de tomate com 1.039,7 mil toneladas seguido de Goiás (GO) (1 021,7 mil) (IBGE 2023).

Dentre os patógenos que acometem a cultura, destacam-se os vírus, principalmente begomovírus (gênero *Begomovirus*, família *Geminiviridae*). Este gênero é o mais numeroso da família, com 445 espécies descritas até o momento (ICTV 2024). Os begomovírus apresentam genomas de DNA circular de fita simples constituídos de uma única molécula (espécies monopartidas) contendo apenas o segmento genômico DNA–A ou de duas moléculas (espécies bipartidas), contendo os segmentos genômicos DNA–A e DNA–B (Brown et al. 2015; ICTV 2024). Os begomovírus podem ser separados em dois grandes grupos: begomovírus do Velho Mundo (África, Ásia e Europa) e do Novo Mundo (Américas) (Navas-Castillo & Fiallo-Olivé 2020). A gama de hospedeiras dos begomovírus é ampla, incluindo dicotiledôneas cultivadas e não cultivadas (Rojas et al. 2018). A transmissão dos begomovírus é feita de maneira eficiente por um complexo de espécies crípticas relacionadas com a antiga espécie monotípica, *Bemisia tabaci* (família *Aleyrodidae*, ordem *Hemiptera*); predominantemente *B. tabaci Middle East Asia Minor* 1 (MEAM1) e *B. tabaci Mediterranean* (MED) (De Barro et al. 2011; Rosen et al. 2015; Fernandes et al. 2023).

Após o ingresso de *B. tabaci* MEAM1 no Brasil no início da década de 1990 (Lourenção & Nagai, 1994; Ribeiro et al. 1994), houve um aumento na incidência, severidade e diversidade de begomoviroses no tomateiro (Ribeiro et al. 2003). A partir de então, trabalhos de levantamento vêm sendo realizados com objetivo de avaliar a diversidade dentro deste grupo

de patógenos virais. Diferentes estudos de prospecção da diversidade têm sido conduzidos em larga escala geográfica e temporal (Ribeiro et al. 2003, Fernandes et al. 2008, Reis et al. 2020, Souza et al. 2020).

As begomoviroses representam um fator limitante em áreas produtoras de tomate em regiões tropicais e subtropicais, com perdas normalmente superiores a 50% (Giordano et al. 2005b). Em tomateiro, a infecção por begomovírus pode ocasionar os sintomas de mosaicos, clorose apical e encarquilhamento, deformações foliares, desvios de coloração, mosqueado distorções, amarelecimento, bronzeamento em nervuras, rugosidades e epinastias foliares, nanismo (**Figura 1**) (Inoue-Nagata et al. 2016). Alguns begomovírus que infectam a cultura do tomateiro no Brasil apresentam uma prevalência em certas regiões do país, por exemplo, tomato severe rugose virus (ToSRV) e tomato mottle leaf curl virus (ToMoLCV) estão amplamente distribuídos na região central e nordeste do Brasil, respectivamente (Macedo et al. 2016; Gilbertson et al. 2015; Reis et al. 2020). No Brasil Central, tem sido comum a ocorrência de infecções mistas envolvendo múltiplas espécies de begomovírus (Reis et al. 2020). Do ponto de vista epidemiológico, as plantas daninhas desempenham papel relevante, pois atuam como reservatórios destes vírus que, por meio dos insetos vetores, podem infectar o cultivo do tomateiro (Barreto et al. 2013; Duarte et al. 2021; Pereira-Silva et al. 2022).

A geração de variabilidade genética dos begomovírus pode ser alcançada por meio de três mecanismos: mutação, recombinação e pseudorecombinação (Seal et al. 2006). O aumento da diversidade e variabilidade dos begomovírus é um fator que demanda medidas de manejo mais sustentáveis. Entre os métodos de controle disponíveis, a adoção de cultivares com genes de resistência/tolerância tem sido considerada a estratégia mais simples e eficiente, reduzindo os impactos da infecção viral sobre a cultura (Boiteux et al. 2012). Atualmente, oito genes de resistência/tolerância a begomovírus estão caracterizados e/ou mapeados no genoma do tomateiro: *Ty*–1 (Zamir et al. 1994; Verlaan et al. 2013), *Ty*–2 (Hanson et al. 2006; Ji et al. 2009a), *Ty*–3 (Ji e Scott, 2006; Ji et al. 2007), *Ty*–4 (Ji et al. 2009b), *Ty*–5 (Anbinder et al. 2009), *Ty*–6 (Hutton e Scott, 2014; Gill et al. 2019), *tcm*–1 (Giordano et al. 2005a) e *tgr*–1 (Bian et al. 2007). Cultivares de tomateiro portando genes de tolerância a begomovírus causam impacto na dinâmica populacional das espécies virais detectadas na cultura, reduzindo, em termos gerais, a diversidade viral (Reis et al. 2020; Souza et al. 2020). Dados iniciais têm indicado que o uso de cultivares contendo o gene de tolerância *Ty*–1 são capazes de "filtrar" um subgrupo de espécies virais registradas no tomateiro (Reis et al. 2020), influenciando, desta forma, a predominância e frequência das espécies de begomovírus que ocorrem na região do Brasil Central.

Os estudos de diversidade viral em larga escala exigem o uso de abordagens eficientes, gerando dados robustos e de qualidade. Estudos de metagenômica utilizando tecnologia de *High-throughput sequencing* (HTS) vêm se tornando, a mais de uma década, ferramentas tecnológicas extremamente úteis em estudos de virologia vegetal (Adams et al. 2009), permitindo obter uma grande quantidade de dados de forma rápida e com custo-benefício satisfatório (Mehetre et al. 2019). Estudos voltados à dinâmica e diversidade populacional de begomoviroses em tomateiros tolerantes são restritos à região Central do Brasil. Neste contexto, esta pesquisa visa ampliar estas análises a nível nacional, tendo como principais: **(1)** efetuar uma análise metagenômica da diversidade de espécies de *Geminiviridae* e de agentes subvirais, com especial ênfase em populações de begomovírus presentes nas principais regiões produtoras de tomate do Brasil e em diferentes biomas brasileiros; **(2)** conduzir estudos comparativos da diversidade viral em cultivares de tomateiro com e sem fatores de tolerância e **(3)** descrever e caracterizar molecularmente novas espécies de begomovírus.

Os resultados gerados por este estudo contribuem para compreender a distribuição geográfica das espécies virais da família *Geminiviridae* e agentes subvirais associados ao begomovírus e de novas espécies viriais, bem como o impacto dos faltores de tolerância *Ty*–1 e *Ty*–3 sobre a dinâmica e diversidade viral dos begomovírus infectantes do tomateiro distribuídos nas cinco regiões do Brasil, além disso fornece aos melhoristas, e aos programas de melhoramento genético, um panorama mais preciso das espécies de begomovírus que possuem maior relevância epidemiológica para os principais polos produtores de tomate do Brasil.

HIPÓTESES

- O aumento da variabilidade genética/genômica de espécies virais da família *Geminiviridae* e a contínua emergência de novos begomovírus e de novos agentes subvirais sugerem que estes fenômenos biológicos estejam ocorrendo, de maneira ininterrupta, em todas as regiões e biomas onde o cultivo do tomateiro tem sido conduzido no Brasil.
- Begomovírus presentes no território brasileiro podem ter sofrido ou estar sofrendo um processo de regionalização ecológica, tornando-se mais frequentes em determinadas condições climáticas/ambientais do país, tornando a distribuição de alguns desses patógenos restrita ou endêmica a determinadas áreas geográficas do país.
- O emprego de cultivares resistentes/tolerantes pode resultar em processos, ainda não plenamente elucidados, de seleção de populações de begomovírus impactando a diversidade viral nas diferentes regiões do Brasil, contribuindo para formação de um padrão de distribuição e incidência das diferentes espécies de begomovírus.
- A pressão seletiva de fatores de resistência/tolerância pode conduzir para o surgimento de espécies novas ou de novos recombinantes com a capacidade de superar estes mecanismos/fatores de defesa da planta.

OBJETIVO GERAL

Caracterizar e monitorar, via análise metagenômica, a diversidade genética de espécies da família *Geminiviridae* e de agentes subvirais (com ênfase em espécies de begomovirus) associadas com o tomateiro em biomas das cinco regiões brasileiras, afim de fornecer ao melhoramento genético do tomateiro, um panorama mais preciso sobre espécies da família *Geminiviridae* que apresentam relevância epidemiológica no Brasil possibilitando ao programa de melhoramento montar estratégias de manejo integrado das begomoviroses que ocorre no tomateiro que menos impactem negativamente o meio agrícola e a saúde do trabalhador rual.

OBJETIVOS ESPECÍFICOS

- Elucidar aspectos relativos à distribuição geográfica das espécies virais e agentes subvirais, tais como o endemismo de espécies, distribuição das novas espécies virais e agentes subvirais e atualizações da distribuição das espécies já relatadas.

- Caracterizar molecularmente novas espécies de *Begomovirus* associadas com o tomateiro no Brasil por meio de análises de iterons e motivos conservados, reconstruções filogenéticas, comparações nucleotídicas e eventos de recombinações;
- Avaliar os impactos dos fatores de tolerância *Ty*–1 e *Ty*–3 sobre a dinâmica populacional e a diversidade dos begomovírus infectantes dos tomateiros;
- Atualizar a gama de espécies virais do gênero *Begomovirus* associadas à cultura do tomateiro dentro das cinco rregiões brasileiras.

CAPÍTULO 01

REVISÃO DE LITERATURA

1. REVISÃO DE LITERATURA

1.1 A cultura do tomateiro: origem e produção.

O tomateiro (*Solanum lycopersicum* L.) possui como centro de origem as regiões andinas do Peru e Equador. Em decorrência disto, a cultura desenvolve-se bem em condições tropicais e subtropicais de temperaturas amenas, com faixa entre 18 a 25 °C (Boiteux et al. 2016). A domesticação da cultura ocorreu no México, especificamente nas cidades de Puebla e Vera Cruz, e sua distribuição para a Europa ocorreu em 1544. A partir da Europa a cultura alcançou regiões da Ásia meridional e oriental, África e Oriente Médio (Naika et al. 2006).

No âmbito global da produção de tomate (**Figura 1**), a China ocupa a primeira colocação com a produção de 68.241.810,69 milhões de toneladas (ton.), seguida pela Índia (20.694.000), Turquia (13.000.000), Estados Unidos da América (10.199.753), Egito (6.275.380), Itália (6.136.380), México (4.207.889,22), Brasil (3.809.986), Nigéria (3.684.546,41) e Espanha (3.651.940) (FAOSTAT 2024).

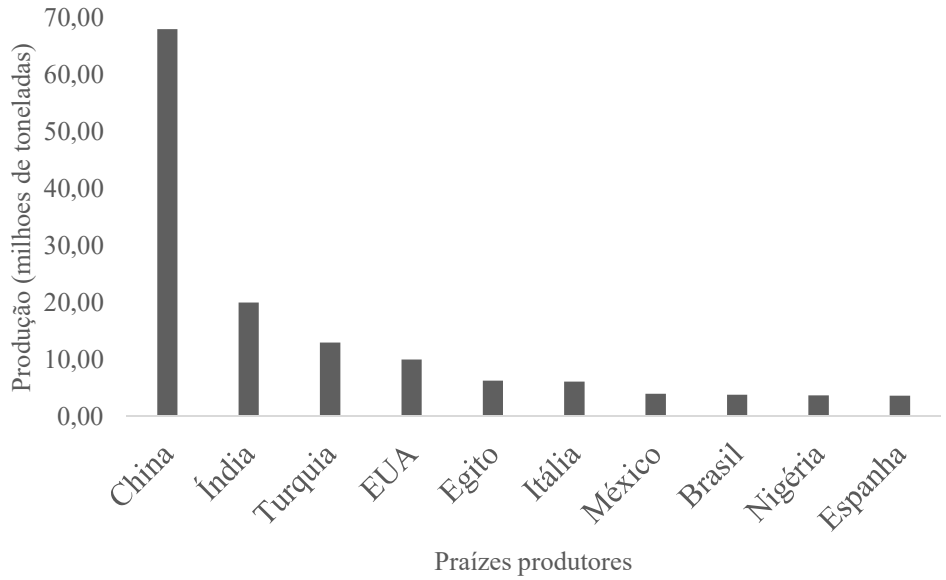

Figura 1. Dez maiores países produtores de tomate do mundo (FAOSTAT 2024).

De acordo com o último levantamento sistemático da produção agrícola brasileira o cenário da produção nacional de tomate mostra uma estimativa de 3,9 milhões de toneladas produzidas (IBGE 2023). Entre os estados brasileiros com maior produção destaca-se São Paulo (SP) como maior produtor, com 1.039,7 mil toneladas, seguido por Goiás (GO) (1.021,7), Minas Gerais (MG) (536,4), Paraná (PR) (238,8), Ceará (CE) (183,5), Bahia (BA) (179,6), e Rio de Janeiro (RJ) (136,6) (**Figura 2**).

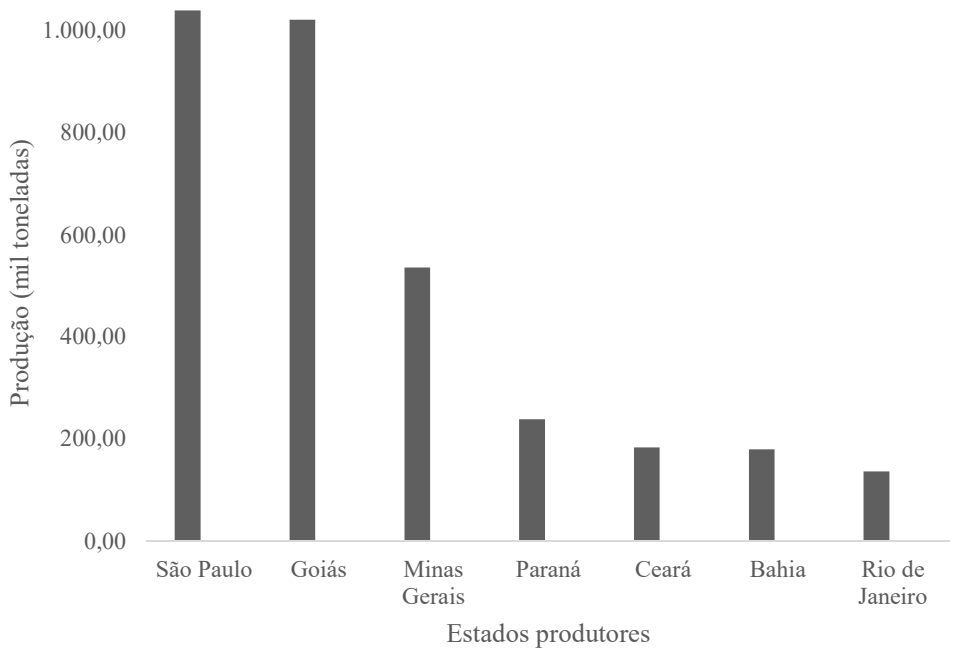

Figura 2. Sete maiores estados brasileiros produtores de tomate (IBGE 2023).

A cultura do tomateiro possui grande importância para a economia de diversos países, gerando lucros, empregos e crescimento econômico. Diante disso, esforços vêm sendo realizados por programas de melhoramento do tomateiro com objetivo de tornar a cultura menos vulnerável ao ataque de fitopatógenos e estresse abióticos com intuito de reduzir custos e garantir a manutenção da qualidade e produtividade da cultura (Acharya et al. 2018; Melomey et al. 2019).

1.2. Doenças de etiologia viral do tomateiro nas condições brasileiras.

Dentre várias doenças que acometem a cultura do tomateiro destacam-se as viroses como um fator limitante para a produção. Os principais vírus que infectam e destacam-se em importância para cultura do tomateiro no Brasil encontram-se classificados nos gêneros *Crinivirus*, *Orthotospovirus*, *Potyvirus* e *Begomovirus* (Inoue-Nagata et al. 2016; ICTV 2024).

Gênero *Crinivirus* – Os crinivírus (família *Closteroviridae*) constituem-se de vírus de RNA de fita simples bipartido, senso positivo, contudo há também representantes desse gênero com genoma tripartido, sendo *Lettuce infectious yellows virus* espécie-tipo do gênero (ICTV 2024; Kiss et al. 2013) e possuem uma considerável gama de hospedeiros, entre as plantas cultivadas. Esses vírus podem infectar melão, beterraba, alface, morango, batata-doce, feijoeiro, batata e

tomate (Tzanetakis et al. 2013). No Brasil o primeiro relato de tomato chlorosis virus (ToCV), membro do gênero *Crinivirus,* ocorreu no estado de São Paulo (Barbosa et al. 2008). Levantamentos em anos posteriores revelaram a presença do patógeno em outros cinco estados brasileiros: Bahia, Espírito Santo, Goiás, Minas Gerais e Rio de Janeiro (Barbosa et al. 2011). A presença do vírus em tomateiro no Distrito Federal foi também foi relata (Nogueira et al. 2011). Além do tomateiro, outras espécies do gênero *Solanum* também são hospedeiras do ToCV, ampliando a gama de hospedeiras e as potenciais fontes de inóculo do vírus (Fonseca et al. 2016; Boiteux et al. 2018). A transmissão do ToCV ocorre de maneira não circulativa semi-persistente através de insetos vetores dos gêneros *Trialeurodes* e *Bemisia* (Tzanetakis et al. 2013; Vargas-Asencio et al. 2013). Entre os sintomas observados pela infecção viral está a clorose internerval (muito similar os sintomas de deficiência de magnésio) e espessamento do limbo foliar, bronzeamento e necrose foliar, declínio do vigor e queda de produção (Fiallo-Olivé & Navas-Castilho 2019). Fontes de tolerância contra isolados sul-americanos de ToCV têm sido detectadas em acessos de *Solanum* (*Lycopersicon*) (González-Arcos et al. 2018).

Gênero *Orthotospovirus* – Os orthotospovírus (família *Tospoviridae*) são vírus trisegmentados que possuem genoma de RNA fita simples senso negativo ou ambisenso, divididos em segmentos L, M e S (Nigam & Garcia-Ruiz 2021). No tomateiro, os orthotospovírus causam a doença conhecida como "vira-cabeça" ou "spotted wilt", sendo considerada uma das principais viroses da cultura. Os vetores de orthotospovírus são insetos denominados tripes (ordem *Thysanoptera*) (Gupta et al. 2018; Zhang et al 2021; Jorge et al. 2023). O Brasil é considerado um centro de diversidade dos orthotospovírus. Um estudo das populações de orthotospovírus que infectam hortaliças no Brasil revelou que as espécies que mais prevalecem no país são: *Orthotospovirus arachianuli* (anteriormente denominado de groundnut ringspot virus – GRSV), *Orthotospovirus tomatomaculae* (tomato spotted wilt virus – TSWV) e *Orthotospovirus tomatoflavi* (tomato chlorotic spot virus – TCSV) (Martínez et al. 2019). No mundo, além de TSWV, GRSV e TCSV, outros orthotospovirus também já foram relatadas como capazes de infectar o tomateiro naturalmente e/ou experimentalmente, tais como: *Orthotospovirus tomatanuli* (tomato yellow ring virus – TYRV), *Orthotospovirus arachinecrosis* (groundnut bud necrosis virus – GBNV) e *Orthotospovirus tomatozonae* (tomato zonate spot virus – TZSV) (EFSA, 2012; Ong et al. 2020). O gene *Sw*-5b (identificado na espécie selvagem *S. peruvianum*) passou a ser amplamente utilizado em programas de melhoramento genético devido ao fato de a resistência ser mais estável e sem evidências de respostas do tipo isolado-específicas (Boiteux & Giordano, 1993; Dianese et al. 2011; Oliveira et al. 2018; Jorge et al. 2023). No entanto, no

continente europeu, alguns isolados de TSWV capazes superar a resistência conferida pelo gene *Sw*-5b vêm sendo relatados, demonstrando a importância de monitoramento constante da diversidade viral (Batuman et al. 2017; Oliveira et al. 2018). Marcadores moleculares funcionais e codominantes foram desenvolvidos para o gene *Sw*-5b (Dianese et al. 2010), facilitando o trabalho de incorporação desse fator de resistência em cultivares comerciais de tomateiro.

Gênero *Potyvirus* – São vírus de RNA fita simples senso positivo de genoma com poliproteínas (Untiveros et al. 2016). Dois potyvírus (família *Potyviridae*) são importantes para a cultura do tomateiro: potato virus Y (PVY) e tobacco etch virus (TEV) (Ong et al. 2020). Além disto, uma espécie ainda endêmica do Brasil, pepper yellow mosaic virus – PepYMV (Inoue-Nagata et al. 2002), tem apresentado uma elevada prevalência em campos de produção de tomate na região central do país (Dianese et al. 2008; Oliveira et al. 2018). O PVY é responsável pela doença da risca do tomateiro, mas também infecta outras plantas cultivadas e não cultivadas da família Solanaceae. O vírus é transmitido de maneira não circulativa não persistente por afídeos (Gadhave et al. 2020). Como estratégia de controle, a busca por fontes de resistência e uso de cultivares de tomateiro resistentes ao PVY tem mostrado eficiência (Lourenção et al. 2005; Oliveira et al. 2018). O emprego desses materiais resistentes aliados à prevalência de outros potyvírus do tomateiro, como (PepYMV) (Dianese et al. 2008), reduziram os surtos de PVY em alguns estados produtores de tomate (Ávila et al. 2004). Novas fontes de resistência contra ambas espécies de potyvírus foram detectadas dentro do gênero *Solanum* (Oliveira et al. 2018).

1.3. Família *Geminiviridae*: Gênero *Begomovirus*.

Atualmente a família *Geminiviridae* é composta por 520 espécies distribuídas em 14 gêneros virais: *Becurtovirus* (3 espécies), *Begomovirus* (445), *Capulavirus* (4), *Citlodavirus* (4), *Curtovirus* (3), *Eragrovirus* (1), *Grablovirus* (3), *Maldovirus* (3), *Mastrevirus* (45), *Mulcrilevirus* (2), *Opunivirus* (1), *Topilevirus* (2), *Topocuvirus* (1) e *Turncurtovirus* (3) (ICTV 2024). Destes, o gênero *Begomovirus* é o único que apresenta uma conformação bipartida e é também o gênero de maior importância dentro da família.

Os begomovírus merecem grande importância entre as doenças de etiologia viral do tomateiro. Estes vírus apresentam uma (espécies monopartidas) ou duas (espécies bipartidas) moléculas de DNA circular. O genoma dos begomovírus bipartidos possui dois componentes

de DNA denominados DNA–A e DNA–B, com tamanhos variando entre 2,5 a 2,7 kb. Os dois componentes apresentam uma região genômica comum de aproximadamente 200 nucleotídeos. No componente DNA–A, no sentido viral, estão as ORFs para o gene AV1/V1 que codifica a proteína da capa proteica viral (CP) e o gene AV2/V2 da proteína de movimento viral (MP). A ORF AV2 está presente apenas em begomovírus do Velho Mundo. No sentido complementar encontram-se as ORFs: AC1/C1 que codificam proteínas associadas à replicação viral (Rep), AC2/C2 que codificam a proteína ativadora da transcrição (TrAP), AC3/C3 codifica para proteína potenciadora da replicação (Ren), AC4/C4 relacionado aos sintomas e AC5, presente em alguns begomovírus do Novo Mundo, é responsável pela patogenicidade e supressão do silenciamento gênico pós-transcricional (Li et al. 2015). No componente DNA–B, estão as ORFs para BV1 que codifica para proteína de transporte nuclear (NSP) e BC1 que codifica para a proteína de movimento (MP). O genoma dos begomovírus monopartidos (**Figura 3, painel C**) é semelhante ao componente DNA–A dos begomovírus bipartidos do Velho Mundo (**Figura 3, painel B**). Estudos recentes relataram novas ORFs nos genomas de isolados de algumas espécies de begomovírus. Wang et al. (2021) relataram a presença de uma nova ORF (V3) em Malvastrum yellow vein Honghe virus (MaYVHhV). A nova ORF V3 encontra-se a jusante da V2, responsável por codificar uma proteína de 7,4 kDa ainda sem função descrita. Wang et al. (2022) identificaram uma nova proteína (C6) expressa por tomato leaf curl China virus (ToLCCNV) quando infectando N. *benthamiana*. A nova ORF C6 está no sentido viral sobrepondo parcialmente as ORFs CP e V2 (Wang et al. 2022). A nova proteína C6 desempenha um papel de direcionamento para mitocôndrias da planta infectada, onde nesta organela a proteína é expressa, contudo sem contribuir para a virulência de ToLCCNV em *N. benthaminana* (Wang et al. 2022). Mais recentemente, foi identificada uma outra proteína (C7) codificada por uma ORF C7 no sentido complementar do genoma tomato yellow leaf curl virus (ToYLCV) (Liu et al. 2023). A proteína C7 interage com as proteínas das ORF V2 e C2, além disso inibe o silenciamento de RNA e quando mutada, as infecções por ToYLCV foram amenizadas, sugerindo que a proteína C7 atua como fator de patogenicidade (Liu et al. 2023).

O gênero *Begomovirus* possui 445 espécies descritas, sendo o mais numeroso na família *Geminiviridae* (ICTV 2024; Brown et al. 2015). Isolados de begomovírus infectam uma ampla gama de espécies hospedeiras dicotiledôneas, entre plantas cultivadas, daninhas, nativas e ornamentais (Rojas et al. 2018). Dois grupos distintos de begomovírus são observados, um composto por espécies com genomas mono ou bipartidos no Velho Mundo (África, Ásia e Europa) e um grupo composto predominantemente por espécies com genomas bipartidos no Novo Mundo (Américas) (Navas-Castillo e Fiallo-Olivé, 2020). Os begomovírus do Novo

Mundo diferenciam-se dos begomovírus do Velho Mundo pela ausência da ORF AV2 (Zerbini et al. 2017; ICTV 2024). O atual critério de demarcação para o gênero *Begomovirus* considera como sendo de uma mesma espécie viral isolados com uma identidade igual ou superior a 91% de identidade do genoma completo do componente DNA–A (Brown et al. 2015).

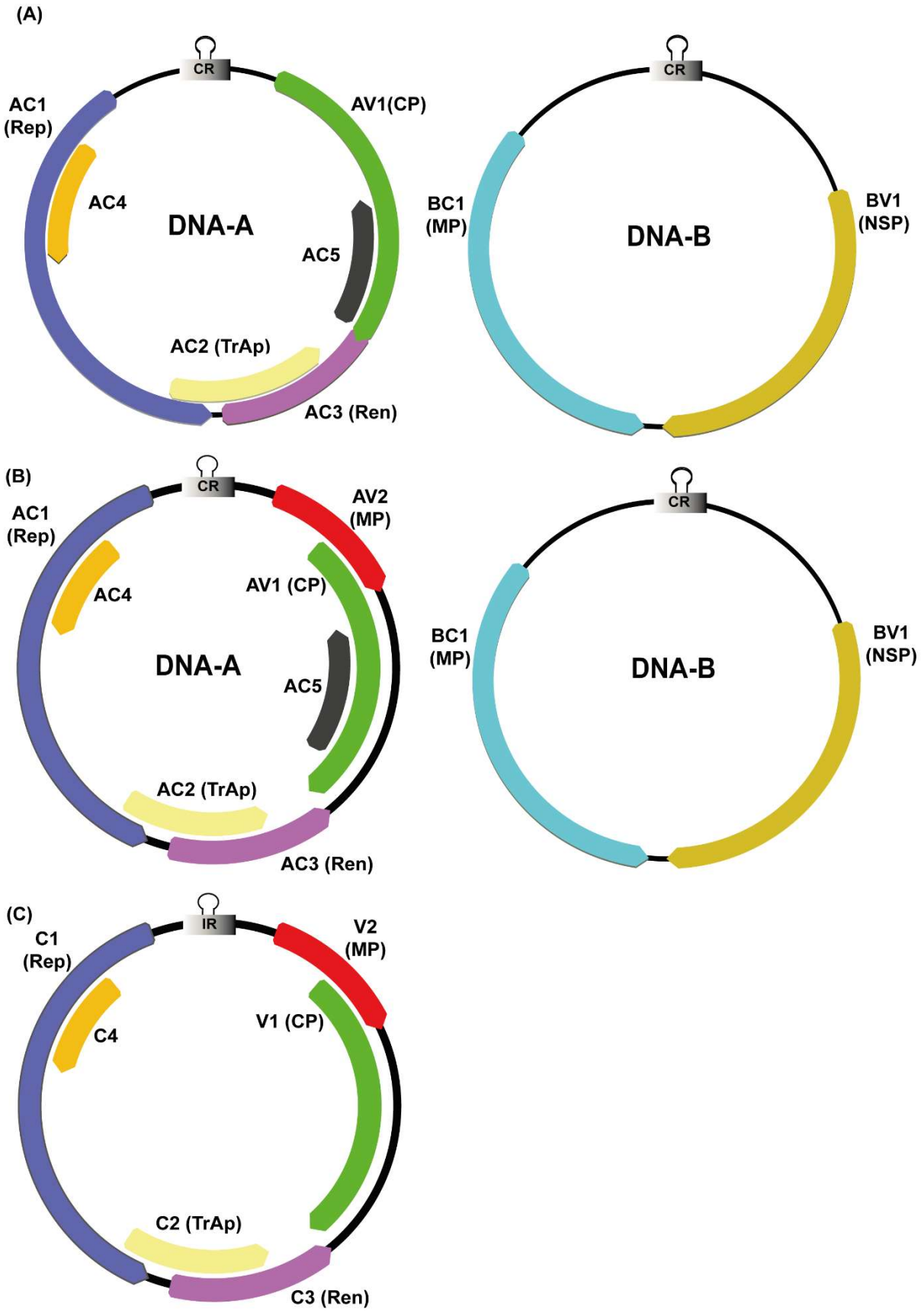
(A)
CR
AC1
(Rep)
AV1(CP)
AC4
DNA-A
AC5
AC2 (TrAp)
AC3 (Ren)
CR
BC1
(MP)
DNA-B
BV1
(NSP)
(B)
CR
AC1
(Rep)
AV2
(MP)
AV1 (CP)
AC4
DNA-A
AC5
AC2 (TrAp)
AC3 (Ren)
CR
BC1
(MP)
DNA-B
BV1
(NSP)
(C)
IR
C1
(Rep)
V2
(MP)
V1 (CP)
C4
C2 (TrAp)
C3 (Ren)

Figura 3. Organização genômica dos begomovírus bipartidos do Novo Mundo **(A)**, bipartidos do Velho Mundo **(B)** e monopartidos **(C)**. A ORF (*Open Reading Frame*) V1/AV1, codifica a capa proteica viral (CP); V2/AV2, responsável pela proteína de movimento (MP); C1/AC1, proteína associada à replicação viral (Rep); C2/AC2, codifica a proteína ativadora da transcrição (TrAp); C3/AC3, codifica a proteína potencializadora da replicação (Ren) C4/AC4, relacionada com os sintomas; AC5, relacionada à supressão do silenciamento gênico e patogenicidade; BV1, responsável por codificar a proteína de transporte nuclear (NSP) e BC1, a proteína de movimento (MP).

Os begomovírus são transmitidos por um complexo de espécies crípticas de *Bemisia tabaci* (família *Aleyrodidae*, ordem *Hemiptera*) conhecida como mosca-branca. Atualmente a espécies *B. tabaci* Gennadius *Middle East Asia Minor* 1 – MEAM-1 (anteriormente denominado biótipo B) e *B. tabaci* Mediterranean MED (antigo biótipo Q) são as predominantes no Brasil (Rosen et al 2015; De Barro et al. 2011; Watanabe et al. 2019; Fernandes et al. 2022). A relação entre begomovírus e inseto vetor é do tipo circulativa não propagativa, contudo, estudos de transmissão do tomato yellow leaf curl virus (ToYLCV) têm demonstrado a capacidade desse begomovírus também se replicar no inseto vetor, em algumas condições específicas (Pakkianathan et al. 2015), porém os estudos realizado por Sánchez-Campos et al. (2016) não comprovaram a replicação de ToYLCV em *B. tabaci*. Estudos de transmissão revelaram que a proteína capsidal (CP) tem papel importante na transmissão dos begomovírus por espécies do complexo *B. tabaci*, pois mutações na proteína da CP são capazes de alterar significativamente as taxas de transmissão do vírus pelo inseto vetor (Pan et al. 2020).

O processo de transmissão dos begomovírus pela mosca-branca envolve as etapas de ingestão, fixação entrada, translocação, circulação, retenção e por fim a liberação do vírus (Ghosh e Ghanim 2021; Naveed et al. 2023). A quisiação dos vírus pela mosca-banca ocorre quando o inseto se alimenta da ceiva do floema de plantas infectadas a partir do aparelho bucal sugador, alcançando o intestino médio do inseto, onde é fixado e permanece por vários dias (Ghosh e Ghanim 2021; Naveed et al. 2023). Após a aquisição, os vírus translocam-se intracelularmente dentro das membranas do intestino médio do vetor. Ao romper as membranas do intestino médio, os vírus circulam pela homolinfa até atingirem as periferias das glândulas salivares primárias (Ghosh e Ghanim 2021). A partir das glândulas salivares primárias, os vírus cruzam as lâminas basal e células secretoras, alcançando o lúmem central, que por sua vez faz conexão com o ducto da glândula salivar (Ghosh e Ghanim 2021; Naveed et al. 2023). Os vírus são então liberados nas células hospedeiras juntos com a saliva pelo canal salivar do estilete durante o processo de salivação do inseto ao se alimentar (Ghosh e Ghanim 2021; Naveed et al. 2023).

A separação das espécies crípticas dentro do complexo *B. tabaci* possui uma abordagem genética, utilizando a informação derivada da sequência do gene *mitochondrial citrocromo oxidase* 1 (mtCOI-1). O limite de divergência de 3,5% tem sido empregado com critério para demarcação de novas espécies dentro do complexo *B. tabaci* (Dinsdale et al. 2010; Boykin & De Barro 2014; Marubayashi et al. 2013). Também são considerados como critérios adicionais de separação de espécies do complexo *B. tabaci* as propriedades biológicas do inseto, incluindo variabilidade no círculo de espécies hospedeiras, distúrbios fisiológicos induzidos nas plantas, amplitude na capacidade de dispersão, resistência a inseticidas e transmissão de diferentes espécies virais (De Barro et al. 2011; Mahmood et al. 2022). O complexo de espécies crípticas de *B. tabaci* é composto por 11 grupos geneticamente distintos e 34 espécies morfologicamente indistinguíveis (De Barro et al. 2011; Boykin e De Barro 2014). No Brasil, predomina o grupo genético MEAM-1, anteriormente denominado biótipo B (Fernandes et al. 2022). Algumas características deste grupo genético incluem a plasticidade genética com variantes apresentando resistência a inseticidas, altas taxas de oviposição e colonização de amplo círculo de plantas hospedeiras (hábito polífago) (De Barro et al. 2011; Gilbertson et al. 2015).

Os primeiros relatos de infecções por begomovírus no tomateiro no Brasil foram feitos entre as décadas de 1950 e 1970 (Flores et al. 1960; Costa 1974; Matyis et al. 1975). Destes primeiros relatos até início da década de 1990 as doenças causadas por espécies de begomovírus eram esporádicas e sem importância econômica. No entanto, a partir de 1990, simultaneamente com a entrada no país de *Bemisia tabaci* MEAM-1 (biótipo B) foram observadas a eclosão de diversas epidemias e um súbito aumento na incidência das begomoviroses (Ribeiro et al. 2003; Fernandes et al. 2008). O primeiro relato de uma epidemia de begomovírus em tomateiro data de 1993 em Brasília-DF (Ribeiro et al. 1994) e, desde então, várias espécies de begomovírus foram relatadas em diferentes estados do país (**Figura 4**) (Ribeiro et al. 2003; Colariccio et al. 2003; Albuquerque et al. 2010; Macedo et al. 2018; Quadro et al. 2019; Reis et al. 2020; Reis et al. 2023, Oliveira et al. 2024). Em tomateiro, a infecção por begomovírus pode ocasionar os sintomas de mosaicos, clorose apical e encarquilhamento, deformações foliares, desvios de coloração, mosqueado distorções, amarelecimento, bronzeamento em nervuras, rugosidades e epinastias foliares, nanismo (Inoue-Nagata et al. 2016) (**Figura 5**).

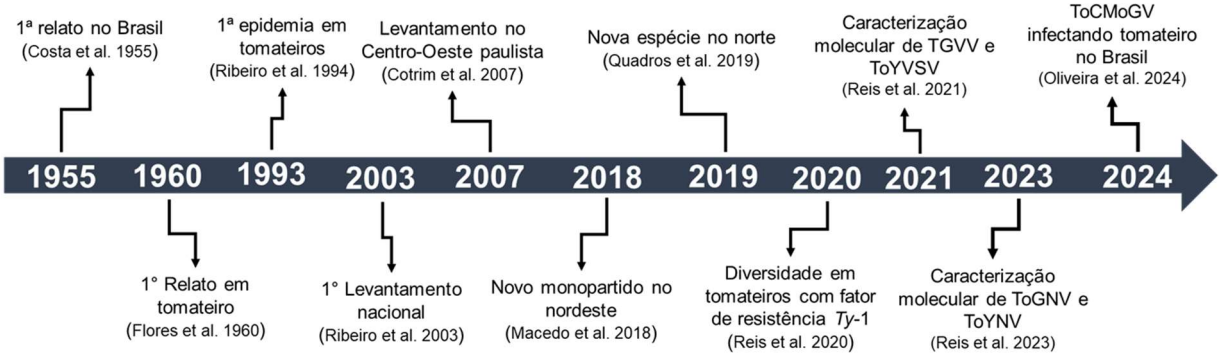

Figura 4. Linha do tempo para os principais relatos históricos dos begomovirus associados ao tomateiro no Brasil.

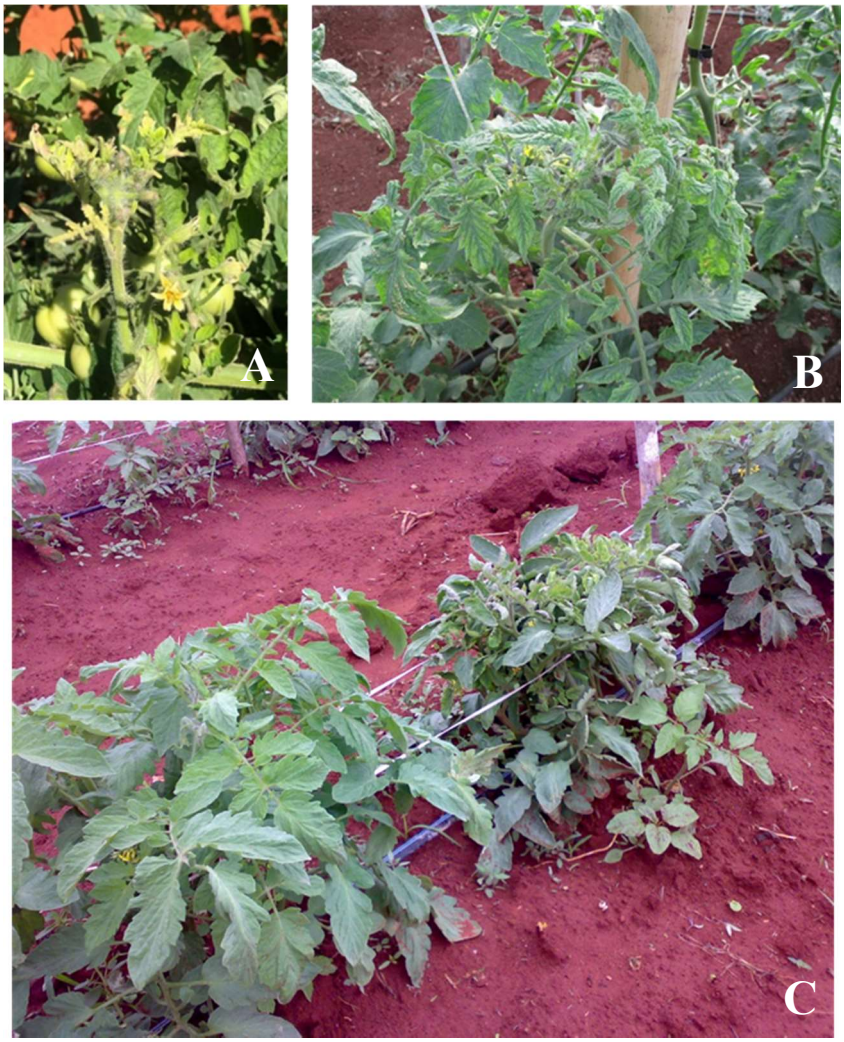

Figura 5. Tomateiros exibindo sintomas de infecções causadas por begomovírus (**A**): Clorose apical e encarquilhamento. (**B**): Deformações foliares e desvios de coloração, incluindo mosqueado e clorose nas folhas apicais em Brasília-DF. (**C**): Nanismo e clorose apical (ao lado de uma planta não infectada). Fotos: Leonardo Silva Boiteux.

Os begomovírus se replicam nas células dos hospedeiros por meio do mecanismo de replicação por círculo rolante ou *rolling circle replication* (RCR), e seu ciclo replicativo inicia com a entrada na da partícula viral na célula e posterior remoção da capa proteica e exposição do ssDNA viral no citoplasma da célula hospedeira (**Figura 6**) (Yadava et al. 2010; Pradhan et al. 2017). Em seguida o DNA viral é transportado para o núcleo da célula, onde se inicia o processo de replicação. O início da replicação dos begomovírus ocorre com a ligação da proteína Rep com a região comum (RC) e o corte da fita de DNA (Pradhan et al. 2017). A primeria faze do ciclo replicativo consiste na conversão do ssDNA viral em dsDNA intermediário, denominada forma replicativa (FR) (Gutierrez, 2000; Yadava et al. 2010). Na segunda etapa, a FR dsDNA é usada como molde para síntese de novos ssDNA através da ação da Rep pelo mecanismo de RCR (Gutierrez, 2000; Yadava et al. 2010). Os ssDNA são transcritos em RNA mensageiros que por sua vez são transportados para o citoplasma onde ocorre a tradução em proteínas virais envolvidas no ciclo replicativo, das quais a proteína de transporte nuclear (NSP) e a replicase viral (Rep) retornam para o núcleo celular (Pradhan et al. 2017). A NSP atua no transporte do ssDNA viral através da membrana nuclear, transportando-o para o citoplasma onde a proteína de movimento (MP) o tansporta para uma nova célula através dos plasmodesmas, podendo ser também por meio da partícula viral montada (Hanley-Bowdoin et al. 2013).

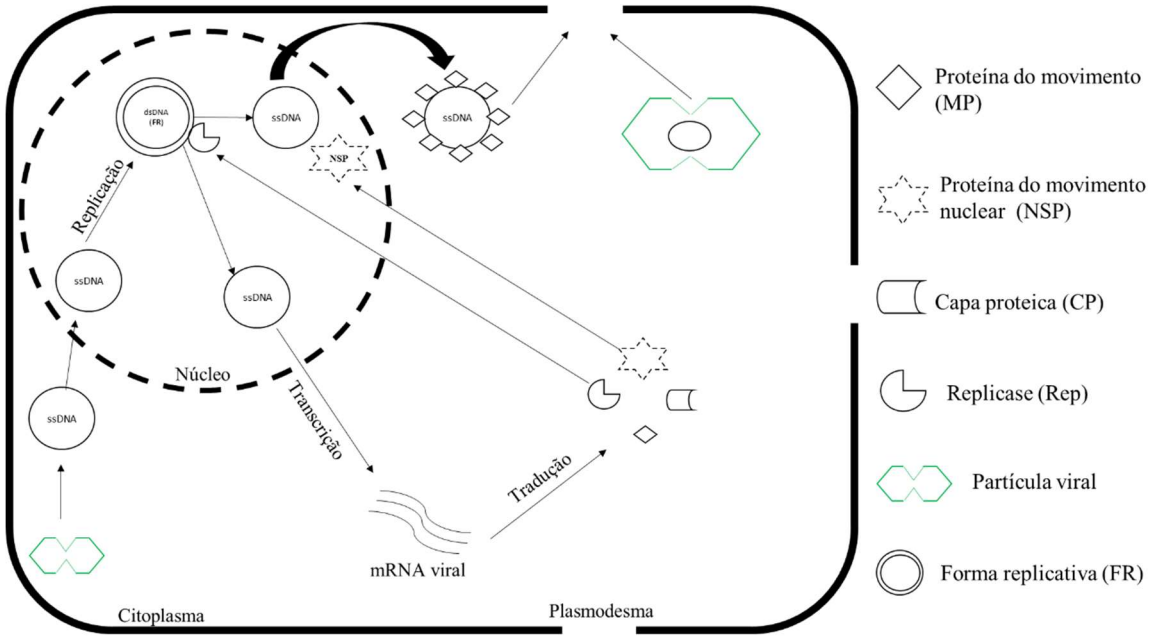

Figura 6. Estratégia de replicação dos begomovírus na célula da planta hospedeira. O ssDNA viral perde a capa proteica e é exposto no citoplasma de onde é levado para o núcleo onde é convertido em dsDNA (forma replicativa – FR), o qual é usado como molde para a síntese de novos ssDNA que são transportados para o citoplasma pela proteína de

transporte nuclear (NSP) ou são transcritos em RNA mensageiros (mRNA) e em sequência são transportados para o citoplasma para serem traduzidos em proteínas. A replicase viral (Rep) e NSP retornam para o núcleo celular e a proteína de movimento (MP) atua no transporte do ssDNA viral para outras células pelos plasmodesmas. Proteína de movimento (MP), proteína de transporte nuclear (NSP), capa proteica (CP), replicase viral (Rep), forma replicativa dsDNA (FR).

Família *Geminiviridae*: Gênero *Topilevirus* – Esse gênero também pertence à família *Geminiviridae* e possui apenas duas espécies: *Tomato apical leaf curl virus* (Vaghi-Medina et al. 2018; Batista et al. 2019) e *Tomato associated geminivirus* 1 (Fontenele et al. 2017). Os genomas desses vírus são de DNA fita simples circular com três ORFs no sentido viral (V1, V2 e V3) e três ORFs no sentido complementar (C1, C2 e C3) e duas regiões intergênicas, uma região intergênica curta (SIR) e outra regiõa itergênica longa (LIR) e um intron entre as ORFs C1/C2 (Vaghi Medina et al. 2018, ICTV 2024). Isolados de vírus classificados nas duas espécies são endêmicos da América do Sul, estando presentes infectando o tomateiro na Argentina e no Brasil. De acordo com ICTV (2024), o atual critério de demarcação de espécies é de identidade nucleotídica do genoma igual ou superior a 78% (Vaghi-Medina et al. 2018). Plantas de tomate inoculadas por biobalística com tomato apical leaf curl virus (ToALCV) apresentaram amarelecimento internerval, enrolamento apical das folhas e hipertrofia radicular. A cigarrinha *Micrutalis maleifera* (família *Membracidae*) tem sido especulada como sendo o inseto vetor do ToALCV (Vaghi Medina et al. 2018). Além de tomateiro, Fontenele et al. (2017) também detectaram o tomato associated geminivirus 1 (TaGV1) em plantas daninhas do gênero *Cleome* spp. O genoma apresenta o nonanucleotídeo conservado TAATATTAC, com seis ORFs (*Open Reading Frames*), sendo três no sentido viral (V1, V2 e V3) e três no sentido complementar (C1, C2 e C3). A C1 codifica uma proteína A associada a replicação viral (RepA), C1:C2 codifica para a replicase viral (Rep), C3 está contida dentro da ORF C1 e sua função ainda não foi definida; V1 codifica para a capa proteica (CP), V2 possivelmente codifica para a proteína de movimento (MP) e V3 não possui função definida (Vaghi-Medina et al. 2018; ICTV 2024).

1.4. DNA satélites associados a vírus da família *Geminiviridae*.

Os begomovírus monopartidos do Velho Mundo possuem associação com agentes subvirais de DNA de fita simples, também circulares (**Figura 5**), denominados de alfa satélites e betassatélites, cujo tamanho é de ≈ 1400 pb (Rojas et al. 2018).

Os **alfassatélites**, quando associados ao vírus auxiliar, modulam a patogênese viral, retardando e atenuando sintomas e reduzindo o acúmulo viral na hospedeira (Kumar et al. 2021). Os alfassatélites codificam uma REP (**Figura 7, painel A**) que os possibilitam a auto-replicação independente do vírus auxiliar (Zhou et al. 2013). De acordo com ICTV (2024), os alfassatélites estão inseridos na família *Alphasateliitidae*. Nesta, por sua vez, é constituída de três subfamílias (*Geminialphasatellitinae*, *Nanoalphasatellitinae* e *Petromoalphasatellitinae*), englobando 18 gêneros e 85 espécies. Os alfassatélites associados aos geminivírus estão contidos na subfamília *Geminialphasatellitinae*, gêneros *Ageyesisatellite*, *Clecrusatellite*, *Colecusatellite* e *Gosmusatellite*. O critério de demarcação de espécie dentro da família *Geminialphasatellitinae*, considera uma identidade de nucleotídeos inferior a 88% em comparação com genomas completos de outros alfassatélites e 70% para critérios de demarcação de gêneros (Briddon et al. 2018). Mais recentemente no Brasil, Reis et al. (2020) detectaram um novo alfassatélite associado com plantas de tomateiro que ainda permanece não plenamente caracterizado.

Os **betassatélites** são responsáveis pela expressão típica dos sintomas virais (Zhou et al. 2013) possuem a ORF βC1 (**Figura 7, painel B**) que está relacionada com a supressão do silenciamento gênico de RNA, resultando em uma maior virulência ao vírus auxiliar (Cui et al. 2005). Mais recentemente, uma nova molécula (denominada βV1) foi identificada em um betassatélite associado a begomovírus (Hu et al. 2020). A βV1 pode provocar a morte celular programada do tipo resposta de hipersensibilidade (HR) e clorose em plantas de *Nicotiana bentahamiana*. Ensaios de co-infecções entre tomato yellow leaf curl China virus (ToYLCCV) e o betassatélite mostrou que há um aumento da patogenicidade e acúmulo viral quando comparada a infecção de ToYLCCV e o betasatélite mutante para βV1, além disso foi desmonstrado que βV1 contribui para a virulência em complexos de infecção geminivirus/betassatélites, contudo seu papel biológico exato e seus papel moleculares continuam a serem indefinidos (Hu et al. 2020). Ao contrário dos alfassatélites, os betassatélites não podem se autorreplicar, dependendo inteiramente do vírus auxiliar para esse processo, bem como para encapsidação e transmissão pelo vetor (Zhou et al. 2013). Os betassatélites estão classificados na família *Tolecusatellitidae*, que possui dois gêneros (*Betasatellite* e *Deltasatellite*). O número atual de espécies dentro do gênero *Betasatellite* é de 119. O critério em vigência para demarcação de espécies dentro do gênero considera uma identidade de nucleotídeos inferior a 91% (ICTV 2024).

Outros agentes subvirais são os **deltassatélites**, de ≈ 700 pb (**Figura 7, painel C**), sendo reportados em associação tanto a begomovírus monopartidos do Velho Mundo (Dry et

al. 1997) quanto aos begomovírus bipartidos do Novo Mundo (Fiallo-Olivé et al. 2012). Essa classe de satélite não sintetiza nenhuma proteína sendo inteiramente dependente do seu vírus auxiliar, podendo ser transreplicado pelo begomovírus (Ferro et al. 2020; ICTV 2024). O deltassatélites não afetam os sintomas induzidos pelo vírus, porém, dependendo da relação entre o vírus auxiliar e a planta hospedeira, reduzem o acúmulo viral e ainda podem ser transmitidos por *B. tabaci* (Fiallo-Olivé et al. 2016; Ferro et al. 2020). Os deltassatélites possuem atualmente 12 espécies catalogadas dentro do gênero *Deltasatelite* e família *Tolecusatellitidae*. O critério utilizado para demarcação de espécies é de identidade de nucleotídeos do genoma completo inferior a 91% (ICTV 2024).

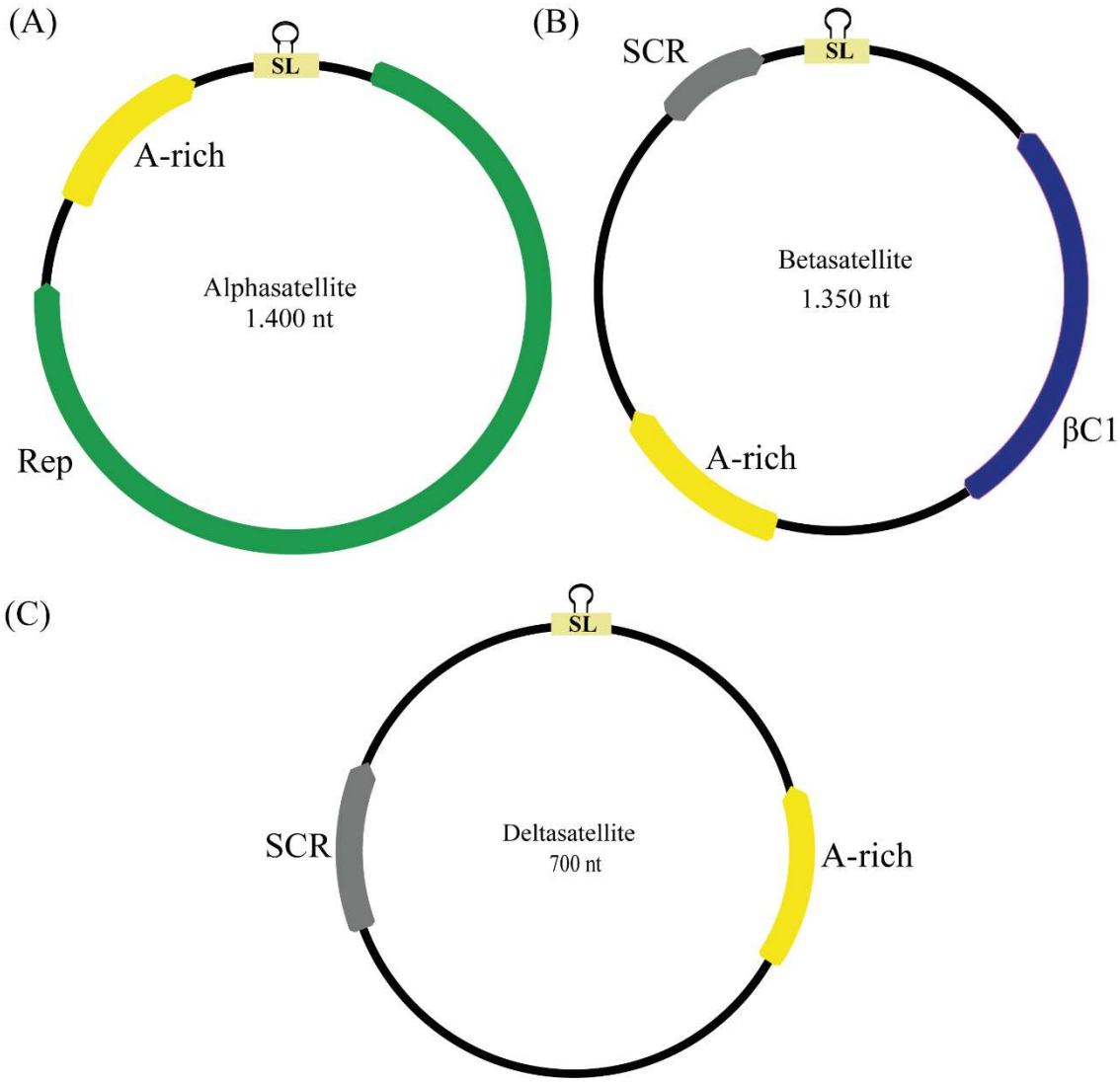

Figura 7. Representação esquemática dos DNAs satélites associados aos begomovírus: Alphasatellite **(A)**, Betasatellite **(B)** e Deltasatellite **(C)**. Região rica em adenina (A-rich), proteína associada a replicação (Rep), proteína associada supressão do silenciamento gênico (βC1), região conservada dos satélites (SCR) e stem-loop (SL).

1.5. Caracterização molecular de begomovírus por iterons e por motivos conservados.

Com objetivo de caracterizar a diversidade genômica de begomovírus, Arguello-Astorga et al. (2001) usaram os iterons como um critério de investigação, avaliando os potenciais determinantes de especificidades de ligação da proteína REP aos motivos específicos no DNA viral. Neste estudo, subdomínios da REP foram identificados por se mostrarem variáveis entre vírus de iterons diferentes, mas estando conservados em vírus com iterons idênticos, mesmo entre vírus de diferentes hospedeiros, vetores, origem geográfica e estrutura genômica (Arguello-Astorga et al. 2001). A esses subdomínios conservados foram denominados de "*Iteron-Related Domain*" (IRD). Para definir a potencial correlação entre os geminivírus, a estrutura primária da Rep-IRD e a sequência nucleotídica do iteron cognato foram analisadas. O resultado dessas análises permitiu agrupar os begomovírus com iterons únicos, tanto em espécies do Velho Mundo quanto do Novo Mundo, estabelecendo uma correlação clara e consistente (Arguello-Astorga et al. 2001).

Em um estudo adicional, Arguello-Astorga et al. (2004) conduziram a caracterização de uma região genômica conservada do fator de replicação de geminivírus (AC1) que interage com retinoblastoma vegetal (pBRB). Um motivo conservado de 11 aminoácidos foi detectado na AC1 de todos geminivírus. Mutações nesta sequência resultaram em comprometimento na interação com a pRBR celular, confirmando o papel crucial desse motivo nas interações entre a proteína de replicação dos geminivírus e a pBRB das plantas hospedeiras (Arguello-Astorga et al. 2004).

Estes domínios conservados têm sido empegados na caracterização mais amplas de genomas e funções de ORFs em begomovírus (Li et al. 2015). Dois grandes grupos de begomovírus foram discriminados: begomovírus bipartidos do Novo Mundo, mono e bipartidos do Velho Mundo ("grupo a") e outro grupo composto exclusivamente com begomovírus do Velho Mundo ("grupo b"). Com auxílio da CDART (*Conserved Domain Architecture Retrieval Tool*) foi detectado em ambos os grupos um domínio conservado Gemini AC5-1 (pfam04807). No entanto, apenas os begomovírus do "grupo b" possuem um domínio adicional Gemini AC5-2 (pfam08464) (Li et al. 2015). Esse trabalho também demonstrou o envolvimento da região C-terminal da ORF AC5 na supressão do silenciamento gênico transcricional e na indução e intensificação dos sintomas virais (Li et al. 2015).

Mais recentemente, Cantú-Iris et al. (2019) detectaram e caracterizaram uma nova espécie de begomovírus: *Blechum interveinal chlorosis virus*. Neste estudo, foi confirmada a

presença do nonanucleotídeo TAATATTAC e de três iterons GGGGGA em arranjos característicos dos begomovírus. Além disso, análise das sequências da nova espécie revelou duas repetições de 15 nts, incluindo um motivo (G) GGACCAC. Ao realizar análises mais sistemáticas utilizando ≈ 130 sequências de begomovírus do Novo Mundo, Cantú-Iris et al. (2019) observaram regiões promotoras à CP viral que são curtas e que apresentavam três características: **(1)** a presença de uma sequência central ACTT-N7-AAGT; **(2)** uma sequência heptanucleotídica (N7) rica em GC e altamente variável e **(3)** uma invariável associação ao TATA-box, denominando-o TATA-*Associated Composite Element* (TACE).

Análises direcionadas aos Rep-IRD (motivos relacionados à proteína AC1 dos geminivírus) e a sequência quase-palindrômica do DNA (ACTT-N7-AAGT) foram empregadas por Reis et al. (2021) para caracterização de 45 isolados denominados como tomato golden vein virus (TGVV) e tomato yellow vein streak virus (ToYVSV). As análises relacionadas à REP-IRD mostraram que esses dois vírus possuem distintos domínios relacionados à proteína REP, reforçando a ideia de que são, de fato, duas espécies distintas. Dois isolados divergentes da Argentina e Uruguai foram reclassificados como sendo semelhantes ao ToYVSV com base na análise do motivo da AC1. Divergência entre nucleotídeos da sequência de DNA quase-palindrômica (ACTT-N7-AAGT) foi constatada entre isolados brasileiros de TGVV, resultados semelhantes foram observados entre os isolados de ToYVSV da Argentina, Uruguai e Chile (Reis et al. 2021). Estas análises permitiram aos autores identificar um número relevante de isolados com nomenclatura errônea e propor uma reavaliação na classificação de isolados depositados no *GenBank* correspondendo a estas duas espécies Sul-Americanas.

1.6. Panorama global de espécies virais associadas com o tomateiro.

Em termos globais, mais de 300 vírus foram relatados infectando a cultura do tomateiro de acordo com levantamentos mais recentes feitos com base em dados do *GenBank* (2022), *Host Data Base* (2022) e Kitajima (2020) (**Figura 8**). O maior número de vírus que infectam o tomateiro está contido no gênero *Begomovirus* (família *Geminiviridae*), com 221, ou seja, o gênero comporta 66,97% dos mais de 300 vírus que infectam o tomateiro (**Figura 9**).

1.7. Diversidade de begomovírus associados ao tomateiro no Brasil e no Mundo.

Os trabalhos de levantamento têm sido amplamente conduzidos desde a entrada de *B. tabaci* MEAM 1 no Brasil, revelando a existência de um complexo extremamente diverso de begomovírus infectando o tomateiro no país. Atualmente, 30 espécies de begomovírus já foram reportados associados com a cultura do tomateiro no Brasil (**Tabela 1**). Os vírus que já foram

relatados infectando o tomateiro nas condições brasileiras incluem: tomato golden mosaic virus (**TGMV**) (Matyis et al. 1975), tomato rugose mosaic virus (**ToRMV**) (Ribeiro et al. 2003), tomato chlorotic mottle virus (**ToCMoV**) (Ribeiro et al. 2007), tomato yellow spot virus (**ToYSV**) (Calegario et al. 2007), tomato severe rugose virus (**ToSRV**) (Cotrim et al. 2007), tomato common mosaic virus (**ToCmMV**) (Castillo-Urquiza et al. 2008), tomato mild mosaic virus (**ToMMV**) (Castillo-Urquiza et al. 2008), tomato leaf distortion virus (**ToLDV**) (Castillo-Urquiza et al. 2008), tomato yellow vein streak virus (**ToYVSV**) (Albuquerque et al. 2010), tomato mottle leaf curl virus (**TMoLCV**) (Albuquerque et al. 2012), tomato golden vein virus (**TGVV**) (Reis et al. 2020), tomato interveinal chlorosis virus (**ToICV**) (Albuquerque et al. 2012), tomato interveinal chlorosis virus-2 (**ToICV2**) (Rêgo-Machado et al. 2019), tomato chlorotic leaf curl virus (**ToCLCV**) (Quadros et al. 2019), tomato leaf curl purple vein virus (**ToLCPVV**) (Macedo et al. 2018); chino del tomate Amazonas virus (**CdTAV**) (Fonseca et al. 2011); tomato bright yellow mosaic virus (**ToBYMV**) (Fonseca et al. 2013), tomato bright yellow mottle virus (**ToBYMV**) (Fonseca et al. 2013), tomato golden leaf distortion virus (**ToGLDV**) (Fonseca et al. 2013), tomato golden leaf spot virus (**ToGLSV**) (Fonseca & Boiteux, 2013) e tomato rugose yellow leaf curl virus (**TRYLCV**) (Fonseca et al., 2016), tomato mottle leaf distortion virus (**ToMLDV**) (Martins et al. 2021), tomato chlorotic mottle Guyane virus (**ToCMoGV**), (Oliveira et al., 2024); tomato golden net virus (**ToGNV**) e tomato yellow net virus (**ToYNV**) (Reis et al. 2023) Além disso, registros têm sido feitos da infecção natural de tomateiro com isolados relacionados com as espécies virais que infectam hospedeiras alternativas ou plantas daninhas, incluindo neste grupo: sida mottle virus (**SiMoV**) (Cotrim et al. 2007), sida micrantha mosaic virus (**SimMV**) (Calegario et al. 2004), Euphorbia yellow mosaic virus (**EuYMV**) (Duarte et al. (2020), sida common mosaic virus (**SiCMV**) (Duarte et al. 2021a) e sida yellow net virus (**SiYNV**) (Duarte et al. 2021a).

Esta enorme diversidade de espécies de begomovírus associados com o tomateiro foi catalogada após diversos trabalhos de levantamento, tanto em âmbito nacional como em âmbito regional, mesmo antes do advento das plataformas de HTS. O primeiro grande levantamento nacional foi conduzido por Ribeiro et al. (2003) com o sequenciamento parcial de 23 isolados coletados entre 1994 e 1999 em diferentes regiões produtoras do Distrito Federal, do Nordeste e do Sudeste. Neste estudo, pelo menos sete novas espécies foram reportadas no país. Outro importante estudo investigando a diversidade de espécies de begomovírus em tomateiro foi conduzido com 138 isolados coletados (principalmente em tomateiro para processamento industrial) entre os anos de 2004 e 2005 no Brasil Central (Fernandes et al. 2008). Foram identificados seis begomovírus previamente registrados: ToSRV, TGVV, TMoLCV, ToYVSV

mais duas potenciais novas espécies (Fernandes et al. 2008). Em levantamentos conduzidos na região de Coimbra (MG) três novas espécies foram também identificadas infectando tomateiro: ToCmMV, ToMlMV e ToLDV (Castillo-Urquiza et al. 2008). Macedo et al. (2018) detectaram um novo vírus, ToLCPVV, infectando tomateiro no Nordeste do Brasil. Rego-Machado et al. (2019) utilizando HTS detectaram um novo begomovírus bipartido ToICV2 em amostras foliares de tomateiro no Goiás. Quadros et al. (2019) relataram um novo vírus, ToCLCV, infectando tomateiros na região norte brasileira. Mituti et al. (2019) realizaram um levantamento em estados do Nordeste, Centro-Oeste e Sudeste e relataram a presença de quatro begomovírus já previamente reportados: ToSRV; ToCmMV, ToMoLCV e ToYVSV. Um levantamento da diversidade de begomovírus que infectam o tomateiro no bioma da Mata Atlântica realizada por Duarte et al. (2020a), indicou uma predominância de ToCmMV e a crescente presença de ToSRV, além de ocorrências de ToICV, ToMoLCV, EuYMV, SiYNV e SiCMV. Duarte et al. (2020b) ao realizarem o levantamento de vírus em quatro estados brasileiros, demonstraram a infecção natural de tomateiros por EuYMV. Martins et al. (2021), detectaram uma nova espécie de begomovírus infectando tomateiro no Centro-Oeste brasileiro e propuseram o nome tomato mottle leaf distortion virus (ToMoLDiV). Reis et al. (2023) detectaram mais dois novos begomovírus monopartidos em tomateiros no Brasil: tomato golden net virus (ToGNV) e tomato yellow net virus (ToYNV).

Neste cenário de extrema variabilidade, eventos de recombinação e pseudorecombinação têm sido detectados (Silva et al. 2014). De fato, os begomovírus apresentam três mecanismos geraradores de variabilidade genética: mutação, recombinação e pseudorecombinação (Roossinck 1997; Seal et al. 2006). Para Lima et al. (2017), a mutação é o principal mecanismo impulsionador da diversidade de populações de begomovírus. No entanto, a recombinação genética em vírus permite que os genomas parentais transmitam informações genéticas para os seus recombinantes. Essa transferência de informações possibilita uma evolução da organização genômica de tal modo a maximizar a capacidade adaptativa e reduzir efeitos deletérios nesses vírus (Lefeuvre & Moriones 2015). A principal função da recombinação em populações virais seria reparar os defeitos do DNA resultantes de mutações (Seal et al. 2006). Para o tomato yellow leaf curl Sardinia virus (TYLCSV), os principais mecanismos para o ganho de variabilidade são a mutação e a recombinação entre estirpes (Díaz-Pendon et al. 2019). Na Argentina, um novo begomovírus (tomato mottle wrinkle virus – ToMoWrV) foi caracterizado e análises demonstraram se tratar de um novo recombinante. Os eventos de recombinação detectados no componente DNA–A do ToMoWrV, envolvem, como parentais, o soybean blistering mosaic virus (SoBlMV) e ToYVSV, dois

begomovírus presentes em regiões meridionais da América do Sul (Vaghi-Medina et al. 2015; Reis et al. 2021).

A pseudorecombinação permite que os begomovírus troquem e/ou compartilhem componentes genômicos entre si, esses rearranjos genéticos permitem uma maneira rápida de mudança evolutiva viral (Seal et al. 2006). O mecanismo da pseudorecombinação permite que isolados de ToRMV e ToSRV compartilhem o mesmo componente DNA–B em infecções em tomateiro, sugerindo que o surgimento do ToRMV envolveu a recombinação e pseudorecombinação como mecanismos de variabilidade e adaptação evolutiva (Silva et al. 2014; Fiallo-Olivé & Navas-Castillo 2023).

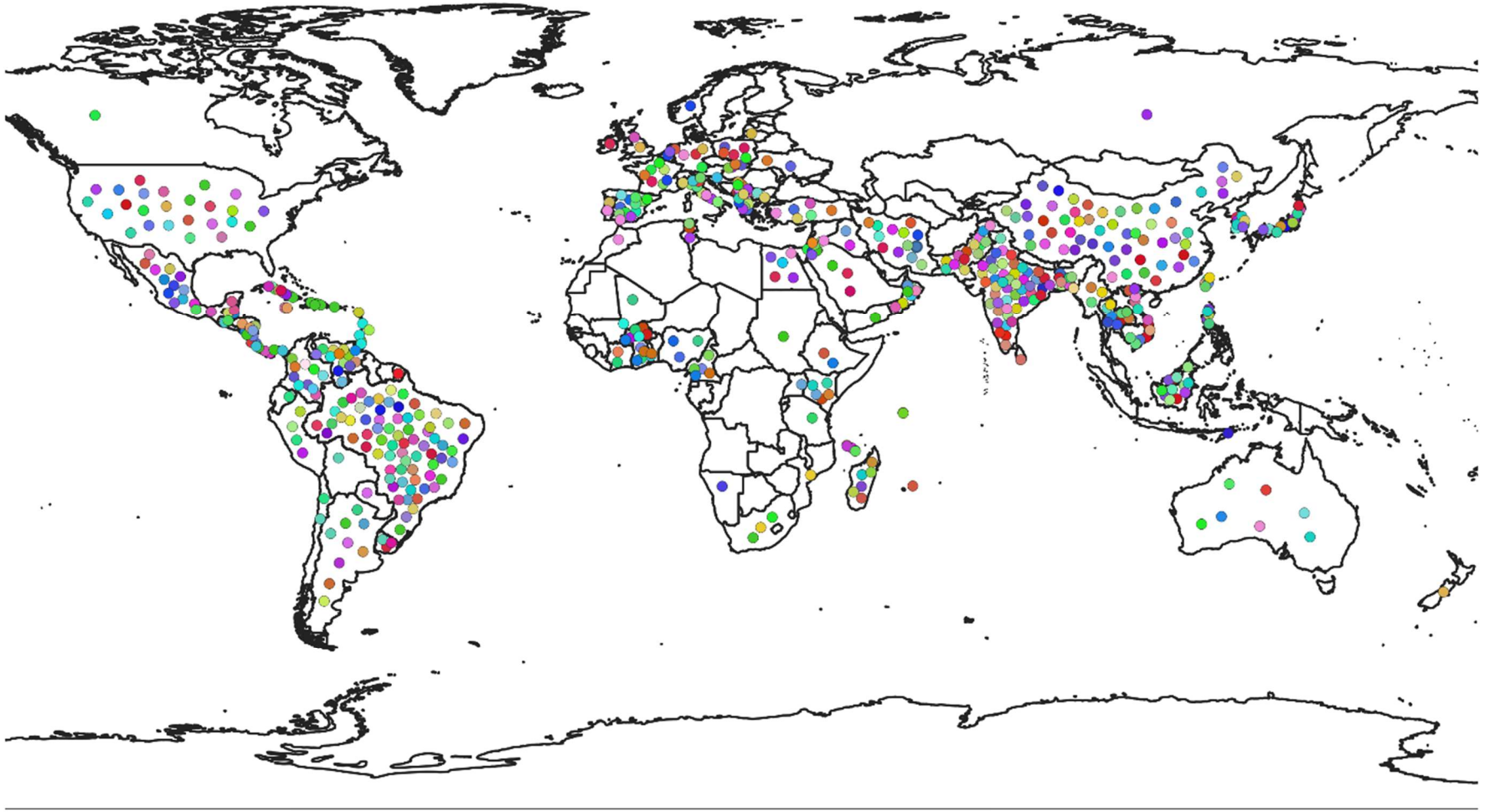

Figura 8. Distribuição mundial dos vírus que infectam a cultura do tomateiro com base no banco de dados *GenBank* (2022), *Host DataBase* (2022), Kitajima (2020) e sites de pesquisas relacionados à virologia vegetal. Ageratum enation virus (AEV), Amalgavirus não identificado (ANI), alfalfa mosaic virus (AMV), alstroemeria necrotic streak orthotospovirus (ANSV), arabis mosaic virus (ArMV), Ageratum yellow vein China virus (AYVCNV), Ageratum yellow vein Hualian virus (AYVHuaV), Ageratum yellow vein virus (AYVV), broad bean wilt virus 2 (BBWV-2), beet curly top Iran virus (BCTIV), beet curly top virus (BCTV), Begomovirus begomovirus não identificado (BeNC), bean golden mosaic virus (BGMV), bell pepper alphaendornavirus (BPEV), bidens pilosa leaf crumple virus (BPLCrV), brasilian tomato curly top virus (BrTCTV), beet

severe curly top virus (BSCTV), beet western yellows virus (BWYV), bhendi yellow vein mosaic virus (BYVMV), Capsicum chlorosis orthotospovirus (CaCV), chino del tomate Amazonas virus (CdTAV), chino del tomate virus (CdTV), Colombian datura virus (CDV), Chilli leaf curl Índia virus (CINV), Chilli leaf curl virus (ChiLCV), Chilli veinal mottle virus (ChiVMV), cotton leaf curl Burewala virus (CLCBV), cotton leaf curl Rajasthan virus (CLCRV), cotton leaf curl Gezira virus (CLCuGeV), Cleome leaf crumple virus (CleLCrV), Cucumber mosaic virus (CMV), Cucumber necrosis virus (CNV), chickpea chlorotic dwarf virus (CpCDV), cherry rasp leaf virus (CRLV), croton yellow vein mosaic virus (CroYVMV), Chrysanthemum stem necrosis orthotospovirus (CSNV), cherry tomato necrotic ringspot virus (CTNRSV), chilli yellow ringspot virus (CYRSV), eggplant mottled dwarf nucleorhabdovirus (EMDV), "Encarquilhamento" (Encarq), Engrujo (Engrujo), Eupatorium yellow vein virus (EpYVV), Erectites yellow mosaic virus (ErYMV), Euphorbia mosaic virus (EuMV), Euphorbia yellow mosaic virus (EuYMV), grapevine Algerian latent virus (GALV), groundnut bud necrosis orthotospovirus (GBNV), gemygorvirus poaspe1 (GemP1), groundnut ringspot orthotospovirus (GRSV), henbane mosaic virus (HMV), honeysuckle yellow vein virus (HYVV), infectious chlorosis of malvaceae complex (ICMC), impatiens necrotic spot orthotospovirus (INSV), lenaf leaf curl virus (KLCV), Lettuce chlorosis virus (LCV), Lettuce ring necrosis ophiovirus (LRNV), Malvastrum yellow vein Honghe virus (MaYVHV), milk vetch dwarf virus (MDV), Merremia mosaic virus (MerMV), Moroccan pepper virus (MPV), Malva vein clearing virus (MVCV), mungbean yellow mosaic India virus (MYMIV), olive latent virus 1 (OLV1), Okra mottle virus (OMoV), plant associated genomovirus 1 (PAG1), papaya leaf curl China virus (PaLCuCNV), papaya leaf curl virus (PaLCuV), Pedilanthus leaf curl virus (PeLCV), Pedilanthus leaf curl virus (PeLCV), pepper golden mosaic virus (PepGMV), pepper leaf curl Lahore virus (PepLCLaV), pepper leaf curl Yunnan virus (PepLCYnV), pepper leafroll virus (PepLRV), pepper mottle virus (PepMoV), pepino mosaic virus (PepMV), pepper ringspot virus (PepRSV), pepper yellow leaf curl Aceh virus (PepYLCAV), pepper yellow leaf curl Indonesia virus (PepYLCIV), pepper yellow leaf curl Thailand virus (PepYLCTHV), pepper yellow mosaic virus (PepYMV), pepper yellow vein Mali virus (PepYVMLV), pepper vein yellows virus (PeVYV), Physostegia chlorotic mottle alphanucleorhabdovirus (PhCMoV), pepper huasteco yellow vein virus (PHYVV), potato leafroll virus (PLRV), pepper mild mottle virus (PMMoV), Parietaria mottle virus (PMV), pepper necrotic spot virus (PNSV), pepper virus A (PepVA), potato yellow mosaic Trinidad virus (PoYMTV), Peru tomato mosaic virus (PTV), potato virus H (PVH), potato virus M (PVM), pepper veinal mottle virus (PVMV), potato virus S (PVS), potato virus X (PVX), potato virus Y (PVY), potato yellow mosaic Panama virus (PYMPV), potato yellow mosaic virus (PYMV), potato yellow vein virus (PYVV), Pelargonium zonate spot virus (PZSV), Radish leaf curl virus (RLCV), Ranunculus white mottle ophiovirus (RWMV), soybean chlorotic blotch virus (SbCBV), Sida leaf curl virus (SiLCuV), Sida micrantha mosaic virus (SimMV), Sida mottle virus (SiMoV), Sida mosaic Sinaloa virus (SiMSiV), Sida yellow net virus (SiYNV), Squash leaf curl China virus (SLCCNV), Squash leaf curl virus (SLCuV), Solanum mosaic Bolivia virus (SoMBoV), Spinach latent virus (SpLV), southern tomato virus (STV), Synedrella leaf curl virus (SyLCV), squash yellow mild mottle virus (SYMMV), tomato associated geminivirus 1 (TAG1), tomato associated geminivirus 2 (TAG2), tomato aspermy virus (TAV), Tobacco curly shoot virus (TbCSV), Tobacco leaf curl Japan virus (TbLCJV), Tobacco leaf curl Pusa virus (TbLCPuV), Tobacco leaf curl Thailand virus (TbLCTHV), Tobacco leaf curl Yunnan virus (TbLCYnV), tomato black ring virus (TBRV), tomato bushy stunt virus (TBSV), Tobacco bushy top virus (TBTD), tomato chlorotic spot orthotospovirus

(TCSV), tomato golden mosaic virus (TGMV), tomato golden vein virus (TGVV), tomato infectious chlorosis virus (TICV), tomato leaf curl Burkina Faso virus (TLCBFV), Tobacco leaf curl Kochi virus (TLCKV), tomato matilda virus (TMaV), Tobacco mild green mosaic virus (TMGMV), Tobacco mosaic virus (TMV), tomato necrotic ringspot virus (TNRV), Tobacco necrosis virus (TNV), tomato apical leaf curl virus (ToALCV), tomato begomovirus Colômbia (ToBC), tomato begomovirus Cuba (ToBCu), tomato blistering mosaic tymovirus (ToBMV), tomato brown rugose fruit virus (ToBRFV), tomato blunervirus 1 (ToBV1), tomato bright yellow mottle virus (ToBYMoV), tomato bright yellow mosaic virus (ToBYMV), tomato chino La Paz virus (ToChLPV), tomato chocolate virus(ToChV), tomato chlorotic leaf curl virus (ToCLCV), tomato chlorotic leaf distortion virus (ToClLDV), tomato common mosaic virus (ToCmMV), tomato chlorotic mottle Guyane virus (ToCMoGV), tomato chlorotic mottle virus (ToCMoV), tomato crinkle leaf yellows virus (ToCrLYV), tomato crinkle virus (ToCrV), tomato chocolate spot virus (ToCSV), tomato curly stunt virus (ToCSV), tomato curly top virus (ToCTV), tomato chlorosis virus (ToCV), tomato chlorotic vein virus (ToCVV), tomato dwarf leaf virus (ToDfLV), tomato dwarf leaf curl virus (ToDLCV), tomato enation leaf curl virus (ToELCV), tomato fruit blotch virus (ToFBV), tomato geminivirus (ToGe), tomato begomovirus Tulear 1 (ToGeT1), tomato golden leaf distortion virus (ToGLDV), tomato golden leaf spot virus (ToGLSV), tomato golden mottle virus (ToGMoV), tomato golden net virus (ToGNV), tomato interveinal chlorosis virus (ToICV), tomato interveinal chlorosis virus-2 (ToICV2), tomato infectious yellows virus (ToInYV), tomato interveinal yellowing virus (ToIYV), tomato leaf curl Anjouan virus (ToLCAnV), tomato leaf curl Arusha virus (ToLCArV), tomato leaf curl Antsiranana virus (ToLCAV), tomato leaf curl Bangalore virus (ToLCBaV), tomato leaf curl Bhatinda virus (ToLCBhV), tomato leaf curl Barka virus (ToLCBrV), tomato leaf curl Bangladesh virus (ToLCBV), tomato leaf curl Cameroon virus (ToLCCaV), tomato leaf curl Cebu virus (ToLCCeV), tomato leaf curl Chuxiong virus (ToLCChV), tomato leaf curl China virus (ToLCCNV), tomato leaf curl Cotabato virus (ToLCCoV), tomato leaf curl Diana virus (ToLCDiV), tomato leaf curl Fontem virus (ToLCFV), tomato leaf curl Guangdong virus (ToLCGdV), tomato leaf curl Gandhinagar virus (ToLCGNV), tomato leaf curl Gujarat virus (ToLCGUV), tomato leaf curl Ghana virus (ToLCGV), tomato leaf curl Guangxi virus (ToLCGxV), tomato leaf curl Hainan virus (ToLCHaiV), tomato leaf curl Hanoi virus (ToLCHaV), tomato leaf curl Hsinchu virus (ToLCHsV), tomato leaf curl Iran virus (ToLCIRV), tomato leaf curl Java virus (ToLCJaV), tomato leaf curl Japan virus (ToLCJV), tomato leaf curl Joydebpur virus (ToLCJV), tomato leaf curl Karnataka virus (ToLCKaV), tomato leaf curl Kerala virus (ToLCKeV), tomato leaf curl Comoros virus (ToLCKMV), tomato leaf curl Kunene virus (ToLCKunV), tomato leaf curl Karnataka virus 2 (ToLCKV2), tomato leaf curl Karnataka virus 3 (ToLCKV3), tomato leaf curl Sri Lanka virus (ToLCLKV), tomato leaf curl Las Playitas virus (ToLCLPV), tomato leaf curl Laos virus (ToLCLV), tomato leaf curl Liwa virus (ToLCLwV), tomato leaf curl Mahe virus (ToLCMahV), tomato leaf curl Madagascar virus (ToLCMGV), tomato leaf curl Mindanao virus (ToLCMiV), tomato leaf curl Mali virus (ToLCMLV), tomato leaf curl Moheli virus (ToLCMohV), tomato leaf curl Mayotte virus (ToLCYTV), tomato leaf curl Mumbai virus (ToLCMuV), tomato leaf curl Malaysia virus (ToLCMYV), tomato leaf curl Namakely virus (ToLCNaV), tomato leaf curl New Delhi virus (ToLCNDV), tomato leaf curl New Delhi virus 2 (ToLCNDV2), tomato leaf curl New Delhi virus 4 (ToLCNDV4), tomato leaf curl Nigeria virus (ToLCNGV), tomato leaf curl Oman virus (ToLCOMV), tomato leaf curl Palampur virus (ToLCPalV), tomato leaf curl Patna virus (ToLCPatV), tomato leaf curl Pune virus (ToLCPuV), tomato leaf curl Philippines virus (ToLCPV), tomato leaf curl purple vein

virus (ToLCPVV), tomato leaf curl Rajasthan virus (ToLCRaV), tomato leaf curl Seychelles virus (ToLCSCV), tomato leaf curl Sudan virus (ToLCSDV), tomato leaf curl Sinaloa virus (ToLCSiV), tomato leaf curl Sulawesi virus (ToLCSuV), tomato leaf curl Sirsa virus (ToLCSV), tomato leaf curl Togo virus (ToLCTgV), tomato leaf curl Toliara virus (ToLCToV), papaya leaf curl China virus (ToLCV), tomato leaf curl Taiwan virus (ToLCTV), tomato leaf curl Uganda virus (ToLCUV), Tobacco leaf curl virus (TLCV), tomato leaf curl virus (ToLCV), tomato leaf curl Vietnam virus (ToLCVV), tomato leaf deformation virus (ToLDeV), tomato leaf distortion virus (ToLDV), tomato latent virus (ToLV), tomato marchitez virus (ToMarV), tomato mosaic Barbados virus (ToMBV), tomato mosaic Havana virus (ToMHaV), tomato mild leaf curl virus (ToMiLCV), tomato mosaic leaf curl virus (ToMLCV), tomato mild mosaic virus (ToMMV), tomato mottle mosaic virus (ToMMV), tomato necrotic streak virus (TomNSV), tomato mottle leaf curl virus (ToMoLCV), tomato mottle leaf distortion virus (ToMoLDV), tomato mottle Taino virus (ToMoTaV), tomato mottle virus (ToMoV), tomato mottle wrinkle virus (ToMoWV), tomato mosaic severe dwarf virus (ToMSDV), tomato mosaic Trujillo virus (ToMTrV), tomato mosaic virus (ToMV), tomato mild yellow leaf curl Aragua virus (ToMYLCAV), tomato necrotic dwarf virus (ToNDV), tomato necrotic spot associated virus (ToNeAV), tomato necrotic spot tospovirus (ToNeSV), tomato necrotic stunt virus (ToNStV), tomato necrotic spot virus (ToNSV), tomato rugose mosaic virus (ToRMV), tomato ringspot virus (ToRSV), tomato severe leaf curl Kalakada virus (ToSLCKV), tomato severe leaf curl virus (ToSLCV), tomato severe mosaic virus (ToSMV), tomato severe rugose virus (ToSRV), tomato twisted leaf virus (ToTLV), tomato torrado virus (ToTV), tomato Venezuela begomovirus (ToVB), tomato vein clearing leaf deformation virus (ToVCLDeV), tomato wrinkled mosaic virus (ToWMV), tomato white ringspot virus (ToWRV), tomato yellow blotch virus (ToYBV), tomato yellow leaf deformation dwarf virus (ToYLDeDV), tomato yellow leaf distortion virus (ToYLDV), tomato yellow margin leaf curl virus (ToYMLCV), tomato yellow mottle virus (ToYMoV), tomato yellow mosaic virus (ToYMV), tomato yellow net virus (ToYNV), tomato yellow spot virus (ToYSV), tomato yellow vein streak virus (ToYVSV), tomato rugose yellow leaf curl virus (TRYLCV), Tobacco streak virus (TSV), tomato spotted wilt orthotospovirus (TSWV), tomato spotted wilt tospovirus (TSWV), tomato yellow fruit ring virus (TYFRV), tomato yellow leaf curl Axarquia virus (TYLCAxV), tomato yellow leaf curl China virus (TYLCCNV), tomato yellow leaf curl Guangdong virus (TYLCGdV), tomato yellow leaf curl Indonesia virus (TYLCIDV), tomato yellow leaf curl Kanchanaburi virus (TYLCKaV), tomato yellow leaf curl Malaga virus (TYLCMaV), tomato yellow leaf curl Mali virus (TYLCMLV), tomato yellow leaf curl Saudi virus (TYLCSauV), tomato yellow leaf curl Sardinia virus (TYLCSaV), tomato yellow leaf curl Shuangbai virus (TYLCShV), tomato yellow leaf curl Thailand virus (TYLCTHV), tomato yellow leaf curl virus (TYLCV), tomato yellow leaf curl Vietnam virus (TYLCVV), tomato yellow leaf curl Yunnan virus (TYLCYnV), tomato yellow mottle-associated cytorhabdovirus (TYMaV), tomato yellow ring orthotospovirus (TYRV), tomato zonate spot orthotospovirus (TZSV), watermelon bud necrosis orthotospovirus (WBNV), watermelon chlorotic stunt virus (WmCSV), wild potato mosaic virus (WPMV), watermelon silver mottle orthotospovirus (WSMoV), yellow net (YN), zucchini yellow mosaic virus (ZYMV).

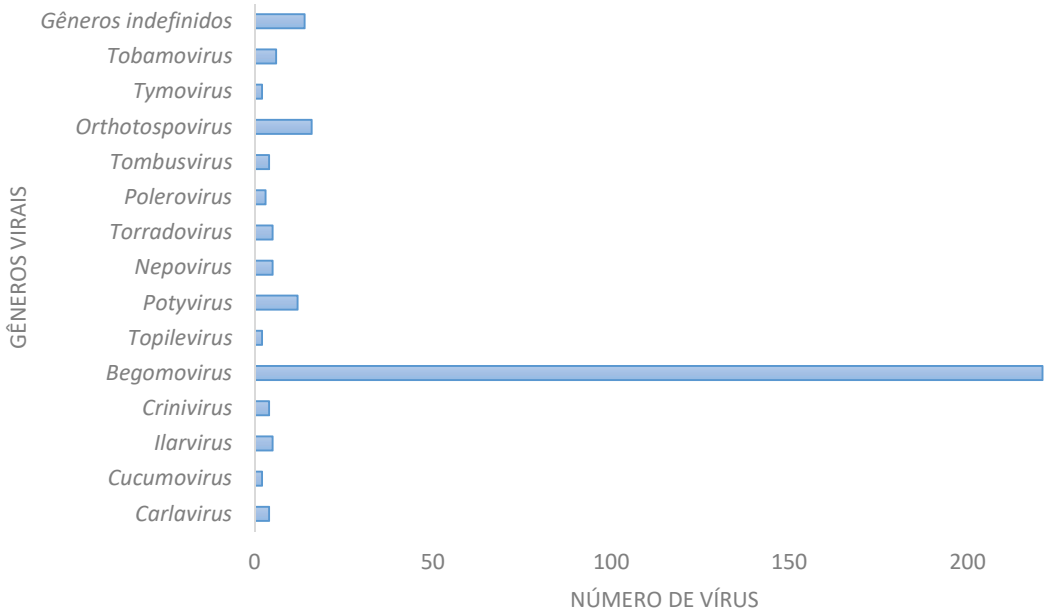

Figura 9. Gêneros virais com membros reportados em associação com a cultura do tomateiro no mundo. Fonte: *GenBank* (2022), *Host DataBase* (2022), Kitajima (2020) e sites de buscas relacionados à virologia vegetal.

Tabela 1. Distribuição das atuais 30 espécies de begomovírus descritas em associação com o tomateiro (*Solanum lycopersicum*) no Brasil.

Espécie viral	Estado da Federação	Referências
*Chino del tomate Amazonas virus**	AM	Fonseca et al. (2011)
Euphorbia yellow mosaic virus	DF, GO, SP & MG	Barreto et al. (2013); Macedo et al. (2018) e Duarte et al. (2020)
Leonurus mosaic virus = Tomato yellow spot virus	MG	Calegario et al. (2007) e presente tese
Sida common mosaic virus	RJ	Duarte et al. (2021a)
Sida micrantha mosaic virus	MG, DF & GO	Calegario et al. (2004) e Reis et al. (2020)
Sida mottle virus	SP	Cotrim et al. (2007)
Sida yellow net virus	AM & RJ	Fernandes (2015) e Duarte et al. (2021a)
*Tomato bright yellow mosaic virus**	BA	Fonseca et al. (2013)
*Tomato bright yellow mottle virus**	TO	Fonseca et al. (2013)
Tomato chlorotic leaf curl virus	PA	Quadros et al. (2019)
Tomato chlorotic mottle Guyane virus	AM	Oliveira et al. (2024)
Tomato chlorotic mottle virus	BA, MG, DF, ES, PE & RJ	Ribeiro et al. (2003) e Ribeiro et al. (2007)
Tomato common mosaic virus	MG, RJ & ES	Castillo-Urquiza et al. (2008); Barbosa et al. (2016) e Mituti et al. (2019)
*Tomato golden leaf distortion virus**	TO	Fonseca et al. (2013)
*Tomato golden leaf spot virus**	TO	Fonseca & Boiteux (2013)
Tomato golden mosaic virus	BA, DF, MG, PR, RN, RJ & SP	Matyis et al. (1975) e Hamilton et al. (1984)
Tomato golden net virus	MG	Reis et al. (2023)
Tomato golden vein virus	GO, DF & MG	Albuquerque et al. (2012); Reis et al. (2020) e Reis et al. (2021)
Tomato interveinal chlorosis virus	PE	Albuquerque et al. (2012)
Tomato interveinal chlorosis virus-2	GO	Rêgo-Machado et al. (2019)
Tomato leaf curl purple vein virus	PI	Macedo et al. (2018)
Tomato leaf distortion virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mild mosaic virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mottle leaf curl virus	MG, GO, DF, ES, RJ, SP, BA, CE, PB & PE	Ribeiro et al. (2003); Chaves et al. (2017); Ferro et al. (2017) e Souza et al. (2022)
Tomato mottle leaf distortion virus	GO	Martins et al. (2021)
Tomato rugose mosaic virus	MG, GO, DF, SP, PR & BA	Ribeiro et al. (2003); Fernandes et al. (2006) e Souza et al. (2020)
*Tomato rugose yellow leaf curl virus**	RS	Fonseca et al. (2016)
Tomato severe rugose virus	DF, GO, MG, RJ, SP, PE, SC & RS	Rezende et al. (1997); Cotrim et al. (2007) e Duarte et al. (2021)
Tomato yellow net virus	GO	Reis et al. (2023)
Tomato yellow vein streak virus	DF, GO, MG, RS, RJ & SP	Faria et al. (1997); Albuquerque et al. (2010) e Reis et al. (2021)

* Vírus ainda em fase de caracterização, mas com sequências depositadas no *GenBank*. Amazonas (AM), Distrito federal (DF), Goiás (GO), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Bahia (BA), Tocantins (TO), Espírito Santo (ES), Pernambuco (PE), Pará (PA), Paraná (PR), Rio Grande do Norte (RN), Piauí (PI), Ceará (CE), Paraíba (PB), Rio Grande do Sul (RS), Santa Catarina (SC).

1.8. *High-Throughput Sequencing* (HTS) aplicado à virologia vegetal.

O HTS tornou-se uma ferramenta de grande utilidade no estudo da diversidade viral. O HTS tem sido executado nas chamadas "plataformas de nova geração" tais como: 454, Hiseq, MiSeq, NextSeq – Illumina, SOLiD, Ion Torrent e Nanopore (Ambardar et al. 2016). Essas plataformas propiciam um melhor rendimento, maior quantidade de sequências geradas, maior multiplexagem de amostras, fragmentos de tamanhos variados, mas com boa cobertura, melhor qualidade e menor custo por base sequenciada. As plataformas de HTS são agrupadas em três categorias. Na primeira estão as que usam detecção ótica para identificar a base nitrogenada incorporada (exemplos: Illumina, 454 e SOLiD) e aquelas que registram alterações do pH durante o processo de polimerização. Na segunda categoria estão agrupadas as plataformas de acordo com a fonte do nucleotídeo das moléculas (exemplo: a plataforma Nanopore) e na terceira categoria estão aquelas classificadas de acordo como com a química utilizada no sequenciamento, podendo ser pela enzima Polimerase (Ion Torrent), por ligação dos nucleotídeos (SOLiD) ou leitura direta da molécula (Nanopore) (Levy & Myers 2016; Villamor et al. 2019), já tendo sido, inclusive, empregada para a primeira análise de begomovírus no Brasil (Naito et al. 2019). Com o advento dessas tecnologias ampliou-se o estudo da diversidade, como aplicação da metagenômica no estudo e detecção de vírus de plantas auxiliando na descoberta de novas espécies de vírus de plantas (Blawid et al. 2017; Mehetre et al. 2019).

As etapas para detecção de vírus por meio de análises metagenômicas iniciam pela extração do DNA total ou RNA total, seguido do enriquecimento do ácido nucleico viral extraído. Para vírus de DNA, circular, a técnica de *Rolling Circle Amplification* (RCA), permite o enriquecimento desse tipo de ácido nucleico e também amplificação de DNA satélites. Para o estudo de viromas de plantas também é válida a obtenção dos pequenos RNAs interferentes (siRNA) (Kutnjak et al. 2014; Kutnjak et al. 2015). De forma resumida pode-se descrever os passos (*pipeline*) para descoberta de genomas virais por uma abordagem metagenômica da seguinte forma: **(1)** preparação das amostras e enriquecimento do DNA viral; **(2)** análises da qualidade das sequências obtidas das bibliotecas por *Illumina* Hiseq; **(3)** Eliminar leituras de baixa qualidade obtidas os do HTS; **(4)** Montagem de novo dos contigs; **(5)** submeter ao *tBLASTn*; **(6)** extensão dos *contigs* e montagem de novo; **(7)** montagens (Blawid et al. 2017; Reis et al. 2023).

Metagenômica, usando este tipo de estratégia de sequenciamento, permite o estudo de viromas de diferentes culturas agrícolas (Villamor et al. 2019) e vem sendo utilizada desde 2009

na virologia de plantas (Adams et al. 2009). A partir de então a metagenômica aliada ao HTS e aplicada na virologia vegetal tem possibilitado inúmeras descobertas de novas espécies virais e agentes subvirais e a diversidade de espécies em diferentes hospedeiros vegetais, como por exemplo, obtenção de genoma completos de maize yellow striate virus (MYSV), uma nova espécies de rhabdovírus (Marurino et al. 2018), nova espécie Prunus virus F (PrVF) (Tahzima et al. 2019), quatro novas espécies dos gêneros *Potyvirus* e *Carlavirus* associada ao mamoeiro (Mumo et al. 2020) e novas espécies de potyvirus em videira (Bertazzon et al. 2020).

Reis et al. (2020) utilizaram essa técnica em estudos de diversidade viral em tomateiro com objetivo de revelar a diversidade de begomovírus e agentes subviras que infectam cultivares com e sem o gene de tolerância *Ty*-1. Como resultados relataram um gemycircularvírus, um novo alfassatélite e duas novas espécies de begomovírus em tomateiros sem o gene *Ty–1* e uma nova espécie de begomovírus em tomateiro com *Ty*-1. Souza et al. (2020) utilizando a tecnologia *High-Throughput Sequencing* (HTS) com objetivo de estimar a diversidade genética de begomovírus em tomateiro que portam os genes de resistência *Ty*-like ao longo de 14 anos revelaram a predominância e diversidade genética de dois begomovírus, ToSRV e ToMoLCV em campos de cultivo do tomate e que a abundância relativa e a diversidade genética desses dois vírus alternaram ao longo dos anos amostrados.

1.9. *Rolling Circle Amplification* (RCA)

A amplificação por círculo rolante, do inglês *rolling circle amplification* (RCA), consiste em uma técnica enzimática isotérmica que utiliza DNA polimeraze (Φ29 DNA polimerase) para a síntese de fitas de DNA (ssDNA) (Gu et al. 2018). Para que esse processo de amplificação ocorra se faz necessário a presença dos seguintes componentes: (I) DNA polimerase e seu tampão homóloco; (II) primer para DNA; (III) o DNA molde e (IV) trifosfatos de desoxinucleotídeos (dNTPs) (Gu et al. 2018). Essa técnica molecular vem sendo utilizada nos mais diversos estudos, como por exemplo a detecção de contaminantes em ambientes aquosos (Zhang et al. 2021), detecções de fatores de transcrições (Deng et al. 2017), análises de proteínas e pequenas moléculas ligadas ao DNA (Shi et al. 2017), bem como para o estudo de vírus de plantas, como por exemplo barconding para vírus vegetais baseados em RCA (Jesk 2018). O uso da técnica RCA aplicado a virologia inciou em 2004 para a obtenão de clones de vírus do gênero *Begomovirus* (Inoue-Nagata et al. 2004). A partir de então, vário outros grupos de pesquisa tabém investiram na técnica para estudos de vírus vetetais de genomas de DNA (Haible et al. 2006; Homs et al. 2008; Schubert et al. 2007). Mais recentemente, a técnica de

RCA foi utilizada em estudos estudo da diversidade viral e dinâmica espaço-temporal de vírus da família geminivirdae associada à cultura do tomateiro no Brasil (Macedo et al. 2017; Reis et al. 2020; Souza et al. 2020) e também na descoberta de novas espécies virais do gênero *Begomovirus* (Reis et al. 2023; Oliveira et al. 2024) além de relatos de novas hospedeiras em plantas não cultivadas (Pereira-Silva et al. 2022). Ao mesmo tempo, protolocolo para sequenciamento de alto desempenho para estudo de populações virais de DNA basedas em enriquecimento por RCA estão sendo aplicados afim de melhor aproveitamento das informações obtidas pelo sequenciamento (Aimone et al. 2022).

1.10. Fontes de resistência genética a begomovírus em tomateiro

Para o manejo de begomovírus algumas estratégias podem ser adotadas, tais como: eliminação de tomateiros espontâneos e de plantas daninhas (hospedeiras alternativas) que sirvam de fonte de inóculo desses vírus e de abrigo para a mosca branca, não realização de plantios escalonados, monitoramento e manutenção da população do inseto vetor em baixo nível durante todo o ciclo da cultura e utilização de híbridos ou variedades de tomateiro resistentes e/ou tolerantes aos begomovírus (Inoue-Nagata et al. 2009). Dentre as estratégias de manejo, o uso de cultivares ou híbridos de tomateiro contendo genes de resistência é a estratégia de controle mais eficiente, pois permite a redução das perdas causadas pelos begomovírus à cultura do tomateiro (Boiteux et al. 2012). Além disso, quando se trata do controle da transmissão viral, o emprego de inseticidas para reduzir a pressão do vetor *B. tabaci* se mostra pouco eficiente. De fato, o uso indiscriminado dessa estratégia representa um fator de seleção a favor de populações de insetos com resistência aos principais princípios ativos (Silva et al. 2009; Yao et al. 2017; Horowitz et al. 2020).

Os programas de melhoramento genético visando a resistência a doenças vêm utilizando várias espécies do gênero *Solanum* para introgressão de genes de resistência (Pereira-Carvalho et al. 2014). A diversidade do germoplasma de espécies selvagens do gênero *Solanum* (sect. *Lycopersicon*) tem sido uma rica fonte de fatores resistência contra begomovírus. Entre as espécies utilizadas pelos programas de melhoramento estão: S. *pimpinellifolium*, S. *peruvianum*, S. *chilense*, S. *habrochaites* e S. *cheesmaniae* (Dhaliwal et al. 2019). Oito genes de resistência foram mapeados no genoma do tomateiro e serão descritos a seguir. Nenhum destes genes confere respostas do tipo imunidade completa às infecções virais, mas sim uma resposta de tolerância (Cooper & Jones 1983). Uma planta é dita como resistente quando a infecção, replicação e/ou invasão do vírus é restringida pela hospedeira, enquanto tolerância é

classificada quando uma planta é infectada por vírus e este replica-se na hospedeira sem causar grandes danos severos (Cooper & Jones 1983). Por sua vez, suscetibilidade é definida quando os mecanismos da planta que buscam restringir a infecção, replicção e/ou invasão viral são superados ou insuficientes. Quanto à estes mecanismos que a planta utiliza para combater o processo de infecção pelo vírus, podem ser, por exemplo, através do silenciamento gênico transcricional, do inglês, *transcriptional gene silencing* (TGS); silenciamento gênico pós-transcricional (*post transcriptional gene silencing* – PTGS); imunidade antiviral mediata por micro RNA (*MicroRNAs in antiviral immunity* – miRNA) ou mesmo a imunidade desencadeada por efetores (*effector-triggered immunity* – ETI) (Gupta et al. 2021; Zhang et al. 2023). O TGS envolve uma sequência complexa interação entre hospedeiro e vírus que ocorrem em diferentes estágios da infecção viral, essa resposta da planta à infecção viral é meidiada a metilação do DNA dirigida por RNA, suprimindo replicação viral e silenciando os genes virais (Gupta et al. 2021). O mecanismo de PTGS atua sobre os transcritos virais, o mecanismo direciona iniciamente os dsRNA devirados dos transcritos virais complementares, é uma resposta da planta essencial para a defesa, desenvolvimento e expressão dos genes do hospedeiro (Rodríguez-Negrete et al. 2009; Gupta et al. 2021). Os miRNA desempenham papel importante quanto às respostas das plantas à estresse biótico e abiótico, contudo há poucas respostas esclarecedoras quanto esse mecanismo contra a infecção por geminivirus (Gupta et al. 2021). O uso de miRNA direcionado as ORFs AV1 e AV2 tornaram tomateiros tolerantes à infecção viral de tomato leaf curl New Delhi virus (ToLCNDV), mostrando ser o miRNA uma estratégia eficaz de controle de geminivirus (Vu et al. 2013). O ETI constitui um sistema da imunidade vegetal que desenvolveu sistemas receptores para dectar os sinais da infecção viral e posteriormente induzir uma resposta ao patógeno, essa resposta pode ser do tipo hipersensibilidade (HR) o que leva a restrição local da infecção ao induzir a morce celular programada (Zhang et al. 2023).

O gene *Ty*–1 está localizado no cromossomo 6 do tomateiro e é responsável por codificar uma RNA polimerase dependente de RNA (RdRp) (Zamir et al. 1994; Verlaan et al. 2013). Híbridos de tomateiro que portam o gene quando desafiados por espécies de begomovírus em campo demonstraram que o locus *Ty*–1 confere uma resposta de tolerância aos vírus (Boiteux et al. 2007). O mecanismo de tolerância desse gene está envolvido com o silenciamento gênico transcricional por RNAi, mais precisamente na metilação do DNA viral, contudo infecções mistas com vírus de RNA reduzem o mecanismo de tolerância conferido pelo gene (Butterbach et al. 2014). O gene *Ty*–2, proveniente de *S. habrochiates*, está localizado no cromossomo 11 (Ji et al. 2009a). Linhagens que portavam o gene mostraram elevados níveis

de resistência a begomovírus monopartidos oriundos da Índia, Vietnã, Japão e Taiwan, já sendo implementado em programas de melhoramento (Hanson et al. 2006). Yamaguchi et al. (2018) relataram que o *Ty*–2 pertence à classe genes que codificam para proteína com domínios de ligação de nucleotídeos contendo repetições ricas em leucina (NB-LRR), denominando-o como *TYNBS*1. Mais recentemente, Shen et al. (2020) mostraram que a expressão das proteínas Rep/C1 do tomato yellow leaf curl virus (TYLCV) desencadeia a resposta de hipersensibilidade em *Nicotiana benthamiana* que co-expressavam o gene *Ty*–2, indicando que os genes da Rep/C1 representam um determinante de avirulência para resistência mediada por *Ty*–2. Ji et al. (2007) mapearam o gene *Ty*–3 no cromossomo 6. Posteriormente, Verlaan et al. (2013) mostraram que o gene *Ty*–3 é alelo do gene *Ty*–1 e que ambos codificam para uma RdRp pertencente a classe RDRγ. Alguns isolados de TYLCV conseguem silenciar os genes *Ty*–1 e *Ty*–3 em tomateiro e comprometer a resistência em linhagens derivadas de *Solanum chilense* (Caro et al. 2015). Ji et al. (2009b) ao realizarem o mapeamento genômico de S. *chilense* identificaram um novo *locus* de resistência aos begomovírus contido no cromossomo 3, chamando-o de *Ty*–4. Os autores demonstraram que *Ty*–4 desempenha um papel complementar à resistência ao TYLCV conferida por *Ty*–3, pois o *Ty*–4 possui um efeito menor de resistência ao TYLCV quando comparada à resistência atribuída ao *Ty*–3, contudo, linhagens que portam ambos os genes tiveram a resistência ao ToYLC aumentada (Ji et al. 2009b). O gene *ty*–5 está localizado em um QTL (*Quantitative Trait Loci*) no cromossomo 4 de linhagens de *S. peruvianum*, esse gene confere resistência ao TYLCV que varia de 39,7 a 46,6 % em linhagens segregantes (Anbinder et al. 2009). Os estudos realizados por Lapidot et al. (2015) permitiram identificar o gene *Pelo* ou *Pelota*, que está envolvido na fase de reciclagem da biossíntese de proteínas e de reciclagem de ribossomos, é o responsável pela resistência recessiva ao TYLCV atribuída ao locus *ty*–5. Mais recentemente, o estudo de Wang et al. (2018) corroborou com as descobertas dos autores supracitados, que o gene recessivo *Pelota* contido no *locus ty*–5 é o responsável por conferir resistência ao TYLCV. O locus *Ty*–6 está localizado no cromossomo 10, quando este encontra-se em heterozigose sua resistência ao TYLCV é dita como tolerância, porém quando combinado com os genes *Ty*–3 e *ty*–5, sua resistência é aperfeiçoada, portanto, a resistência conferida por *Ty*–6 é mais bem aproveitada como resistência complementar aos genes *Ty*–3 e *ty*–5(Scott et al. 2015; Gill et al. 2019). O gene *tcm*–1 foi obtido de uma linhagem (TX 468-RG) de *S. lycopersicum* (Giordano et al. 2005; Pereira-Carvalho et al. 2015). Contido no cromossomo 6, foi o primeiro gene monogênico recessivo caracterizado no tomateiro (Machado et al. 2013). Linhagens de tomate que portavam o gene *tcm*–1 mostraram resistência a outros begomovírus bipartidos, de forma semelhante à observada na fonte original (TX 468-

RG) de resistência (Giordano et al. 2005). O mecanismo de resistência conferido pelo gene está relacionado à redução do acúmulo viral em plantas resistentes e menor expressão de sintomas (García-Cano et al. 2008; Pereira-Carvalho et al. 2015). O gene *tgr*–1 consiste em um alelo recessivo oriundo da linhagem de tomate FLA-653 com resistência ao begomovírus monopartido tomato yellow leaf curl virus (TYLCV). A resistência conferida pelo gene leva à redução do acúmulo viral e prejudica a translocação do vírus célula a célula, limitando a infecção sistêmica (Bian et al. 2007).

No Brasil, diferentes estudos e grupos de pesquisa têm evidenciado o uso promissor dos genes de tolerância em tomateiro contra às begomoviroses. Giordano et al. (2005) mostraram que a incorporação do gene *tcm*–1 em cultivares comerciais de tomateiro conferiu resistência ao ToMoLCV e as linhagens de reprodução também foram resistentes a outras espécies de begomovírus. Boiteux et al. (2007) em ensaios de campo com tomateiros heterozigotos e homozigotos para o gene *Ty*–1 demonstraram que este gene confere à planta uma reação de tolerância à infecção por espécies de begomovírus, com sintomas mais suaves em plantas heterozigotas (*Ty*–1/*ty*–1). Hurtado et al. (2012), avaliaram 11 genótipos de tomateiros que portavam os genes *Ty*–1 e *Ty*–2 frente à infecção por ToRSV e TYLCV evidenciaram que um subgrupo de genótipos se mostrou promissor devido à ausência de sintomas e baixas concentrações virais.

A incorporação de genes de resistência a vírus em cultivares comerciais tem sido uma busca constante pelos programas de melhoramento. Várias estratégias vêm sendo aplicadas para alcançar esses objetivos. A técnica de piramidação de genes foi demonstrada recentemente como uma ferramenta eficiente no controle de tomato leaf curl virus (ToLCV). A piramidação dos genes *Ty*–1, *Ty*–2, *ty*–5 e *Ty*–6 resultou em um nível de tolerância de amplo espectro ao ToLCV, superior aos seus parentais individuais (Prabhandakavi et al. 2021).

O fenômeno descrito como “quebra” ou “superação” da resistência tem sido registro com begomovírus. Variantes virais capazes de tornar um fator de resistência ineficiente já têm sido detectadas na Espanha, onde recombinantes do TYLCV conseguiram causar sintomas severos em tomateiros que portavam os genes *Ty*–1 e *Ty*–3 (Torre et al. 2019). No Brasil, existem evidencias ainda preliminares que isolados virais capazes de quebrar a resistência conferida pelo gene *Ty*–1 (Reis et al. 2020).

Além da redução dos impactos às culturas, os programas de melhoramento também buscam impedir ou minimizar o fenômeno de “quebra” ou “superação” da resistência. Para isso, são cruciais as ações de pesquisa envolvendo monitoramento constante e intensivo da dinâmica populacional das espécies virais dentro das lavouras bem como a avaliação do impacto das

cultivares resistentes nessa dinâmica populacional e as respostas dessas cultivares resistentes em diferentes regiões do Brasil. A informação gerada por estes estudos fornece um panorama mais específico e mais preciso da diversidade deste grupo de patógenos, dando suporte aos programas de melhoramento que visam compreender a amplitude dos fatores de tolerância a begomovírus.

REFERÊNCIAS

Acharya B, Dutta S, Dutta S, Chattopadhyay A (2018) Breeding tomato for simultaneous improvement of processing quality, fruit yield, and dual disease tolerance. International Journal of Vegetable Science 5:407–423

Adams IP, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene, M., Samuitiene M, Boonham, N (2009) Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. Molecular Plant Pathology 10:537–545

Aimone CD, Hoyer JS, Dye AE, Deppong DO, Duffy S. Carbone I, Hanley-Bowdoin L (2022) An experimental strategy for preparing circular ssDNA virus genomes for next-generation sequencing. Journal of Virological Methods 300:1–10

Albuquerque LC, Martin DP, Avila AC, Inoue-Nagata AK (2010) Characterization of tomato yellow vein streak virus, a begomovirus from Brazil. Virus Genes 40:140–147

Albuquerque LC, Varsani A, Fernandes FR, Pinheiro B, Martin DP, Ferreira PTO, Lemos TO, Inoue-Nagata AK (2012) Further characterization of tomato-infecting begomoviruses in Brazil. Archives of Virology 157:747–752

Ambardar S, Gupta R, Trakroo, Lal R, Vakhlu J (2016) High Throughput Sequencing: An Overview of sequencing chemistry. Indian Journal of Microbiology 56:394–404

Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, Chen L, Lapidot M, Levin I (2009) Molecular dissection of tomato leaf curl virus resistance in tomato line TY172 derived from *Solanum peruvianum*. Theoretical and Appllied Genetics 119:519–530

Andrade IAD (2020) Levantamento de novas espécies de begomovírus no Distrito Federal e em Goiás e caracterização molecular de uma nova espécie associada a tomateiro contendo *Ty*–1 (Dissertação de Mestrado), Universidade de Brasília, 2020.

Arguello-Astorga G, Lopez-Ochoa L, Kong LJ, Orozco BM, Settlage SB, Hanley-Bowdoin L (2004) A novel motif in geminivírus replication proteins interacts with the plant retinoblastoma-related protein. Journal of Virology 78:4817–4826

Arguello-Astorga G, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminivíruses: Identification of potential interacting amino acid-base pairs by a comparative approach. Archives of Virology 146:1465–1485

Ávila ACD, Inoue-Nagata AK, Costa H, Boiteux LS, Neves LODQ, Prates RS, Bertini LA (2004) Ocorrência de viroses em tomate e pimentão na região serrana do estado do Espírito Santo. Horticultura Brasileira 22:655–658

Barbosa JC, Costa H, Gioria R, Rezende JAM (2011) Occurrence of tomato chlorosis virus in tomato crops in five Brazilian states. Tropical Plant Pathology 36:256–258

Barbosa JC, Teixeira APM, Moreira AG, Camargo LEA, Filho AB, Kitajima EW, Rezende JAM (2008) First report of Tomato chlorosis virus infecting tomato crops in Brazil. Plant Disease, 92:1709–1709

Barreto SS, Hallwass M, Aquino OM, Inoue-Nagata AK (2013) A study of weeds as potential inoculum sources for a tomato-infecting begomovirus in central Brazil. Phytopathology 103:436–444

Batuman O, Turini TA, Oliveira PV, Rojas MR, Macedo M, Mellinger HC, Adkins S, Gilbertson RL (2017) First report of a resistance-breaking strain of Tomato spotted wilt virus infecting tomatoes with the *Sw*-5 tospovirus-resistance gene in California. Plant Disease 4:637–637

Blawid R, Morgado FS, Souza CA, Resende RO, Boiteux LS, Pereira-Carvalho RC (2015) Fluorescence *in situ* hybridization analysis of endosymbiont genera reveals novel infection patterns in a tomato-infesting *Bemisia tabaci* population from Brazil. Tropical Plant Pathology 40:233–243

Blawid R, Silva JMF, Nagata T (2017) Discovering and sequencing new plant viral genomes by next-generation sequencing: Description of a practical pipeline. Annals of Applied Biology 170:301–314

Bertazzon N, Chitarra W, Angelini E, Nerva L (2020) Two new putative plant viruses from wood metagenomics analysis of an esca diseased vineyard. Plants 9:1–10

Bian XY, Thomas MR, Rasheed MS, Saeed M, Hanson P, De Barro PJ, Rezaian MA (2007) A recessive allele (*tgr*-1) conditioning tomato resistance to geminivírus infection is associated with impaired viral movement. Phytopathology 97:930–937

Boiteux LS, Giordano LB (1993) Genetic basis of resistance against two *Tospovirus* species in tomato (*Lycopersicon esculentum*). Euphytica 71:151–154.

Boiteux LS, Fonseca MEN, Giordano LB, Melo PTC (2012) Melhoramento genético. Produção de tomate para processamento industrial. Embrapa p. 31–50

Boiteux LS, Fonseca MEN, González-Arcos M (2016) Melhoramento de Tomate para Processamento Industrial. In: Nick C e Borém A (Org.). Melhoramento de Hortaliças. 1ed.Viçosa-MG: Editora UFV, v. 1, p. 432–464

Boiteux LS, Lima MF, Fonseca MEN, Mendonça JL, Costa AF, Silva-Filho JG, Fontes MG, González-Arcos M (2018) Identification of eight *Solanum* (subgenus *Leptostemonum*) species as novel natural hosts of tomato chlorosis virus in Brazil. Plant Disease 102:16–73

Boiteux LS, Oliveira VR, Silva CH, Makishima N, Inoue-Nagata AK, Fonseca MEN, Giordano LB (2007) Reaction of tomato hybrids carrying the *Ty–1* locus to Brazilian bipartite *Begomovirus* species. Horticultura Brasileira 25:20–23

Boykin LM, De Barro PJ (2014) A practical guide to identifying members of the *Bemisia tabaci* species complex: and other morphologically identical species. Frontiers in Ecology and Evolution 2:1–45

Briddon RW, Martin DP, Roumagnac P, Navas-Castillo J, Fiallo-Olivé E, Moriones E, Lett JM, Zerbini FM, Varsani A (2018) Alphasatellitidae: a new family with two subfamilies for the classification of geminivírus-and nanovirus-associated alphasatellites. Archives of Virology 163:2587–2600

Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JCF, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of begomovirus taxonomy based on pairwise sequence comparisons. Archives of Virology 160:1593–1619

Butterbach P, Verlaan MG, Dullemans A, Lohuis D, Visser RG, Bai Y, Kormelink R (2014) Tomato yellow leaf curl virus resistance by *Ty*–1 involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. Proceedings of the National Academy of Sciences 35:12942–12947

Calegário RF, Ferreira SS, Andrade EC, Zerbini FM (2007) Characterization of Tomato yellow spot virus, a novel tomato-infecting begomovirus in Brazil. Pesquisa Agropecuária Brasileira 42:1335–1343

Calegário, RF, Ferreira SS, Andrade EC, Zerbini FM (2004) Caracterização de um isolado do Begomovirus Sida micrantha virus (SimMV) obtido de tomateiro. Fitopatologia Brasileira 29:150

Cantú-Iris M, Pastor-Palacios G, Mauricio-Castillo JA, Bañuelos-Hernández B, Avalos-Calleros JA, Juárez-Reyes A, Rivera-Bustamante R, Argüello-Astorga GR (2019) Analysis of a new begomovirus unveils a composite element conserved in the CP gene promoters of several Geminiviridae genera: Clues to comprehend the complex regulation of late genes. PloS One, 14:1–24

Caro M, Verlaan MG, Julián O, Finkers R, Wolters AMA, Hutton SF, Scott JW, Kormelink R, Visser RGF, Díez MJ, Castro AP, Bai Y (2015) Assessing the genetic variation of *Ty*–1 and *Ty–3* alleles conferring resistance to tomato yellow leaf curl virus in a broad tomato germplasm. Molecular Breeding 35:1–13

Castillo-Urquiza GP, Beserra JR JEA, Bruckner FP, Lima ATM, Varsani A, Alfenas-Zerbini P, Zerbini FM (2008) Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Archives of Virology 153:1985–1989

Cooper JI, Jones AT (1983) Responses of plants to viruses: Proposals for the use of terms. Phytopathology 73:127–128

Colariccio A, Eiras M, Chaves ALR, Bergmann JC, Zerbini FM, Harakava R, Chagas CM (2007) Tomato yellow vein streak virus, a new Begomovirus on tomato from Brazil: Complete DNA–A sequence and some molecular and biological features. Journal of Plant Pathology 89:385–390

Costa AS (1974) Moléstias do tomateiro no Brasil transmitidas pela mosca-branca *Bemisia tabaci*. Fitopatologia 9:1–47

Cotrim AA, Krause-Sakate R, Narita N, Zerbini FM, Pavan MA (2007) Diversidade genética de begomovírus em cultivos de tomateiro no Centro-Oeste Paulista. Summa Phytopathologica 33:300–303

Cui X, Li G, Wang D, Hu D, Zhou X (2005) A Begomovirus DNA beta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. Journal of Virology 79:10764–10775

De Barro PJ, Liu S-S, Boykin LM, Dinsdale AB (2011) *Bemisia tabaci*: A statement of species status. Annual Review of Entomology 56:1–19

Dianese EC, Resende RO, Inoue-Nagata AK (2008) Alta incidência de pepper yellow mosaic virus em tomateiro em região produtora no Distrito Federal. Tropical Plant Pathology 33:67–68

Dianese EC Fonseca MEN, Goldbach R, Kormelink R, Inoue-Nagata AK, Resende RO, Boiteux LS (2010) Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw*-5 (*Tospovirus* resistance) gene cluster in a wide range of tomato accessions. Molecular Breeding 25:133–142

Dianese EC Fonseca MEN, Inoue-Nagata AK, Resende RO, Boiteux LS (2011) Search in *Solanum* (section *Lycopersicon*) germplasm for sources of broad-spectrum resistance to four *Tospovirus* species. Euphytica 180:307–319

Díaz-Pendón JA, Sánchez-Campos S, Fortes IM, Moriones E (2019) Tomato yellow leaf curl sardinia virus, a begomovirus species evolving by mutation and recombination: A challenge for virus control. Viruses 11:1–45

Dhaliwal M, Jindal S, Sharma A, Prasanna H (2019) Tomato yellow leaf curl virus disease of tomato and its management through resistance breeding: A review. The Journal of Horticultural Science and Biotechnology 95:1–20

Dinsdale A, Cook L, Riginos C, Buckley YM, De Barro P (2010) Refined global of *Bemisia tabaci* (*Hemiptera: Sternorryncha: Aleyrodoidea: Aleyrodidae*) mitocondrial cytochrome oxidase 1 to identify species level genetic boundaries. Annals of the Entomological Society of America 103:196–208

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7 Molecular Biology and Evolution 29:1969–1973

De Barro PJ, Liu SS, Boykin LM, Dinsdale AB (2011) *Bemisia tabaci*: A statement of species status. Annual review of entomology 56:1–19

Deng K, Li C, Huang H, Li X (2017) Rolling circle amplification based on signal-enhanced electrochemical DNA sensor for ultrasensitive transcription factor detection. Sensors and Actuators B: Chemical 238:1302–1308

Dry IB, Krake LR, Rigden JE, Ali Rezaian M (1997) A novel subviral agent associated with a geminivírus: The first report of a DNA satellite. Proceedings of the National Academy of Sciences 94:7088–7093

Duarte MF, Fonseca ME, Costa H, Fernandes NA, Reis A, Boiteux LS, Pereira-Carvalho RC (2021a) Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. Virus Genes 57:83–93

Duarte MF, Pereira-Carvalho RC, Reis LNA, Rojas MR, Gilbertson RL, Costa H, Boiteux LS, Fonseca MEN (2021b) Natural infection of tomatoes (*Solanum lycopersicum*) by euphorbia yellow mosaic virus isolates across four Brazilian states. Plant Disease 105:517

Edgar RC (2004) MUSCLE: A multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:1–19

EFSA Panel on Plant Health (PLH) (2012) Scientifc opinion on the pest categorisation of the tospoviruses. EFSA J 10:2772

FAOSTAT (2024) Statistics Division of the Food and Agricultural Organization of the United Nations. https://www.fao.org/faostat/es/#data/QCL/visualize. Acessado 20 de janeiro de 2024

Faria JC, Souza-Dias J, Slack S, Maxwell D (1997) A new geminivírus associated with tomato in the State of São Paulo, Brazil. Plant Disease 81:423

Ferro CG, Zerbini FM, Navas-Castillo J, Fiallo-Olivé E (2021) Revealing the complexity of sweepovirus-deltasatellite–plant host interactions: Expanded natural and experimental helper virus range and effect dependence on virus-host combination. Microorganisms 9:1–9

Fernandes NAN. 2015. Begomoviroses no cultivo do tomateiro no Brasil: Variabilidade e caracterização de novas espécies virais e diversidade do vetor *Bemisia tabaci*. (Tese de Doutorado) Universidade de Brasília (UnB), 2020.

Fernandes DDS, Okuma D, Pantoja-Gomez LM, Cuenca A, Corrêa AS (2023) *Bemisia tabaci* MEAM1 still remains the dominant species in open field crops in Brazil. Brazilian Journal of Biology 84:1–8

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

Fernandes JJ, Carvalho MG, Andrade EC, Brommonschenkel SH, Fontes EPB, Zerbini FM (2006) Biological and molecular properties of Tomato rugose mosaic virus (ToRMV), a new tomato-infecting begomovirus from Brazil. Plant Pathology 55:513–522

Fiallo-Olivé E, Navas-Castillo J (2020) Molecular and biological characterization of a New World mono-/bipartite begomovirus/deltasatellite complex infecting *Corchorus siliquosus*. Frontiers in Microbiology 11:1–14

Fiallo-Olivé E, Navas-Castillo J (2019) Tomato chlorosis virus, an emergent plant virus still expanding its geographical and host ranges. Molecular Plant Pathology 20:1307–1320

Fiallo-Olivé E, Tovar R, Navas-Castillo J (2016) Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. New Phytologist 212:680–692

Fiallo-Olivé E, Zubiaur YM, Moriones E, Navas-Castillo J (2012) A novel class of DNA satellites associated with New World begomoviroses. Virology 426:1–6

Flores E, Silberschmidt K, Kramer M (1960) Observações de "clorose infecciosa" das malváceas em tomateiros do campo. O Biológico 26:65–69

Fonseca MEN, Boiteux LS, Fernandes N (2010) Tomato golden leaf distortion virus, a new begomovirus infecting tomato in Tocantins state, Brazil. [Genbank]. Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/NC_043122.1 Acessado em: 10 julho de 2022

Fonseca MEN, Boiteux LS, Fernandes N (2011) Genomic organization of Chino del tomate Amazonas virus a bipartite begomovirus endemic to the Amazon region of Brazil. [Genbank]. Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/NC_038443.1 Acessado em: 10 de julho de 2022

Fonseca MEN, Boiteux LS, Fernandes N (2013) Tomato bright yellow mosaic virus and Tomato bright yellow mottle virus: two new tomato-infecting begomoviruses in Brazil. [Genbank]. Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/KC791690.1 Acessado em: 10 de julho de 2022

Fonseca MEN, Boiteux LS, Costa AF (2016) Tomato rugose yellow leaf curl virus (Geminiviridae) in tomatoes associated with biological invasion of *Bemisia tabaci* Mediterranean (biotype Q) into subtropical Brazil. [Genbank] Acessado em: 10 de julho de 2022

Fonseca, MEN, Boiteux, LS, Lima, MF, Mendonça, JL, Costa, AF, Fontes, MG, Costa H, González-Arcos, M (2016). First report of tomato chlorosis virus infecting eggplant and scarlet eggplant in Brazil. Plant Disease 100:867

Fontenele RS, Lamas NS, Lacorte C, Lacerda ALM, Varsani A, Ribeiro SG (2017) A novel geminivírus identified in tomato and cleome plants sampled in Brazil. Virus Research 240:175–179

Gadhave KR, Gautam S, Rasmussen DA, Srinivasan R (2020) Aphid transmission of Potyvirus: The largest plant-infecting RNA virus genus. Viruses 12:1–22

García-Cano E, Resende RO, Boiteux LS, Giordano LB, Fernández-Muñoz R, Moriones E (2008) Phenotypic expression, stability, and inheritance of a recessive resistance to monopartite begomoviruses associated with tomato yellow leaf curl disease in tomato. Phytopathology 98:618–627

Ghosh S, Ghanim M (2021) Factors determining transmission of persistent viruses by Bemisia tabaci and emergence of new virus–vector relationships. Viruses 13:1–13

Gilbertson RL, Batuman O, Webster CG, Adkins S (2015) Role of the insect supervectors Bemisia tabaci and Frankliniella occidentalis in the emergence and global spread of plant viruses. Annual Review of Virology 2:67–93

Gill U, Scott JW, Shekasteband R, Ogundiwin E, Schuit C, Francis DM, Sim SC, Smith H, Hutton SF (2019) *Ty*-6, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus. Theoretical and Applied Genetics 132:1543–1554

Giordano LB, Silva-Lobo VL, Santana FM, Fonseca MEN, Boiteux LS (2005) Inheritance of resistance to the bipartite Tomato chlorotic mottle begomovirus derived from *Lycopersicon esculentum* cv. 'Tyking'. Euphytica 143:27–33

González-Arcos M, Fonseca MEN, Arruabarrena A, Lima MF, Michereff-Filho M, Moriones E, Fernández-Muñoz, Boiteux LS (2018) Identification of genetic sources with attenuated tomato chlorosis virus-induced symptoms in *Solanum* (section *Lycopersicon*) germplasm. Euphytica 214:1–16

Gu L, Yan W Liu L, Wang S, Zhang X, Lyu M (2018) Research progress on rolling circle amplification (RCA)-based biomedical sensing. Pharmaceuticals 11:1–19

Gupta N, Reddy K, Bhattacharyya D, Chakraborty S (2021) Plant responses to geminivirus infection: guardians of the plant immunity. Virology Journal 18:1–25

Gupta R, Kwon S-Y, Kim ST (2018) An insight into the Tomato spotted wilt virus (TSWV), tomato and thrips interaction. Plant Biotech Rep 12:157–163

Gutierrez, C (2000) DNA replication and cell cycle in plants: learning from geminiviruses. The EMBO Journal 19:792–799

Haible D, Kober S, Jeske H (2006) Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. Journal of virological methods 135:9–16

Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S (2013) Geminiviruses: masters at redirecting and reprogramming plant processes. Nature Reviews Microbiology 11:777–788

Hanson P, Green S, Kuo G (2006) *Ty*-2, a gene on chromosome 11 conditioning geminivírus resistance in tomato. Report of the Tomato Genetics Cooperative 56:17–18

Homs M, Kober S, Kepp G, Jeske H (2008) Mitochondrial plasmids of sugar beet amplified via rolling circle method detected during curtovirus screening. Virus Resarch 136:124–129

Horowitz AR, Ghanim M, Roditakis E, Nauen R, Ishaaya I (2020) Insecticide resistance and its management in *Bemisia tabaci* species. Journal of Pest Science 93:893–910

Host Data Base (2022) Disponível em: https://www.genome.jp/virushostdb/view/?VirusHostLineage=ssDNA|Viruses. Acessado em: 15 de junho de 2022.

Hu T, Song Y, Wang Y, Zhou X (2020) Functional analysis of a novel βV1 gene identified in a geminivírus betasatellite. Science China Life Sciences 63:688–696

Hurtado FD, Gil MA, Zubiaur YM, Aguilera JG, Xavier CAD, Zerbini Junior FM, Da Silva DJ H (2012) Sources of resistance in tomato to bipartite begomoviruses Tomato yellow spot virus and Tomato severe rugose virus. Horticultura Brasileira 30:639–644.

Hutton S, Scott J (2014) *Ty*-6, a major begomovirus resistance gene located on chromossome 10. Research Reports 64:14–16

Hutton SF, Scott JW, Schuster DJ (2012) Recessive resistance to Tomato yellow leaf curl virus from the tomato cultivar Tyking is located in the same region as *Ty*-5 on chromosome 4. HortScience 47:324–327

International Committee on Taxonomy of Viruses (ICTV). Disponível em: https://ictv.global/taxonomy. Acessado em: 09 de novembro de 2023

IGBE (2023) Instituto Brasileiro de Geografia e Estatística. Levantamento sistemático da Produção Agrícola: Estatítica da produção agrícola 151 p

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. Journal of Virological Methods 116:209–211

Inoue-Nagata AK, Fonseca MEN, Resende RO, Boiteux LS, Monte DC, Dusi AN, Avila AC, Van der Vlugt RAA (2002) *Pepper yellow mosaic virus*, a new potyvirus in sweetpepper, Capsicum annuum. Archives of Virology 147: 849–855.

Inoue-Nagata AK, Ávila AC, Bôas GLV (2009) Os geminivírus em sistema de produção integrada de tomate indústria. Circular Técnica 71, p. 1–11

Inoue-Nagata AK, Lopes C, Reis A, Pereira R, Quezado-Durval A, Pinheiro J, Lima M (2016) Doenças do Tomateiro. In: Amorim LBFA, Rezende JAM, Camargo LEA (Eds) Manual de Fitopatologia. Ceres. p. 697–729

Jeske, H (2018) Barcoding of plant viruses with circular single-stranded DNA based on rolling circle amplification. Viruses 10:1–22

Jones DR (2003) Plant viruses transmitted by whiteflies. European Journal of Plant Pathology 109:195–219

Ji Y, Schuster DJ, Scott JW (2007) *Ty–3*, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus *Ty–1* on chromosome 6 of tomato. Molecular Breeding 20:271–284

Ji Y, Scott JW (2006) *Ty–3*, a begomovirus resistance locus linked to *Ty–1* on chromosome 6 of tomato. Tomato Genetics Cooperative 56:22–25

Ji Y, Scott JW, Schuster DJ (2009a) Toward fine mapping of the tomato yellow leaf curl virus resistance gene *Ty–2* on chromosome 11 of tomato. HortScience 44:614–618

Ji Y, Scott JW, Schuster DJ, Maxwell DP (2009b) Molecular mapping of *Ty*-4, a new tomato yellow leaf curl virus resistance locus on chromosome 3 of tomato. Journal of the American Society for Horticultural Science 134:281–288

Jorge TS, Queiroz LN, Lima MF, Fonseca MEN, Fontes MG, Pereira-Carvalho RC, Kitajima EW, Aragão FJL, Boiteux LS (2023) Classical and biotechnological breeding of tomato, *Capsicum*, and lettuce for resistance to orthotospoviruses in Brazil. *In*: Silva HR; Almeida LC, Rios JA, Michereff SJ. (Org.). Manejo de doenças de plantas: Controle genético, químico e biológico, nas perspectivas acadêmica e empresarial. 1ª ed. Recife-PE: Universidade Federal Rural de Pernambuco, volume 1, p. 7–26.

Kitajima EW (2020). An annotated list of plant viruses and viroids described in Brazil (1926-2018). Biota Neotropica, 20:2–21

Kiss, ZA. Medina, V, Falk, BW (2013) Crinivirus replication and host interactions. Frontiers in Microbiology, 4:1–11

Kumar M, Zarreen F, Chakraborty S (2021) Roles of two distinct alphasatellites modulating geminivírus pathogenesis. Virology Journal 18:1–11

Kutnjak D, Rupar M, Gutierrez-Aguirre I, Curk T, Kreuze JF, Ravnikar M (2015) Deep sequencing of virus-derived small interfering RNAs and RNA from viral particles shows highly similar mutational landscapes of a plant virus population. Journal of Virology 89:4760–4769

Kutnjak D, Silvestre R, Cuellar W, Perez W, Müller G, Ravnikar M, Kreuze J (2014) Complete genome sequences of new divergent potato virus X isolates and discrimination between

strains in a mixed infection using small RNAs sequencing approach. Virus Research 191:45–50

Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y, Makhbash Z, Nahon S, Shlomo H, Chen L, Reuveni M, Levin, I (2015) A novel route controlling begomovirus resistance by the messenger RNA surveillance factor pelota. PLoS Genetics 11:1–19

Lefeuvre P, Moriones E (2015) Recombination as a motor of host switches and virus emergence: Geminiviruses as case studies. Current Opinion in Virology 10:14–19

Levy SE, Myers RM (2016) Advancements in next-generation sequencing. Annual Review of Genomics and Human Genetics 17:95–115

Li F, Xu X, Huang C, Gu Z, Cao L, Hu T, Zhou X (2015) The AC 5 protein encoded by Mungbean yellow mosaic India virus is a pathogenicity determinant that suppresses RNA silencing-based antiviral defenses. New Phytologist 208:555–569

Lima A, Silva JC, Silva FN, Castillo-Urquiza GP, Silva FF, Seah YM, Mizubuti ESG, Duffy S, Zerbini FM (2017) The diversification of begomovirus populations is predominantly driven by mutational dynamics. Virus Evolution 3:1–14

Liu H, Chang Z, Zhao S, Gong P, Zhang M, Lozano-Durán R, Yan H, Zhou X, Li, F (2023) Functional identification of a novel C7 protein of tomato yellow leaf curl virus. Virology 585: 117–126

Lopes CA, Ávila AC (2005) Doenças do Tomateiro, Embrapa Hortaliças. Brasília, p. 151

Lourenção AL, Siqueira WJ, Melo AM, Palazzo SR, Melo PC, Colariccio A (2005) Resistência de cultivares e linhagens de tomateiro a tomato chlorotic spot virus e a potato virus Y. Fitopatologia Brasileira 30:609–614

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

Macedo M, Albuquerque L, Maliano M, Souza J, Rojas M, Inoue-Nagata A, Gilbertson R (2018) Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. Archives of Virology 163:737–743

Macedo MA, Costa TM, Barbosa JC, Pereira JL, Michereff-Filho M, Gilbertson R L, Inoue-Nagata AK, Bergamin Filho A (2017) Temporal and spatial dynamics of begomovirus disease in tomatoes in central Brazil. Plant Pathology 66:529–538

Machado M, Fonseca MEN, Ferro DDX, Boiteux LS (2013). Disponível em: https://www.alice.cnptia.embrapa.br/handle/doc/1001734. Acessado em: 10 de outubro de 2021

Mahmood MA, Ahmed N, Hussain S, Muntaha ST, Amin I, Mansoor S (2022) Dominance of Asia II 1 species of *Bemisia tabaci* in Pakistan and beyond. Scientific Reports 12:1–13

Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B (2021) RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evolution 7:1–3

Martins TP, Souza TA, da Silva PS, Nakasu EY, Melo FL, Inoue-Nagata AK, Nagata T (2021) Nanopore sequencing of tomato mottle leaf distortion virus, a new bipartite begomovirus infecting tomato in Brazil. Archives of Virology 166:3217–3220

Martínez-Marrero N, Avalos-Calleros JA, Chiquito-Almanza E, Acosta-Gallegos JA, Ambriz-Granados S, Anaya-López JL, Argüello-Astorga GR (2020) A new begomovirus isolated from a potyvirus-infected bean plant causes asymptomatic infections in bean and *N. benthamiana*. Archives of Virology 165:1659–1665

Martínez RT, Almeida MM, Rodriguez R, Cayetano X, Oliveira AS, Silva JM, Silva JMF, Melo FL, Resende, RO (2019) Analyses of orthotospovirus populations and dispersion under different environmental conditions in Brazil and in the Dominican Republic. Tropical Plant Pathology 6:511–518

Matyis JC, Silva DM, Oliveira AAR, Costa AS (1975) Purificação e morfologia do vírus do mosaico dourado do tomateiro. Summa Phytopathologica 1:267–274

Maurino F, Dumón AD, Llauger G, Alemandri V, Haro LA, Mattio MF, Vas M, Laguna IG, Pecci MPG (2018) Complete genome sequence of maize yellow striate virus, a new cytorhabdovirus infecting maize and wheat crops in Argentina. Archives of Virology 163:291–295

Maxwell DP, martin C, salus MS, montes L, mejía L (2006) Tagging begomovirus resistance genes. Disponível em: www.plantpath.wisc.edu/. Acessado em: 10 de outubro de 2021

Melgarejo TA, Rojas MR, Gilbertson RL (2019). A bipartite begomovirus infecting *Boerhavia erecta* (family Nyctaginaceae) in the Dominican Republic represents a distinct phylogenetic lineage and has a high degree of host specificity. Phytopathology 109:1464–1474

Melomey LD, Danquah A, Offei SK, Ofori K, Danquah E, Osei M (2019) Review on tomato (*Solanum lycorpersicum*) improvement programmes in Ghana. In: Nyaku ST e Danquah A (Eds.) Recent advances in tomato breeding and production, IntechOpen. London. pp. 50-69

Mehetre GT, Leo VV, Singh G, Sorokan A, Maksimov I, Yadav MK, Upadhyaya K, Hashem A, Alsaleh, AN, Dawoud TM, Almaary KS, Singh BP (2021) Current Developments and Challenges in Plant Viral Diagnostics: A Systematic Review. Viruses 13:1–31

Mituti T, Moura MF, Macedo MA, Silva TN, Pinto LR, Costa H, Krause-Sakate R, Inoue-Nagata A, Nunes GG, Lima MF, Rezende JA (2019) Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil. Tropical Plant Pathology 44:468–472

Muhire BM, Varsani A, Martin DP (2014) SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. Plos One 9:1–8

Mumo NN, Mamati GE, Ateka EM, Rimberia FK, Asudi GO, Boykin LM, Machuka EM, Njuguna N, Pelle R, Stomeo F (2020) Metagenomic analysis of plant viruses associated with papaya ringspot disease in *Carica papaya* L. in Kenya. Frontiers in Microbiology 11:1–16

Nagata T, Almeida ACL, Resende RO, de Avila AC (2004) The competence of four thrips species to transmit and replicate four tospoviruses. Plant Pathology 53:136–140

Naika S, Jeude JVL, Goffau M, Hilmi M, Dam BV (2006) A cultura do tomate: Produção, processamento e comercialização. Wageningen Agrodok 17

Naito FY, Melo FL, Fonseca MEN, Santos CA, Chanes CR, Ribeiro BM, Gilbertson RL, Boiteux LS, Pereira-Carvalho RC (2019). Nanopore sequencing of a novel bipartite New World begomovirus infecting cowpea. Archives of Virology 164: 1907–1910

Navas-Castillo J, Fiallo-Olivé E (2020) Geminiviruses (*Geminiviridae*). Encyclopedia of Virology 3:411–419

Naveed H, Islam W, Jafir M, Andoh V, Chen L, Chen K (2023) A review of interactions between plants and whitefly-Transmitted Begomoviruses. Plants 12:2–20

Nogueira I, Vieira BG, Pereira-Carvalho RC, Dianese EC, Resende RO, Boiteux LS, Fonseca MEN (2011) Detecção de Tomato chlorosis virus (Crinivirus, Closteroviridae) em tomateiro no Distrito Federal. Disponível em: https://www.alice.cnptia.embrapa.br/bitstream/doc/911057/3/1556.pdf. Acessado em: 14 setembro de 2021

Nigam D, Garcia-Ruiz, H (2020) Variation profile of the orthotospovirus genome. Pathogens, 9: 1 – 26

Oliveira AS, Boiteux LS, Kormelink R, Resende RO (2018) The *Sw*-5 gene cluster: Tomato breeding and research toward orthotospovirus disease control. Frontiers in Plant Science 9:1–7

Oliveira IA, Reis LNA, Nery FMB, Fernandes NAN, Goncalves VPMR, Rojas MR, Gilbertson RL, Reis A, Boiteux LS, Fonseca MEN, Pereira-Carvalho RC (2024) Genomic characterization of a highly divergent strain of tomato chlorotic mottle Guyane virus in the Brazilian Amazon River Basin. Phytoparasitica 52: online, 2024.

Oliveira RM, Dianese EC, Lima MF, Resende RO, Inoue-Nagata AK, Boiteux LS (2018) Sources of resistance to potato virus Y and pepper yellow mosaic virus in *Solanum* (section *Lycopersicon*) germplasm. European Journal of Plant Pathology 3:691–699

Ong SN, Taheri S, Othman RY, Teo CH (2020) Viral disease of tomato crops (*Solanum lycopesicum* L.): An overview. Journal of Plant Disease and Protection 127:725–739

Pakkianathan BC, Kontsedalov S, Lebedev G, Mahadav A, Zeidan M, Czosnek H, Ghanim M (2015) Replication of Tomato yellow leaf curl virus in its whitefly vector, *Bemisia tabaci*. Journal of Virology 89:9791–9803

Pan LL, Chi Y, Liu C, Fan Y-Y, Liu S-S (2020) Mutations in the coat protein of a begomovirus result in altered transmission by different species of whitefly vectors. Virus Evolution 6:1–12

Pereira-Carvalho R, Tobar L, Dianese E, Fonseca M, Boiteux LS (2014) Melhoramento genético do tomateiro para resistência a doenças de etiologia viral: Avanços e perspectivas. Revisão Anual de Patologia de Plantas 22:280–361

Pereira-Carvalho, RC, Díaz-Pendón, JA, Fonseca, MEN, Boiteux, LS, Fernández-Muñoz, R, Moriones, E, Resende, RO (2015). Recessive resistance derived from tomato cv. Tyking-limits drastically the spread of tomato yellow leaf curl virus.Viruses 7:2518–2533

Pereira-Silva J, Boiteux LS, Fonseca MEN, Reis LNA, Souza AS, Nery FMB, Madeira NR, Pereira-Carvalho RC (2022) Novel natural hosts of tomato severe rugose virus (ToSRV) in the Fabaceae, Solanaceae, and Oxalidaceae families. Journal of Plant Diseases and Protection 129:425–431

Pradhan B, Van Tien V, Dey N, Mukherjee SK (2017) Molecular biology of geminivirus DNA Replication. Avid Science 1:1–33

Prabhandakavi P, Pogiri R, Kumar R, Acharya S, Esakky R, Chakraborty M, Pinnamaneni R, Palicherla SR (2021) Pyramiding *Ty*-1/*Ty–3*, *Ty*-2, *ty*-5 and *ty*-6 genes into tomato hybrid to develop resistance against tomato leaf curl viruses and recurrent parent genome recovery by ddRAD sequencing method. Journal of Plant Biochemistry and Biotechnology 30:462–476

Quadros AF, Silva JP, Xavier CA, Zerbini FM, Boari AJ (2019) Two new begomoviruses infecting tomato and *Hibiscus* sp. in the Amazon region of Brazil. Archives of Virology 164: 1897–1901

Reis LNA, Metagenomic analysis of the begomovirus diversity in tomatoes in Central Brazil and impact of the *Ty*–1 tolerance gene on viral evolutionary dynamics (Tese de doutorado), Universidade de Brasília, 2020

Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RC (2021) Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome–wide pairwise identity of multiple isolates. Virus Genes 57:127–131

Reis LNA, Boiteux LS, Fonseca MEN, Rojas MR, Gilbertson RL, Pereira-Carvalho RC (2023) Tomato golden net virus and tomato yellow net virus: Two novel New World begomoviruses with monopartite genomes. Archives of Virology 168:1-6

Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RC (2020) Metagenomics of Neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty*–1 gene. Viruses 8:1–22

Rêgo-Machado CM, Nakasu EY, Blawid R, Nagata T, Inoue-Nagata AK (2019) Complete genome sequence of a new bipartite begomovirus infecting tomato in Brazil. Archives of Virology 164:2873–2875

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 begomoviruses in Brazil. Archives of Virology 148:281–295

Ribeiro SG, Martin DP, Lacorte C, Simões IC, Orlandini DRS, Inoue-Nagata AK (2007) Molecular and biological characterization of tomato chlorotic mottle virus suggests that recombination underlies the evolution and diversity of Brazilian tomato begomoviruses. Phytopathology 97:702–711

Ribeiro SG, Melo LV, Boiteux LS, Kitajima EW, Faria JC (1994) Tomato infection by a geminivírus in the Federal District, Brazil. Fitopatologia Brasileira 19:330

Rodríguez-Negrete EA, Carrillo-Tripp J, Rivera-Bustamante RF (2009) RNA silencing against geminivirus: complementary action of posttranscriptional gene silencing and transcriptional gene silencing in host recovery. Journal of Virology 83:1332–40

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

Rojas MR, Macedo M, Maliano M, Soto-Aguilar M, Souza J, Briddon R, Kenyon L, Rivera Bustamante R, Zerbini F, Adkins S (2018) World management of geminivíruses. Annual Review of Phytopathology 56:637–677

Ronquist F, Huelsenbeck JP (2015) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Rosen R, Kanakala S, Kliot A, Pakkianathan BC, Farich BA, Santana-Magal N, Elimelech M, Kontsedalov S, Lebedev G, Cilia M (2015) Persistent, circulative transmission of begomoviruses by whitefly vectors. Current Opinion in Virology 15:1–8

Roossinck MJ (1997) Mechanisms of plant virus evolution. Annual Review of Phytopathology, 35:191–209

Sánchez-Campos S, Rodríguez-Negrete EA, Cruzado L, Grande-Pérez A, Bejarano ER, Navas-Castillo J, Moriones E (2016) Tomato yellow leaf curl virus: No evidence for replication in the insect vector Bemisia tabaci. Scientific Reports 6:1–6

Schubert J, Habekuss A, Kazmaier K, Jeske H (2007) Surveying cereal-infecting geminiviruses in Germany—Diagnostics and direct sequencing using rolling circle amplification. Virus Research 127:61–70

Scott JW, Hutton SF, Freeman JH (2015) Fla. 8638B and Fla. 8624 tomato breeding lines with begomovirus resistance genes *ty*-5 plus *Ty*-6 and *Ty*-6, respectively. HortScience 50:1405–1407

Seal S, VandenBosch F, Jeger M (2006) Factors influencing begomovirus evolution and their increasing global significance: Implications for sustainable control. Critical Reviews in Plant Sciences 25:23–46

Shen X, Yan Z, Wang X, Wang Y, Arens M, Du Y, Visser RGF, Kormelink R, Bai Y, Wolters, AMA (2020) The NLR protein encoded by the resistance gene *Ty*–2 is triggered by the replication-associated protein Rep/C1 of tomato yellow leaf curl virus. Frontiers in Plant Science 11:1–17

Shi H, Mao X, Chen X, Wang Z, Wang K, Zhu X (2017) The analysis of proteins and small molecules based on sterically tunable nucleic acid hyperbranched rolling circle amplification. Biosensors and Bioelectronics 91:136–142

Silva FN, Lima AT, Rocha CS, Castillo-Urquiza GP, Alves-Júnior, M, Zerbini FM (2014) Recombination and pseudorecombination driving the evolution of the begomoviruses tomato severe rugose virus (ToSRV) and tomato rugose mosaic virus (ToRMV): Two recombinant DNA–A components sharing the same DNA–B . Virology Journal 11:1–66

Silva LD, Omoto C, Bleicher E, Dourado P (2009) Monitoring the susceptibility to insecticides in *Bemisia tabaci* (Gennadius) (*Hemiptera: Aleyrodidae*) populations from Brazil. Neotropical Entomology 38:116–125

Souza TA, Silva JMF, Nagata T, Martins TP, Nakasu EYT, Inoue-Nagata AK (2020) A temporal diversity analysis of Brazilian begomoviruses in tomato reveals a decrease in species richness between 2003 and 2016. Frontiers in Plant Science 11:1–13

Souza JO, Melgarejo TA, Vu S, Nakasu YET, Chen LF, Rojas MR, Zerbini FM, Inoue-Nagata AK, Gilbertson RL (2022) How to be a successful monopartite begomovirus in a bipartite-dominated world: Emergence and spread of tomato mottle leaf curl virus in Brazil. Jounal of Virology 96: 1–23

Tahzima R, Foucart Y, Massart S, De Jonghe K (2019) First report of Prunus virus F infecting sweet cherry cultivars using high-throughput sequencing in Belgium. New Disease Reports 40:1–7

Torre C, Agüero J, Aranda MA (2019) First evidence of tomato yellow leaf curl virus-Israel IS76 recombinant isolates associated with severe yellow leaf curl epidemics in resistant tomatoes in Spain. Plant Disease 103:780

Tzanetakis IE, Martin RR, Wintermantel WM (2013) Epidemiology of criniviruses: An emerging problem in world agriculture. Frontiers in Microbiology 4:1–15

Untiveros M., Olspert A., Artola K, Firth A E, Kreuze JF, Valkonen JP (2016) A novel sweet potato potyvirus open reading frame (ORF) is expressed via polymerase slippage and suppresses RNA silencing. Molecular Plant Pathology, 17: 1111–1123.

Vaghi Medina CG, Martin DP, López Lambertini PM (2015) Tomato mottle wrinkle virus, um begomovírus recombinante que infecta o tomate na Argentina. Archives of Virology 160:581–585

Vaghi Medina CG, Teppa E, Bornancini VA, Flores CR, Marino-Buslje C, López Lambertini PM (2018) Tomato apical leaf curl virus: A novel, monopartite geminivírus detected in tomatoes in Argentina. Frontiers in Microbiology 8:1–11

Vargas-Asencio JA, Hernández E, Barboza N, Hammond R, Mora F, Ramírez P (2013) Detection of tomato chlorosis virus and its vector *Trialeurodes vaporariorum* in greenhouse-grown tomato and sweet pepper in the Cartago province, Costa Rica. Journal of Plant Pathology 3:627–630

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG, Scott JW, Edwards JD, Bai Y (2013) The tomato yellow leaf curl virus resistance genes *Ty–1* and *Ty–3* are allelic and code for DFDGD-class RNA–dependent RNA polymerases. PLoS Genetics 9:1–11

Villamor DEV, Ho T, Al Rwahnih M, Martin RR, Tzanetakis IE (2019) High throughput sequencing for plant virus detection and discovery. Phytopathology 109:716–725

Vu TV, Choudhury NR, Mukherjee SK (2013) Transgenic tomato plants expressing artificial microRNAs for silencing the pre-coat and coat proteins of a begomovirus, Tomato leaf curl New Delhi virus, show tolerance to virus infection. Virus Research 172:35–45

Wang Y, Jiang J, Zhao L, Zhou R, Yu W, Zhao T (2018) Application of whole genome resequencing in mapping of a tomato yellow leaf curl virus resistance gene. Scientific Reports 8:1–11

Wang Y, Zhang X, Hu T, Zhou X (2021) Identification of two distinct begomoviruses infecting Malvastrum coromandelianum. Phytopathology Research 8:1–6

Wang Z, Wang Y, Lozano-Duran R, Hu T, Zhou X (2022) Identification of a novel C6 protein encoded by tomato leaf curl China virus. Phytopathology Research 46: 2–9

Watanabe LFM, Bello VH, De Marchi BR, Da Silva FB, Fusco LM, Sartori MM, Pavan MA, Krause-Sakate R (2019) Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on different host plants. Crop Protection 124:1–6

Yadava P, Suyal G, Mukherjee SK (2010) Begomovirus DNA replication and pathogenicity. Current Science 98:360–368

Yamaguchi H, Ohnishi J, Saito A, Ohyama A, Nunome T, Miyatake K, Fukuoka H (2018) An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the *Ty–2* Begomovirus resistance locus of tomato. Theoretical and Applied Genetics 131:1345–1362

Yao FL, Zheng Y, Huang XY, Ding XL, Zhao JW, Desneux N, He YX, Weng QY (2017) Dynamics of *Bemisia tabaci* biotypes and insecticide resistance in Fujian province in China during 2005–2014. Scientific Reports 7:1–12

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, Van-Oss H. 1994. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, *Ty*-1. Theoretical and Applied Genetics 88: 141–146

Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J, Rivera-Bustamante R, Roumagnac P, Varsani A, Consortium, IR (2017) ICTV virus taxonomy profile: Geminiviridae. The Journal of General Virology 98:131–133.

Zhang K, Zhang H, Cao H, Jiang Y, Mao K, Yang Z (2021) Rolling Circle Amplification as an Efficient Analytical Tool for Rapid Detection of Contaminants in Aqueous Environments Biosensors 11:1–30

Zhang J, Ma M, Liu Y, Ismayil A (2023) Plant defense and viral counter-defense during plant–geminivirus interactions. Viruses 15:1–15

Zhang Z, Zheng, K, Zhao, L, Su, X., Zheng, X., Wang, T (2021) Occurrence, distribution, evolutionary relationships, epidemiology, and management of orthotospoviruses in China. Frontiers in Microbiology 12:1 –12

Zhou X (2013) Advances in understanding begomovirus satellites. Annual Review of Phytopathology 51:357–381

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion (Ph.D Thesis) The University of Texas at Austin.

CAPÍTULO 02

Metagenomic analysis of viral species from the *Geminiviridae* family and satellite DNAs associated with the tomato crop across different Brazilian biomes

Metagenomic analysis of viral species from the *Geminiviridae* family and satellite DNAs associated with the tomato crop across different Brazilian biomes

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Work to be submitted to Viruses

Abstract

Metagenomic analyzes of tomato (*Solanum lycopersicum* L.) using HTS (High-Throughput Sequencing) have provided an increasing discovery power of novel viruses, including members of the genus *Begomovirus* (family *Geminiviridae*). Diseases caused by begomoviruses represent an enormous challenge for management via genetic improvement due to the diversity of this group of pathogens. Herein, HTS was carried out using the Illumina NovaSeq-6000 platform using pools with 154 tomato samples (with and without the resistance/tolerance factors *Ty*–1 and *Ty*–3). Two pools were formed, one pool of 73 samples (eight with resistance/tolerance factors and 65 without resistance/tolerance factors) coming from the North (13), Northeast (36) and South (24) regions and a second pool composed of by 81 samples (56 with resistance/tolerance factors and 25 without resistance/tolerance factors) collected in the Southeast (39) and Central-West (42) regions. The results indicated in the pool from the North, Northeast and South regions a total of 17 viral species, 16 of which belonging to the genus *Begomovirus* and one belonging to the genus *Topilevirus*. Considering the current criteria for demarcating species of the genus *Begomovirus*, four contigs showed nucleotide identity of the DNA–A segment (complete sequence) below 91% and they should represent new species for this genus. **New species #1** was reported in the Northeast region of Brazil, in the states of Ceará (CE–001) and Pernambuco (PE–011 and PE–012). Two new species (New species #2 and new species #4) were collected in Paraná state, southern Brazil. The New species #2 was detected in the samples PR–173 and PR–174, the new species #4 was detected in a single sample (PR–144), and in the North, in the state of Tocantins, in sample TO–167, the new species #3 was detected. In the pool of samples collected from the Southeast and Central-West regions, sequencing analyzes allowed the assembly of a total of 14 viruses. A probable new species of the genus Begomovirus was also detected, as well as the sequences of two species of alphasatellites associated with begomoviruses: Alphasatellitidae sp. and Euphorbia yellow mosaic alphasatellite. The New species #5 was detected in eight samples, three samples from the Federal District (DF–209, DF–216, and DF–235) and five from Goiás (GO–121, GO–124, GO–126, GO–127, and GO–218). In PCR assays with species-specific primers, tomato mottle leaf curl virus (ToMoLCV) was the predominant pathogen, being present in all five Brazilian regions and detected in 70 samples. Tomato severe rugose virus (ToSRV) predominated in the South, Southeast and Midwest, being present in 68 samples. The number of viruses and subviral agents detected in samples without the *Ty*–1 and *Ty*–3 factors (16 viruses plus one alphasatellite) was higher than the number detected in samples with resistance/tolerance factors (nine viruses). The number of infected samples and the number of mixed infections were higher

in samples without the *Ty*-1/*Ty*–3 factors, indicating that these genes can impact the diversity of begomoviruses. In addition, all five novel begomoviruses were detected in plants without both resistance/tolerance factors. The present work confirms that the diversity of begomoviruses and their infection capacity are affected by the *Ty*–1 /*Ty*–3 factors present in tomato cultivars.

Keywords: *Geminiviridae*, *Solanum lycopersicum*, high-throughput sequencing, tolerance.

1. Introduction

Begomoviruses (family *Geminiviridae*) have a single-stranded DNA genome in twinned particles. Begomoviruses comprises monopartite species (with a single segment of DNA–A) or bipartite (with two segments named as DNA–A and DNA–B). The DNA–A component comprises six ORFs. One of them in the viral sense (AV1) and five in the complementary sense (AC1 to AC5). The AV1 gene codes for the coat protein (CP). The AV2 gene is responsible for encoding the movement protein (MP), and is present only in Old World begomoviruses (Zerbini et al. 2017). The AC1 gene codes for a protein involved in viral replication (Rep), while the AC2 gene is responsible for coding the transcription activating protein (TrAp). The AC3 gene encodes, in turn, a protein that enhances viral replication (Ren) (Sunter et al. 1990) and the AC4 gene encodes a protein associated with the expression of symptoms (AC4) (Hanley-Bowdoin et al. 2013). The AC5 gene codes for a protein related to pathogenicity by suppressing post-transcriptional gene silencing (Li et al. 2015; Li et al. 2021). The AC5 sequence is distinct for some groups of begomoviruses. Old World and New World begomoviruses have a conserved domain in AC5 (Gemini AC5-1). However, for some Old World begomoviruses an additional domain (named Gemini AC5-2) has been observed and the presence/absence of this domain has allowed discriminating members of these two subgroups (Li et al. 2015). Recently, three new ORFs were related in begomovirus species. In viral sense, V3 located downstream of V2 was reported by Wang et al (2021) in Malvastrum yellow vein Honghe virus (MaYVHhV). V3 is, responsible for code a 7.4 kDa protein without function described. Wang et al. (2022) also identified a new C6 protein expressed by tomato leaf curl China virus (ToLCCNV) in assays with *N. benthamiana*. The C6 ORF is partially overlapping the CP and V2 ORFs. In according to these authors, the new C6 protein plays a role in targeting the mitochondria of the infected plant, where the protein is expressed in this organelle, however without contributing to the virulence of ToLCCNV in *N. benthaminana*. Liu et al (2023) described C7 protein, encoded by a C7 ORF, in the complementary sense of the tomato yellow leaf curl virus (ToYLCV). The C7 protein interacts with ORF V2 and C2 proteins, in addition, it inhibits RNA silencing and when mutated, ToYLCV infections were alleviated, therefore C7 acts as a pathogenicity factor (Liu et al. 2023). In the DNA–B component, there are two ORFs, one in the viral sense (BV1) and the other in the complementary sense (BC1). The BV1 gene is responsible for encoding the NSP protein (nuclear shuttle protein) involved in transporting the virus from the nucleus to the cytoplasm and vice versa (Noueiry et al. 1994) while the BC1 gene encodes the MP protein (movement protein) responsible by viral movement via plasmodesmata (Noueiry et al. 1994).

Bipartite genomes share a common region (CR) of approximately 200 nucleotides with conserved motifs related to virus replication (Arguello-Astorga & Ruiz-Medrano 2001, Fontes et al. 1994). Within the CR is located a conserved nonanucleotide sequence (TAATATTAC) that corresponds to the site of origin of viral replication and iterons responsible for Rep binding (Fontes et al. 1994; Bridon et al. 2010). Studies characterizing begomoviruses have demonstrated the presence of conserved regions in these viruses and the importance of these regions for viral genomic characterization. The Rep of begomoviruses has specificity for sequences associated with motifs in its genomic DNA, called iterons, which play an essential role in the virus replication process (Arguello-Astorga & Ruiz-Medrano 2001). Based on analyzes of domains related to begomovirus iterons, it is possible to identify Rep subdomains that are invariable among viruses that have identical iterons, allowing viruses with cognate iterons to be grouped. Another important domain for begomoviruses was characterized by Arguello-Astorga et al. (2004). These authors identified a conserved domain in the viral replication protein (Rep) that is essential for modulating host gene expression by interacting with the plant retinoblastoma protein (pRBR). Mutations in this domain reduce the efficiency of binding between Rep and pRBR. Subsequently, a new conserved element was located between the origin of replication and the start codon of the begomovirus coat protein (CP) gene (Cantú-Iris et al. 2019). Analysis of the promoter sequence homologous to the CP of New World begomoviruses revealed a nearly palindromic DNA sequence with a conserved core (ACTT-N7-AAGT) that was distinct from Old World begomoviruses (Cantú-Iris et al. 2019). These regions have three characteristics: **(1)** the presence of a central ACTT-N7-AAGT sequence; **(2)** the presence of a heptanucleotide sequence (N7) rich in GC and highly variable in sequence and **(3)** they present an invariable association with the TATA-box, calling it TATA-Associated Composite Element (TACE). Some begomoviruses also present associations with DNA satellites that can attenuate or increase the severity of symptoms depending on the relationship between the satellite and helper virus (Hu et al. 2020; Ferro et al. 2021; Kumar et al. 2021). Transmission of begomoviruses is carried out under natural conditions by a complex of cryptic species (Rosen et al 2015; De Barro et al. 2011) of the insect vector *Bemisia tabaci* (family Aleyrodidae, order Hemiptera) Middle East Asian Minor–1 (MEAM 1) Transmission of the begomoviruses by whiteflies displays a predominant a non-propagative circulative relationship. However, transmission studies with tomato yellow leaf curl virus (ToYLCV) revealed that, in some specific situations, this virus can replicate in the insect (Pakkianathan et al. 2015). Begomoviruses have a wide range of host species among cultivated, non-cultivated and ornamental plants (Rojas et al. 2018). Currently, the genus *Begomovirus* has the largest

number of species within the *Geminiviridae* family, with 445 already accepted by the ICTV (2024). To be considered a new species of the genus, the complete sequence of the DNA–A segment must present a nucleotide identity of less than 91% in relation to other isolates of species of the genus (Brown et al. 2015).

The first reports of begomovirus infections in Brazil in tomato occurred between the 1960s and 1970s, including the characterization of the first Neotropical begomovirus – tomato golden mosaic virus (TGMV) (Costa et al. 1975; Matyis et al. 1975). During this period, begomoviruses occurred sporadically with no economic importance. However, this scenario was modified after the entry of the whitefly *B. tabaci* MEAM 1 (former biotype B) in the 1990s, resulting in an explosion of regional outbreaks and a significant increase in the incidence of begomoviruses in several Brazilian states (Watanabe et al. 2019; Fernandes et al. 2022). In 2003, the occurrence of tomato chlorotic mottle virus (ToCMoV) was reported in the states of Pernambuco (PE), Bahia (BA), Rio de Janeiro (RJ) and Minas Gerais (MG) and tomato rugose mosaic virus (ToRMV) in MG (Ribeiro et al. 2003). Calegario et al. (2004) characterize an isolate of Sida micrantha mosaic virus (SimMV) collected from tomato plants in MG. In 2007, isolates of tomato yellow spot virus (ToYSV) collected from tomato plants in MG were characterized (Calegario et al. 2007). In the same year Colariccio et al. (2007) reported the occurrence of tomato yellow vein streak virus (ToYVSV) and ToRMV in the state of São Paulo (SP). Cotrim et al. (2007) detected Sida mottle virus (SiMoV) infecting tomato plants in the Center-West of São Paulo. Castillo-Urquiza et al. (2008) reported three new begomoviruses associated with tomato in southeastern Brazil, they were: tomato mild mosaic virus (ToMlMV), tomato common mosaic virus (ToCmMV) and tomato leaf distortion virus (ToLDV). Isolates of a begomovirus called tomato golden vein virus (TGVV) were reported in 2004-2005, being a species with a close genetic relationship with ToYVSV (Reis et al. 2021). Fernandes et al. (2008) detected tomato severe rugose virus (ToSRV), TGVV, tomato mottle leaf curl virus (ToMoLCV) and TYVSV infecting tomato plants in Central Brazil. Albuquerque et al. (2010) carried out the complete characterization of ToYVSV, which had been partially described by Faria et al. (1997). Albuquerque et al. (2012) made the first report of tomato interveinal chlorosis virus – (ToICV) in tomato samples collected in PE. Barreto et al. (2013) confirmed experimental infection of tomato plants by Euphorbia yellow mosaic virus (EuYMV), with natural infection later confirmed by Duarte et al. (2021b). Fonseca et al. (2013) make available the complete DNA–A sequence of tomato bright yellow mosaic virus (ToBYMV) detected infecting tomato plants in BA. In 2015, the complete sequences of the DNA–A segment of four new begomoviruses associated with tomato were described: chino del lado Amazonas virus

(CdTAV), tomato golden leaf distortion virus (ToGLDV), tomato golden leaf spot virus (ToGLSV) and tomato bright yellow mottle virus (ToBYMoV). The first record of Sida yellow net virus (SiYNV) isolates infecting tomato plants was made by Fernandes (2015). Fonseca et al. (2016) made available the complete DNA–A sequence of tomato rugose yellow leaf curl virus (ToRYLCV) associated with tomato in Rio Grande do Sul (RS). Macedo et al. (2018) reported a new monopartite begomovirus called tomato leaf curl purple vein virus (ToLCPVV) infecting tomato plants in Piauí (PI). Pereira-Carvalho et al. (2019) made available the complete DNA–A sequence of tomato yellow leaf deformation dwarf virus (ToYLDDV) associated with tomatoes in Tocantins (TO). Quadros et al. (2019) detected the new begomovirus tomato chlorotic leaf curl virus (ToCLCV) infecting tomato plants in the Amazon region, in the same year Rêgo-Machado et al. (2019) detected tomato interveinal chlorosis virus-2 (ToICV2) in tomato plants in the state of Goiás (GO). Tomato mottle leaf distortion virus (ToMoLDiV) was detected in association with tomato cultivation in the State of Goiás by Martins et al. (2021). In 2021 Sida common mosaic virus (SiCMV) was detected infecting tomato plants in the state of RJ (Duarte et al. 2021a). More recently two new monopartite begomoviruses were detected in tomato plants, tomato golden net virus (ToGNV) and tomato yellow net virus (ToYNV) (Reis et al. 2023). Given this history, to date, 30 species of begomoviruses are associated with tomato cultivation. Among these viruses, ToSRV is the most widespread begomovirus (Duarte et al. 2021a).

Due to the great diversity of begomviruses species and the difficult of control, the use of cultivars that carry resistance factors is the most efficient strategy and should be used when available (Boiteux et al. 2012; Rojas et al. 2018). Eight genes conferring tolerance have been mapped in the tomato genome. The genes/allelic variants *Ty*–1 and *Ty*–3 (located on chromosome 6) stand out for their wide use in tomato breeding programs worldwide (Zamir et al. 1994; Ji et al. 2007; Verlaan et al. 2013; Pereira-Carvalho et al. 2015). However, neither of the two alleles confers immunity to viral infections, but rather a tolerance-type response (sensu Cooper and Jones 1983). The *Ty*–1 gene encodes an RDRy-type RNA polymerase (Verlaan et al. 2013), which mechanism of action involves transcriptional silencing via methylation of the genomic DNA of begomoviruses (Butterbach et al. 2014).

The inherent non-targeted approach of HTS (High-Throughput Sequencing) technology and advances in bioinformatics have significantly and on a large scale allowed the rapid discovery of new viruses and new hosts, enabling the characterization of diverse viromes by different methods and protocols detection (Adams et al. 2009; Prabhap et al. 2013; Maclot et al. 2020) and allowed to monitoring the virus diversity in Brazil (Reis et al. 2020). In addition

to understanding ecology and diversity, HTS technology associated with metagenomic analyzes also provides analytical tools for the epidemiological study of plant viruses (Villamor et al. 2019). Similarly, metagenomic analyzes in tomato have led to the discovery of new viruses from the *Genomoviridae* family in association with tomato cultivation (Rêgo-Machado et al. 2019; Reis et al. 2020; Souza et al. 2020) as well as studying the dynamics temporal and spatial begomovirus (Reis et al. 2020; Souza et al. 2020).

Recently, the impact of the tolerance factor *Ty*–1 on the diversity and dynamics of begomoviruses in tomato crops was evaluated using HTS (Reis et al. 2020; Souza et al. 2020). A filtering and viral selection effect was observed in tomato samples containing the *Ty*–1 gene collected in Central Brazil (Reis et al. 2020) and the Federal District (Souza et al. 2020).

In the present work, a wide metagenomic prospecting of members of the *Geminiviridae* family and satellite DNA was conducted, encompassing samples from the main tomato producing centers, located in different biomes and in all five Brazilian macro-regions. From the point of view of tomato genetic improvement, the present work represents a more representative sampling of the state-of-the-art diversity of these viral species and investigates the potential impact of two resistance factors (*Ty*–1 and *Ty*–3) on population dynamics of members of the *Geminiviridae* family.

2. Material and Methods

2.1. Leaf samples from symptomatic tomato plants.

In total, 154 tomato leaf samples exhibiting typical begomovirus symptoms (mosaic dwarf, leaf deformations and mottle) (**Figure 1**) were selected from collections in different producing areas of the five Brazilian regions: North (13), Northeast (36), Midwest (42), Southeast (39) and South (24) (**Table 1**). These samples were selected from the begomovirus collection at the Embrapa Hortaliças in according to representativeness by location and year of collection. The samples were collected in different regions of the country from 2001 to 2017 and are part of a collection of 1400 isolates from Embrapa Hortaliças and are stored at –80º C and –20º C.

2.2. DNA extraction, confirmation of the presence of *Ty*–1 and *Ty*–3 resistance genes.

A part of the samples was stored in a freezer – 20º C with the total DNA extracted using a modified protocol 2X CTAB plus organic solvents as described by Boiteux et al. (1999). Quantification of the DNA of the samples was carried out using nanodrop and the integrity of the molecule visualized on a 1% agarose gel Total DNA from leaf samples was used as a template in PCR reactions using specific primers for genomic regions linked to the *Ty*–1

(Maxwell et al. 2006) and *Ty*–3 resistance genes/loci. Ji et al. 2007). The PCR products were analyzed on a 1% agarose gel, stained in ethidium bromide and visualized under UV light. The samples were selected and grouped using year and collection location criteria.

2.3. Enrichment of circular DNAs by Rolling Circle Amplification – RCA and confirmation of begomovirus infection.

Part of the DNA obtained from each sample was used as a template for RCA (Rolling Circle Amplification) (Inoue-Nagata et al. 2004). Confirmation of begomovirus infection in the samples was performed using the primers PAL1v1978/PAR1c496 for the DNA–A component and PBL1v2040-PRCc1 for the DNA–B component (Rojas et al. 1993).

Table 1. Information on geographical regions, absence and/or presence of molecular markers associated with the resistance factors *Ty*–1 and *Ty*–3, year of collection and code of the 154 leaf samples of tomato cultivars (*Solanum lycopersicum*) used in the present study. Samples were collected across all five Brazilian regions and all of them displayed variable levels of begomovirus-like symptoms.

Pools	Regions	*Ty*–1	*Ty*–3	Collecting year	Isolate code
BP1	North	Absent	Absent	2005	TO–026
				2007	AM–010 and AM–012
				2008	TO–083; TO–167; TO–045; TO–046; TO–094 and TO–095
				2013	RR–003 and RR–004
				2016	AM–035 and AM–037
	North East	Present	Absent	2010	BA–063; CE–046; CE–048 and CE–049
				2011	BA–124
				2014	PE–122
				2016	PE–123
		Absent	Absent	2005	CE–001; CE–011; CE–012; PE–027 and PE–028
				2007	BA–034 and BA–035
				2009	BA–050; PE–011 and PE–012
				2011	BA–100; BA–128; BA–134; BA–143; CE–052; CE–053; CE–057 and CE–058
				2012	PE–099; PE–100; PE–104 and PE–105
				2014	BA–173; BA–174 and PE–121
				2016	CE–072; CE–073; PB–025 and PB–027
	South		Present	2006	PR–112
		Absent	Absent	2005	RS–033
				2006	PR–111; SC–001; SC–002 and SC–015
				2008	PR–079; SC–030 and SC–032
				2009	RS–012; RS–013; RS–014 e RS–015
				2010	SC–034
				2011	RS–040; RS–045; SC–044 and SC–051
				2013	RS–071
				2015	PR–143 and PR–144
				2016	RS–095
				2017	PR–173 and PR–174
BP2	Southeast	Present	Absent	2001	SP–066
				2007	SP–018
				2010	MG–268
				2011	MG–291
				2014	SP–156

			2015	SP–172
			2016	SP–240; SP–252 and SP–265
		Present	2016	SP–260
	Absent	Absent	2001	MG–046
			2002	MG–013 and MG–014
			2003	SP–003 and SP–004
			2006	SP–006 and SP-008
			2007	SP–017
			2008	SP–056 and SP–058
			2010	MG–084; MG–108; MG–109 and MG–267
			2011	MG–292; SP–111 and SP–124
			2013	SP–213
			2014	SP–205; SP–206 and SP–154
			2015	MG–381; SP–173 and SP–201
			2016	SP–230; SP–239; SP–254; SP–259 and SP–274
Midwest	Present	Absent	2002	GO–005
			2003	GO–124
	Absent	Present	2003	GO–229
			2005	DF–155 and DF–216
			2006	GO–342
			2007	DF–235
	Present	Present	2010	DF–338
			2011	GO–499
		Absent	2012	GO–526
			2013	DF–530; DF–546 and DF–541
	Absent	Present	2013	DF–528
	Present	Absent	2014	GO–588
	Absent	Absent	2003	DF–024; DF–027; DF–034; DF–044; DF–054; DF–057; GO–121; GO–033; GO–034; GO–126; GO–127; GO–204; GO–208; GO–211; GO–212 and GO–218
			2005	DF–154; DF–170 and DF–209
			2011	GO–495
			2012	DF–487
			2014	GO–589
			2015	GO–604 and GO–605
			2016	DF–663; GO–617 and GO–618

2.4. Preparation of pools and sending to High-Throughput Sequencing (HTS).

The RCA of the samples were organized and grouped into two pools called BP1 and BP2. Pool BP1 contains samples from the North (13), Northeast (36) and South (24) regions, while pool BP2 contains samples from the Southeast (39) and Central-West (42) regions (Table 1) After the preparation of the pools, they were subjected to HTS on the Illumina NovaSeq-6000 platform in an AB-3500 Genetic Analyzer automatic sequencer.

2.5. Analysis of sequenced samples

The results obtained were initially analyzed using the CLC Genomics Workbench 7.5 program (Qiagen) and subsequently analyzed with the Geneious program (Kearse et al. 2012). The methodology used was essentially that described in Nery et al. (2020) and Reis et al. (2020). The reads obtained were mapped to the contig of a potential virus to obtain the final contig. The genomes of the individual contigs were extended with the help of the Geneious program and the Map to reference tool (90 to 99% minimum overlap identity parameter) with mapping in the reads file provided by HTS. All contigs were subjected to comparisons with viral sequences present in local banks or GenBank using BLASTn algorithms. MUSCLE alignments were performed in the Geneious program to annotate ORFs (Open Reading Frames) based on the reference genome. After de novo assembly, contigs from both pools underwent taxonomic prediction analysis using the Kaiju web server (http://Kaiju.binf.ku.dk/server) (Menzel et al. 2016), with the parameter's classification standards. From this analysis, sequences with taxonomy predicted to be of viral origin were separated. The largest sequences were selected and assembled. The viral sequences were aligned with the reference genomes showing greater identities (Menzel et al. 2016) with the help of the Geneious R11.1 program (Kearse et al. 2012). The Geneious R11.1 program was also used to assemble the viral genome, annotate, and align the assemble.

For potential new species, in addition to the annotation of ORFs, the intergenic region (present in monopartite viruses) and the common region present in bipartite viruses were also analyzed. In the common region, the nonanucleotide motifs and iterons were characterized as well as the REP-IRD (Rep Iteron–Related Domains) domains, which allowed us to confirm that components of DNA–A and DNA–B are cognate (Arguello-Astorga and Ruiz-Medrano, 2001 ; Arguello-Astorga et al. 2004). For comparison between isolates and viral species, the sequences were subjected to pairwise MUSCLE multiple alignment using the Sequence Demarcation Tool (SDT) program (Muhire et al. 2014).

2.6. Detection of viruses in individual samples by PCR.

Based on the sequences obtained by HTS sequencing, open and oppositely directed primer pairs were designed using the primer design function of the Geneious program (Kearse et al. 2012). The primers were used to detect viruses in individual samples using the row *x* column system. Each row and column was formed by grouping of several samples (forming pools) so that each row and column contains a sample in common. If there is a single positive line and single column or a single positive line and two positive columns or two positive lines and a single positive column, the sample in common between them is considered positive for the virus. For more than one positive row and column simultaneously, all samples contained in the respective rows and columns were subjected to PCR individually. A set of specific primers were used to detect viral species in the samples. The sequences of these primers, as well as the conditions of use, are found in **Table 2**.

2.7. PCR conditions used to detect viruses in individual samples.

To recover the viral genomes in each individual DNA sample, PCR assays were used using the species-specific primers as shown in Table 2. In the assays, reactions were carried out in a total volume of 12.5 µL, containing the components: Taq DNA polymerase Buffer 10x (1.25 µL), 50 mM MgCl2 (0.4 µL), 2.5 mM dNTPs (0.25 µL), 10µM each Forward and Reverse Primers (0.25 µL), Milli-Q water (8.0 µL) and *Taq* DNA Polymerase 0.5 U (0.10 µL). The reactions had a total of 35 cycles divided into: initial denaturation (94 °C for 3 minutes), denaturation (94 °C for 30 seconds), annealing (temperatures in Table 2 for 45 seconds), extension (72 °C for 3 minutes) and final extension (72 °C for 10 minutes). The amplicons generated were visualized in agarose gel (1%) stained in ethidium bromide, visualized under UV light and photodocumented.

2.8. Validation via Sanger sequencing of the species-specific primers used in the PCR assays.

To validate the primer pairs used in individual PCR detections (**Table 2**), the amplicons generated by each primer pair were purified using the Ludwig purification kit in accordance with the manufacturers' recommendations and subjected to Sanger sequencing at the company ACTGene. The chromatograms obtained by Sanger sequencing were evaluated and analyzed using the BLASTn algorithm, comparing them with the sequences available in the GenBank database (https://www.ncbi.nlm.nih.gov).

Table 2. List of species-specific primers used to detect different viruses and satellite DNA in tomato (Solanum lycopersicum) samples and details of information regarding the name of the primer, sequences and annealing temperatures (AT ºC). Adapted from Reis et al. (2020) and Batista (2020).

Viral species / DNA component	Primer name	Sequence 5'- 3'	AT (°C)
[1]*Tomato severe rugose virus* DNA–A	ToSRV-For5.1	AGCGTCGTTAGCTGTCTGGCA	58
	ToSRV-Rev5	TGCCGCAGAAGCCTTGAACGCACCT	
[1]*Tomato severe rugose virus* DNA–B	ToSRV-B-For	AAACCCACACGAAAGCAGAGTTT	55
	ToSRV-B-Rev	CACCACGTCTATACATATTGTCCAGG	
[1]*Euphorbia yellow mosaic virus* DNA–A	EuYMV-A-R-For	GGGGTTCCAAGTCCAATAAAGATGA	52
	EuYMV-A-R-Rev	CAGACACCTTATATTTGCCGGATTC	
[1]*Tomato chlorotic mottle virus* DNA–A	ToCMoV-A-For	TTTGGGCCGCTCTTTTGGG	47
	ToCMoV-A-Rev	CAAACTGAATGGGCCTTAAA	
[1]*Tomato chlorotic mottle virus* DNA–B	ToCMoV-B-For	GTATTTGTTCTGGGTGCAATCATAAAAC	55
	ToCMoV-B-Rev	TTGTACTAATGACACATTATTCAATCACGA	
[1]*Tomato golden vein virus* DNA–A	TGVV-A-For1	AAAGGAAGATAATTCAAATATAGGGA	51
	TGVV-A-Rev1	ATCTTCCTTTACTCACGTTCCTGAT	
[1]*Tomato golden vein virus* DNA–B	TGVV-B-S-For	CCCACTTTCCATAACCTACATGAGA	55
	TGVV-B-S-Rev	GGAGAGAAAATTGATAAGATCGGCATC	
[1]*Tomato mottle leaf curl virus* DNA–A	ToMoLCV-For	TGTGGTCCAGTCAATAAATG	47
	ToMoLCV-Rev	TGACTGGACCACATAGTAAA	
[1]*Sida micrantha mosaic virus* DNA–A	SiMMV-For	GATCTCGCTCCCCCCTCT	58
	SiMMV-Rev	AGATCGCACGACAACCAG	
[3]*Tomato yellow spot virus* DNA–A	F1A-ToY	ACGAAATCTTTTAGGAGCTAATGG	53
	R1A-ToY	CGTATTTCTGCAAAAAACTACTTCCT	
[3]*Tomato yellow spot virus* DNA–B	F3B-ToY	AATAAGGCGAAAGGTTAAAAGAATATGGCG	61
	R3B-ToY	GCCTTATTCACTTCACCTTCTTCGATTCAC	
[1]*Tomato yellow net virus* DNA–A	Abuti-A-For	GGACTCCAGGGGGCAAAA	55
	Abuti-A-Rev	AGTCCCGTCCGTACCACTTG	
[3]*Tomato chlorotic mottle Guyane virus* DNA–A	F3A-AM35	GGCGTATGAGTCGTTAGCTGATTGGC	62
	R4A-AM35	ATACGCCAAGGTCTTAAACTCAGAAACAA	
[3]*Tomato bright yellow mottle* virus DNA–A	F1A-TO167	CCCATTATTCCAAGGCCCAACAG	60
	R1A TO167	GGGCCTTTTTTATAGCAACTTAGC	
[3] *New species #1* DNA–A	F1A-C25	AAGTAAGGAAAAAATTCTTGGCTTGG	59
	R1A-C25	ATCCCAAGTGTTCCCTGACGAAAGAG	
[3] *New species #2* DNA–A	F6AC222	TATCAATTCGTCGTCTCCTGATTCCT	60

	R6AC222	AACTTTACCAAACTTAGTGACCAAG	
[3] *New species #3* DNA–A	F1A-C230	GTCTTTTGCTGTGTGGTCCAG	63
	R2A-C230	GCGGGTCGGGGGCATAAAAAAAT	
[3]New species #4 DNA–A	F1A-C16	TATGCTATGAATCGGTAGAACGG	58
	R1A-C16	GCATAAGTTTTCTCTGAATCCC	
[3]*New species #5* DNA–A	F2A-C12	GATTGTGTCCTGGGCGGTTATTTATTTCG	63
	R2A-C12	GTACAACACAGAGCTGCTAAAAAACGAGG	
[1]Alfassatélite	Alfa-For	TGGTGTCCTGGCTTATAT	46
	Alfa-Rev	GGCGGAGTCCTTTTTTTT	
[2]*Tomato associate geminivirus1*	Cap2KpnI-F	GGTACCCCCCTTGGAAATGTAGTCTGCAAC	66
	Cap2KpnI-R	GGTACCTTTGAGGAGAGAGGTATACTTCG	
[2]*Tomato apical leaf curl virus*	Cap1PstI-F	CTGCAGAYTTGCGCGGATCGATTAAT	68
	Cap1PstI-R	CTGCAGAAATGCGTTGTAACTTCTCGGATAT	

[1]Primers designed by Reis et al. (2020); [2]Primers designed by Batista (2020); [3]Primers designed in the present study.

3. Results

3.1. Viral diversity detected in the BP1 pool (with samples from North, Northeast and South Brazilian regions).

The HTS, conducted on the Illumina NovaSeq-6000 platform, provided the BP1 pool (composed of tomato foliar samples collected in the North, Northeast and South regions) with the following raw reads: 7,230,366 reads, 38,575 contigs of which 137 corresponded to genome segments of viruses as indicated by BLASTn analysis. HTS-derived genomic information and assembly of contigs from the BP1 pool allowed the recovery of 15 begomovirus-like genomes, four of these were classified as new species **(Tables 3 and 4)**. A partial genome related to tomato associated geminivirus 1 (genus *Topilevirus*) was also recovered **(Tables 3 and 4)**. Among the 11 previously characterized known *Begomovirus* species, ToMoLCV displayed the highest read coverage (277,339), followed by ToSRV (227,279), ToGLDV (170,461), Tomato chlorotic mottle Guyane virus – ToCMoGV (131,012), and ChdTAV (55,010). The other viruses displayed lower read coverage numbers as shown in **Table 3**. In relation to the number of isolates, the ToMoLCV displayed the highest number (nine isolates), followed by ToGLDV (three) and SimMV (two), the other viruses only one isolate (**Table 3**). Four DNA–A segments exhibited identity levels lower than the threshold of 91% nucleotide identity for the complete DNA–A genome (**Table 3**), which is the current demarcation criterion for a novel species of the genus *Begomovirus* (Brown et al. 2015). The putative new species #1 (41,497 reads) shared 90.4% identity with ToICV (NC_038469.1), new species #2 (153,967 reads) shared 90.17% identity with ToMoLCV (MT215005.1), new species #3 (79.920 reads) 87.10% with ToBYMoV (NC_038468.1) and new species #4 (24.589 reads) 82.80% with ToGLDV (HM357456.2). Sida yellow blotch virus (SiYBV) (contig 6958) was the only formerly described begomovirus that was not yet been reported in association with tomato crops. The HTS results from the BP1 pool (**Table 4**) also allowed the recovery of the complete genome of DNA–B segments of ToCMGV, which displayed the highest coverage (129,354 reads) and the highest number of isolates (=4). A topilevirus-like (contig 8679) showed low reads (82) and only partial genomic recovery (2139 nucleotides) was obtained (**Table 3**). The contig associated with the *Topilevirus*-like partial genome shared a nucleotide identity of 90.27% with tomato associated geminivirus 1 (TAG1; MN527305.1) (**Table 3**).

3.2. Viral diversity present in the BP2 pool (with samples from Southeast and Central-West regions).

The number of reads of the BP2 pool (composed of foliar tomato samples collected in the Southeast and Central-West regions) was in the order of 8,533,058, which generated 34,964 contigs, 145 of which corresponding to putative viral genome segments. HTS-derived genomic information and assembly of contigs from the BP2 pool **(Tables 5 and 6)** allowed the recovery of 14 viruses. Ten of them correspond to begomoviruses previously reported in association with tomato crops viz.: EuYMV (Reis et al. 2020), ToCMoV (Ribeiro et al. 2007), SimMV (Calegario et al. 2004), ToSRV (Cotrim et al. 2007), ToRMV (Reis et al. 2020; Souza et al. 2020), ToYVSV (Albuquerque et al. 2010; Mituti et al. 2019; Reis et al. 2021), ToMoLCV (Fernandes et al. 2008), TGVV (Reis et al. 2020; Reis et al. 2021), ToICV (Albuquerque et al. 2012; Duarte et al. 2021a), and ToYNV (Reis et al. 2023). In addition to these, the genome of a putative new species (New species #5) was recovery. Among the 14 viral genomes recovered, two of them corresponded to members of the *Topilevirus* genus: tomato associated geminivirus 2 – TAG 2 (contig 934) and ToALCV (contig 45). It was also possible to recover by HTS two species of alphasatellites associated with begomoviruses: a putative new *Alphasatellitidae* species and Euphorbia yellow mosaic alphasatellite.

ToRMV displayed the highest number of reads (601,302), followed by ToSRV (590,532 reads), SimMV (431,707), ToMoLCV (412,517) and New species #5 (369,619). Regarding the number of isolates, the ToMoLCV displayed the highest number (11), followed by TGVV (six), SimMV (five), ToSRV, TRMV, and ToCMoV (three isolates of each). The remaining viruses varied from one to two representative isolates (**Table 5**). The new species #5 shares 89.03% with TGVV (MN928612.1) (**Table 5**). The contig 934 shared 97.98% identity with TAG 2 and coverage of 1,694 reads (**Table 5**). The contigs 185 and 45 shared 100% identity with each other and 85.21% identity with ToALCV and 18,143 reads (**Table 5**). Both contigs meet the species demarcation criterion of the genus, minimum nucleotide identity of 78% (Vaghi-Medina et al. 2018; ICTV 2024). Contig 38 corresponds to the satellite DNA of the Alphasatellitidae family, which presented a read coverage of 46,535, while the second satellite DNA recovered was Euphorbia yellow mosaic alphasatellite with 10,481 reads. Among the recovered DNA–B segments, ToSRV presented the highest number of reads (300,551 reads) and recovered sequences (four) (**Table 6**).

Table 3. Code of the contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of the assembled virus, E-value, virus description and GeneBank accession number for the DNA–A segment of *Geminiviridae* viruses and subviral agents obtained by High-Throughput Sequencing (HTS) within the pool BP1 containing 73 foliar tomato samples from the North, Northeast, and South regions of Brazil. Contigs highlighted in gray and bold letter represent putative new viral species.

Code of the contigs	Read coverage	Assembled genome size (nts)	BLASTn coverage (%)	Identity (%)	E-Value	Virus description**	GeneBank accession number
40	227,279	2593	100	99,96	0	Tomato severe rugose virus DNA–A [1]	MW573989.1
*	17,827	2660	100	99,85	0	Tomato yellow leaf deformation dwarf virus DNA–A [2]	NC_055586.1
38	131,012	2630	100	99,73	0	Tomato chlorotic mottle Guyane virus DNA–A [3]	MK878452.1
5	31,367	2694	99	99,52	0	Tomato rugose yellow leaf curl virus DNA–A [4]	KU682839.1
1098	9959	2661	100	98,99	0	Tomato yellow spot virus DNA–A [5]	KX348172.1
63	277,339	2634	100	98,97	0	Tomato mottle leaf curl virus DNA–A [6]	KX896408.1
66	55,010	2603	100	98,47	0	Chino del tomate Amazonas virus DNA–A [7]	NC_038443.1
7	170,461	2623	99	98,25	0	Tomato golden leaf distortion virus DNA–A [8]	HM357456.2
12	236,434	2631	100	98,21	0	Tomato mottle leaf curl virus DNA–A [6]	KX896408.1
125	205,526	2631	100	97,00	0	Tomato mottle leaf curl virus DNA–A [6]	MT215005.1
144	156,309	2623	99	96,85	0	Tomato golden leaf distortion virus DNA–A [8]	HM357456.2
140	77,529	2627	100	96,66	0	Tomato mottle leaf curl virus DNA–A [6]	JF803247.1
507	119,119	2632	100	96,13	0	Tomato mottle leaf curl virus DNA–A [6]	KX896414.1
141	82,22	2629	100	96,09	0	Tomato mottle leaf curl virus DNA–A [6]	JF803247.1
44	194,632	2632	100	95,79	0	Tomato mottle leaf curl virus DNA–A [6]	KX896414.1
552	4407	2686	100	95,38	0	Sida micrantha mosaic virus DNA–A [9]	KC706535.1
105	152,241	2623	99	95,18	0	Tomato golden leaf distortion virus DNA–A [8]	HM357456.2
109	14,715	2661	100	95,11	0	Tomato yellow spot virus DNA–A [5]	KX348172.1
21	44,698	2622	99	94,82	0	Tomato bright yellow mottle virus DNA–A [10]	NC_038468.1
6958	2854	2643	99	94,54	0	Sida yellow blotch virus DNA–A [11]	MT103998.1
67	254,814	2631	100	92,49	0	Tomato mottle leaf curl virus DNA–A [6]	KX896414.1
68	129,337	2631	100	91,66	0	Tomato mottle leaf curl virus DNA–A [6]	JF803250.1
205	32,299	2685	100	91,01	0	Sida micrantha mosaic virus DNA–A [9]	EU908733.1
New species #1	41,497	2604	100	90,40	0	Tomato interveinal chlorosis virus DNA–A	NC_038469.1
8679	82	2139	100	90,27	0	Tomato associated geminivirus 1	MN527305.1
New species #2	153,967	2631	100	90,17	0	Tomato mottle leaf curl virus DNA–A	MT215005.1
New species #3	79,92	2657	99	87,10	0	Tomato bright yellow mottle virus DNA–A	NC_038468.1
New species #4	24,589	2612	97	82,80	0	Tomato golden leaf distortion virus DNA–A	HM357456.2

* Virus obtained from Kaiju online tool. **Viruses with the same number correspond to isolates of the same species.

Table 4. Code of the contigs, read coverage, assembled genome size, assembled genome size, BLASTn coverage, sequence identity of the assembled virus, E-value, virus description and GeneBank accession number for the DNA–B segment of begomoviruses obtained by High-Throughput Sequencing (HTS) within the pool BP1 containing 73 foliar tomato samples from the North, Northeast, and South regions of Brazil.

Code of the contigs	Read coverage	Assembled genome size (nts)	BLASTn coverage (%)	Identity (%)	E-Value	Virus description**	GeneBank accession number
10	129,354	2593	100	99,92	0	Tomato chlorotic mottle Guyane virus DNA–B [1]	MK878451.1
98	3725	2609	100	99,00	0	Tomato yellow leaf deformation dwarf virus DNA–B [2]	NC_060089.1
201	129,354	2593	100	99,87	0	Tomato chlorotic mottle Guyane virus DNA–B [1]	MK878451.1
81	18,916	2535	100	97,09	0	Chino del tomate Amazonas virus DNA–B [3]	MG675220.1
2034	92,661	2662	83	96,76	0	Tomato chlorotic mottle Guyane virus DNA–B [1]	MK878451.1
1156	15,163	2634	100	93,70	0	Tomato yellow spot virus DNA–B [4]	KX348205.1
2913	82,057	2597	100	91,62	0	Tomato chlorotic mottle Guyane virus DNA–B [1]	MK878451.1
1778	1475	2583	100	88,31	0	Tomato crinkle leaf yellows virus DNA–B [5]	JN419011.1
299	2808	2619	99	83,98	0	Tomato yellow leaf deformation dwarf virus DNA–B [2]	NC_060089.1
93	10,358	2565	85	82,05	0	Tomato interveinal chlorosis virus-2 DNA–B [6]	MK087039.1
8	14,354	2656	96	77,95	0	Tomato rugose yellow leaf curl virus DNA–B [7]	JN381822.1

*Viruses with the same number correspond to isolates of the same species.

Table 5. Code of the contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of the assembled virus, E-value, virus description and GeneBank accession number for the DNA–A segment of *Geminiviridae* viruses and subviral agents obtained by High-Throughput Sequencing (HTS) of the pool BP2 containing 81 foliar tomato samples from the Southeast, and Midwest regions. Contig highlighted in gray and bold letter corresponds to a putative new viral species.

Code of the contigs	Read coverage	Assembled genome size (nts)	BLASTn coverage (%)	Identity (%)	E-Value	Virus description**	GeneBank accession number
38	46,535	1322	100	99,92	0	Alphasatellitidae sp.[1]	MT214093.1
107	574,158	2591	100	99,88	0	Tomato severe rugose virus DNA–A [2]	MT733811.1
78	73,475	2631	100	99,81	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
9	590,532	2591	100	99,73	0	Tomato severe rugose virus DNA–A [2]	MW596564.1
36	181,913	2631	100	99,62	0	Tomato mottle leaf curl virus DNA–A [3]	JF803247.1
255	12,05	2628	100	99,51	0	Euphorbia yellow mosaic virus DNA–A [4]	MN782438.1
52	206,835	2622	100	99,50	0	Tomato chlorotic mottle virus DNA–A [5]	MT733804.1
79	57,409	2561	100	99,45	0	Tomato golden vein virus DNA–A [6]	KC706652.1
827	601,302	2698	99	99,32	0	Tomato rugose mosaic virus DNA–A [7]	MT215006.1
83	63,047	2561	100	99,22	0	Tomato golden vein virus DNA–A [6]	NC_038807.1
34	113,869	2561	100	98,83	0	Tomato golden vein virus DNA–A [6]	KC706646.1
14432	113	2636	100	98,79	0	Tomato yellow net virus DNA-ADNA–A [8]	MT214096.1
47	12,561	2610	100	98,74	0	Euphorbia yellow mosaic virus DNA–A [4]	KY559437.1
211	245,699	2631	100	98,29	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
29	335,413	2606	100	98,05	0	Tomato rugose mosaic virus DNA–A [7]	MT215006.1
934	1,694	2574	100	97,98	0	Tomato associated geminivirus 2[9]	MN527305.1
37	154,963	2631	100	97,95	0	Tomato mottle leaf curl virus DNA–A [3]	MT214088.1
*	26,055	2560	100	97,89	0	Tomato yellow vein streak virus DNA–A [10]	KC136337.1
7	232,163	2622	100	97,83	0	Tomato chlorotic mottle virus DNA–A [5]	MT733804.1
268	4530	2671	99	97,78	0	Sida micrantha mosaic virus DNA–A [11]	JX415194.1
73	138,272	2561	100	97,77	0	Tomato golden vein virus DNA–A [6]	JF803259.1
*	4,822	2667	100	97,76	0	Sida yellow mosaic virus DNA–A [12]	AY090558.1
286	134, 472	2561	100	97,70	0	Tomato golden vein virus DNA–A [6]	KC706630.1
363	10,149	2675	100	97,68	0	Sida micrantha mosaic virus DNA–A [11]	KC706535.1
875	278,211	2652	90	97,41	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
101	264,219	2631	100	96,92	0	Tomato mottle leaf curl virus DNA–A [3]	MT215005.1
50	286,731	2637	95	96,83	0	Tomato rugose mosaic virus DNA–A [7]	MT215006.1
74	38,57	2560	100	96,76	0	Tomato yellow vein streak virus DNA–A [10]	MN508216.1

58	253,605	2631	100	96,28	0	Tomato mottle leaf curl virus DNA–A [3]	MT215005.1
40	229,047	2631	100	95,79	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
307	94,086	2676	100	95,70	0	Sida micrantha mosaic virus DNA–A [11]	KC706535.1
67	217,613	2631	100	95,41	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
201	10,481	1365	100	94,59	0	Euphorbia yellow mosaic alphasatellite[13]	FN436008.1
49	203,042	2602	100	93,45	0	Tomato chlorotic mottle virus DNA–A [5]	MT733804.1
23	145,276	2631	100	94,19	0	Tomato mottle leaf curl virus DNA–A [3]	JF803247.1
*	431,707	2676	100	94,18	0	Sida micrantha mosaic virus DNA–A [11]	MT214092.1
*	204,035	2618	100	93,81	0	Tomato interveinal chlorosis virus DNA–A [14]	JF803253.1
56	412,517	2631	100	93,06	0	Tomato mottle leaf curl virus DNA–A [3]	MT733813.1
3065	123,209	2572	100	92,76	0	Tomato severe rugose virus DNA–A [2]	HQ606468.1
357	369,71	2675	100	91,76	0	Sida micrantha mosaic virus DNA–A [11]	MT214092.1
84	210,05	2556	100	91,68	0	Tomato golden vein virus DNA–A [6]	KC706653.1
New species #5	369,619	2561	100	89,03	0	Tomato golden vein virus DNA–A [6]	MN928612.1
45	18,143	2879	100	85,21	0	Tomato apical leaf curl virus[15]	MT135209.1

* Virus obtained from Kaiju online tool. **Viruses with the same number correspond to isolates of the same species.

Table 6. Code of the contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of the assembled virus, E-value, virus description, and GeneBank accession number for the DNA–B segments of begomoviruses obtained by High-Throughput Sequencing (HTS) of the pool BP2 containing 81 foliar tomato samples from the Southeast and Midwest regions of Brazil.

Code of the contigs	Read coverage	Assembled genome size (nts)	BLASTn coverage (%)	Identity (%)	E-Value	Virus description**	GeneBank accession number
103	143,673	2571	100	99,73	0	Tomato severe rugose virus DNA–B [1]	MT215002.1
82	102,342	2597	100	99,58	0	Tomato chlorotic mottle virus DNA–B [2]	MT214087.1
16	137	2570	100	99,57	0	Tomato rugose mosaic virus DNA–B [3]	MT215007.1
86	300,551	2569	100	99,57	0	Tomato severe rugose virus DNA–B [1]	HQ606468.1
14	47,649	2533	100	99,33	0	Tomato golden vein virus DNA–B [4]	MN928611.1
122	48,514	2551	100	98,55	0	Tomato golden vein virus DNA–B [4]	MT733807.1
132	142,262	2554	100	98,26	0	Tomato severe rugose virus DNA–B [1]	MT214085.1
121	147,477	2571	93	97,00	0	Tomato severe rugose virus DNA–B [1]	MT215002.1
*	45,881	2543	100	96,58	0	Tomato mild leaf curl virus DNA–B [5]	DQ336352.1
27613	4	318	100	96,54	0	Sida micrantha mosaic virus DNA–B [6]	AJ557452.1
3050	90,728	2597	85	90,55	0	Tomato rugose mosaic virus DNA–B [3]	MT214091.1
21	37,396	2527	97	95,50	0	Tomato golden vein virus DNA–B [4]	KC706660.1
499	2740	2556	100	94,97	0	Tomato yellow vein streak virus DNA–B [7]	MN508217.1

* Vírus obtido a partir da ferramenta online Kaiju. **Vírus com mesmo número correspondem a isolados da mesma espécie.

3.3. Comparative viral diversity: BP1 pool (North, Northeast, and South regions) versus BP2 pool (Southeast and Central-West regions).

It was possible to notice in the HTS results a greater diversity in the ssDNA the virome of tomato plants from the BP1 pool encompassing samples from the North, Northeast, and South regions (16 viruses) in contrast with the BP2 pool encompassing samples from Southeast and Central-West regions (14 viruses). However, BP2 virome displayed greater quantities of DNA–B segments (**Table 6**) in addition to subviral agents (**Table 5**). Possibly the greater viral diversity in the BP1 pool is related to the greater number of more diverse geographic regions that make up this pool, which leads to greater coverage of ecological niches and biomes. A second hypothesis of the lower number of viruses in the BP2 pool in relation to BP1 could also be the influence of the higher presence of samples carrying the resistance factors *Ty*–1 and *Ty*–3 present in the BP2 samples. Reis et al. (2020) have already demonstrated that the *Ty*–1 factor performs the function of a "filter", reducing the number of viruses and also mixed infections in tomato plants that carried the gene.

It is worth mentioning that the information on tomato-infecting begomoviruses occurring in the Brazilian North region Amazon is yet very limited, as is the knowledge of viral diversity in this geographic area. This can partially explain the greater number of new species occurring in the BP1 pool and makes this region promising for additional virome studies on tomatoes and other crops. Furthermore, the lower relative richness in terms of detection of new species in the Southeast and Central–West regions is also due to the fact that they are the geographic regions of the country is the one most intensely investigated by research groups in plant virology. The high number of DNA–B segments in the BP2 pool may allow us to infer the significant use of these segments in pseudorecombination events, allowing viruses to better adapt in the absence of the cognate DNA–B segment and at the same time increase genetic variability.

3.4. PCR detection with species-specific primers of geminiviruses and subviral pathogens in the BP1 (North, Northeast, and South regions) and in the BP2 (Southeast and Central–West/Central regions) pools

3.4.1 Northern region

In the **Northern region** of Brazil, PCR using species–specific primers allowed the detection of ToMoLCV, SimMV, ToCMoGV, ToYSV, ToBYMoV, and the New species #3 (**Table 7**). ToMoLCV was detected in the states of Amazonas (sample AM–012), Roraima (RR–003) and

Tocantins (TO–088), similarly SimMV in the states of Amazonas (AM–010), Roraima (RR–003 and RR–004), and Tocantins (TO–045 and TO–046). ToCMoGV (a pathogen not yet reported occurring in Brazil) was detected in the state of Amazonas (sample AM–035). In the states of Roraima (samples RR–003 and RR–004) and Tocantins (TO–046), ToYSV infection was found. New species #3 and ToBYMoV were detected in mixed infection in the sample TO–167 (State of Tocantins).

3.4.2 North-East region

In the **Northeast region**, the following viruses were detected: ToMoLCV, SimMV and New species #1 (**Table 7**). Among these, ToMoLCV was the most prevalent, infecting 23 samples. However, there was no confirmation of ToMoLCV infection in tomato plants from the state of Ceará, as was detected in samples CE–001; CE–011 and CE–012 (Table 7). Therefore, the significant predominance of ToMoLCV in this region must be highlighted. SimMV is present in the states of Bahia (BA–100) and Pernambuco (PE–011), while New species #1 was detected in the states of Ceará (CE–001) and Pernambuco (PE–011 and PE–012) (Table 7).

3.4.3 South region Southeast and Central–West regions)

In the **South region**, the following were detected: ToSRV, ToMoLCV and SimMV, in addition to two new species of begomvirus (**Tables 7 and 8**). Among the three viruses, ToSRV was the most prevalent with 16 infected samples, followed by ToMoLCV (nine samples) and only one sample corresponding to SimMV. ToSRV and ToMoLCV were present in samples from the states of Santa Catarina, Rio Grande do Sul and Paraná. SimMV was only present in the state of Paraná (PR–143). New species #2 and New species #4 were detected in the state of Paraná. New species #2 in two (PR–173 and PR–174) and New species #4 in a single sample (PR–144) (Table 7).

3.4.4 Southeast and Mid-West/Central regions

In the pool of tomato samples corresponding to the **Southeast and Mid-West/Central regions** (BP2), seven begomoviruses, two topileviruses and one alphasatellite were detected via PCR (Table 7 and 8) using specific primers. ToSRV, ToMoLCV, ToCMoV, TGVV, SimMV, and EuYMV were detected in both regions. In the **South-East region**, ToSRV was the most prevalent, being present in 26 samples, followed by ToCMoV (19 samples), ToMoLCV (15), TGVV (10) SimMV (2) and EuYMV (2). Tomato samples infected by ToCMoV and TGVV in the state of São Paulo had not been reported in the literature, as well as the presence of the topilevirus ToALCV, present in samples SP–172 and SP–173. For the **Mid-West/Central**

region, the most prevalent virus was also ToSRV (26 samples) followed by ToMoLCV (21), SimMV (16), ToCMoV (14), TGVV (12), New species #5 (8), EuYMV (3) and ToYNV (1). In this region, the alphasatellite was also detected in the Federal District (DF-024, DF–027 and DF–057), in addition to the topileviruses TAGV in the state of Goiás (GO–495), and ToALCV (SP–172 and SP–173) (**Tables 7 and 8**).

Table 7. Positive samples for geminivirus detected via PCR with species-specific primers in tomato samples without *Ty*–1/*Ty*–3 resistance factors collected across the five Brazilian regions.

Virus acronyms (total of positive samples)	Positive samples per geographical region				
	North	**North-East**	**South**	**South-East**	**Mid-West/Central**
ToSRV (15 + 20 + 19 = **54**)	–	–	PR–143; PR–173; PR–174; RS–033; RS–012; RS–013; RS–014; RS–015; SC–001; SC–002; SC–015; SC–030; SC–032; SC–044 & SC–051	MG–013; MG–014; MG–108; MG–109; MG–267; MG–292; SP–003; SP–006; SP–008; SP–017; SP–111; SP–205; SP–206; SP–154; SP–173; SP–201; SP–239; SP–254; SP–259 & SP–274	DF–663; DF–034; DF–209; DF–487; GO–121; GO–033; GO–034; GO–126; GO–127; GO–204; GO–208; GO–211; GO–212; GO–218; GO–589; GO–604; GO–605; GO–617 & GO–618
ToMoLCV (2 + 23 + 9 + 14 + 10 = **58**)	AM–012 & RR–003	BA–034; BA–035; BA–050; BA–100; BA–128; BA–134; BA–143; BA–173; BA–174; CE–001; CE–011; CE–012; PB–025; PB–027; PE–027; PE–028; PE–011; PE–012; PE–099; PE–100; PE–104; PE–105 & PE–121	PR–144; PR–173; PR–174; RS–015; RS–071; RS–095; SC–002; SC–015 & SC–030	MG–013; MG–014; MG–109; MG–292; MG–381; SP–003; SP–056; SP–058; SP–111; SP–213; SP–205; SP–154; SP–173 & SP–201	DF–027; DF–024; DF–054; DF–154; DF–487; GO–495; GO–604; GO–605; GO–617 & GO–618
ToCMoV (16 + 11 = **27**)	–	–	–	MG–046; MG– 013; MG–014; MG–084; MG–109; MG–267; MG–292; MG–381; SP–004; SP–006; SP–008; SP–017; SP–056; SP–205; SP–239 & SP–254	DF–027; DF–024 DF–034; DF–044; DF–054; DF–057; DF–170; DF–209; GO–121; GO–126 & GO–127
TGVV (8 + 8 = **16**)	–	–	–	MG–046; MG–013; MG–014; MG–108; MG–109; SP–003; SP–017 & SP–206	DF–027; DF–024; DF–170; DF–209; GO–121; GO–126; GO–127 & GO–218
SimMV (5 + 2 + 1 + 2 + 11 = **21**)	AM–010; RR–003; RR–004; TO–045 e TO–046	BA–100 & PE–011	PR–143	MG–267 & SP–173	DF–024; DF–034; DF–044; DF–054; DF–170; DF–209; GO–121; GO–033; GO–126; GO–127 & GO–204
EuYMV (1 + 3 = **4**)	–	–	–	SP–003	DF–170; GO–204 & GO–208

ToCMoGV **(1)**	AM–035	–	–	–	–
ToYSV **(3)**	RR–003; RR–004 & TO–046	–	–	–	–
ToBYMoV **(1)**	TO–167	–	–	–	–
New species #1 (3)	**–**	**CE–001; PE–011 & PE–012**	**–**	**–**	**–**
New species #2 (2)	**–**	**–**	**PR–173 & PR–174**	**–**	**–**
New species #3 (1)	**TO–167**	**–**	**–**	**–**	**–**
New species #4 (1)	**–**	**–**	**PR–144**	**–**	**–**
New species #5 (5)	**–**	**–**	**–**	**–**	**DF–209, GO–121, GO–126, GO–127 & GO–218**
Alphasatellite **(3)**	–	–	–	–	DF–024, DF–027& DF–057
ToALCV **(1)**	–	–	–	SP–173	–
TAGV **(1)**	–	–	–	–	GO–495

Tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato chlorotic mosaic virus (ToCMoV) e tomato golden vein virus (TGVV), Sida micrantha mosaic virus (SimMV), Euphorbia yellow mosaic virus (EuYMV) e tomato yellow spot virus (ToYSV), tomato bright yellow mottle virus (ToBYMoV), tomato apical leaf curl virus (ToALCV), and tomato associated geminivirus (TAGV).

Table 8. Positive samples for geminivirus detected via PCR with species-specific primers in tomato samples with *Ty*–1/*Ty*–3 resistance factors collected across the five Brazilian regions.

Virus acronyms (total of positive samples)	Positive samples per geographical region				
	North	**North-East**	**South**	**South-East**	**Mid-West/Central**
ToSRV (1 + 6 + 7 = **14**)	–	–	PR–112	MG–268; MG–291; SP–018; SP–156; SP–240 & SP–252	DF–216; DF–235; DF–338; DF–530; DF–546; DF–528 & DF–541
ToMoLCV (1+ 11 = **12**)	–	–	–	SP–172	DF–155; DF–235; DF–530; DF–546; DF–528; DF–541; GO–124; GO–229; GO–342; GO–499 & GO–526
ToCMoV (3 + 3 = **6**)	–	–	–	MG–268; SP–066 & SP–252	DF–216; DF–235 & GO–124
TGVV (1 + 4 = **5**)	–	–	–	SP–018	DF–216; DF–235; GO–124 & GO–229
ToYNV (**1**)	–	–	–	–	GO–342
SimMV (**5**)	–	–	–	–	DF–216; DF–235; DF–338; GO–005 & GO–124
EuYMV (**1**)	–	–	–	SP–066	–
New species #5 (3)	**–**	**–**	**–**	**–**	**DF–216, DF–235 & GO–124**
ToALCV (**1**)	–	–	–	SP–172	–

Tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato chlorotic mosaic virus (ToCMoV) e tomato golden vein virus (TGVV), tomato yellow net virus (ToYNV), Sida micrantha mosaic virus (SimMV), Euphorbia yellow mosaic virus (EuYMV), tomato yellow spot virus (ToYSV) and tomato apical leaf curl virus (ToALCV).

3.6. Comparative viral diversity of samples with versus without resistance factors

When comparing the detections of different geminiviruses and associated satelellite DNAs in tomato samples with and without the *Ty*–1/*Ty*–3 resistance/tolerance factors (**Figure 1**), it is noteworthy that the amount of viruses detected, the number of infected samples and the number of mixed infections (**Table 7 and Figure 2**) were greater in samples without resistance factors. The number of viruses and subviral agents was also greater in samples without resistance factors (16 viruses and one alphasatellite versus nine viruses) (**Table 7 and Figure 1**).

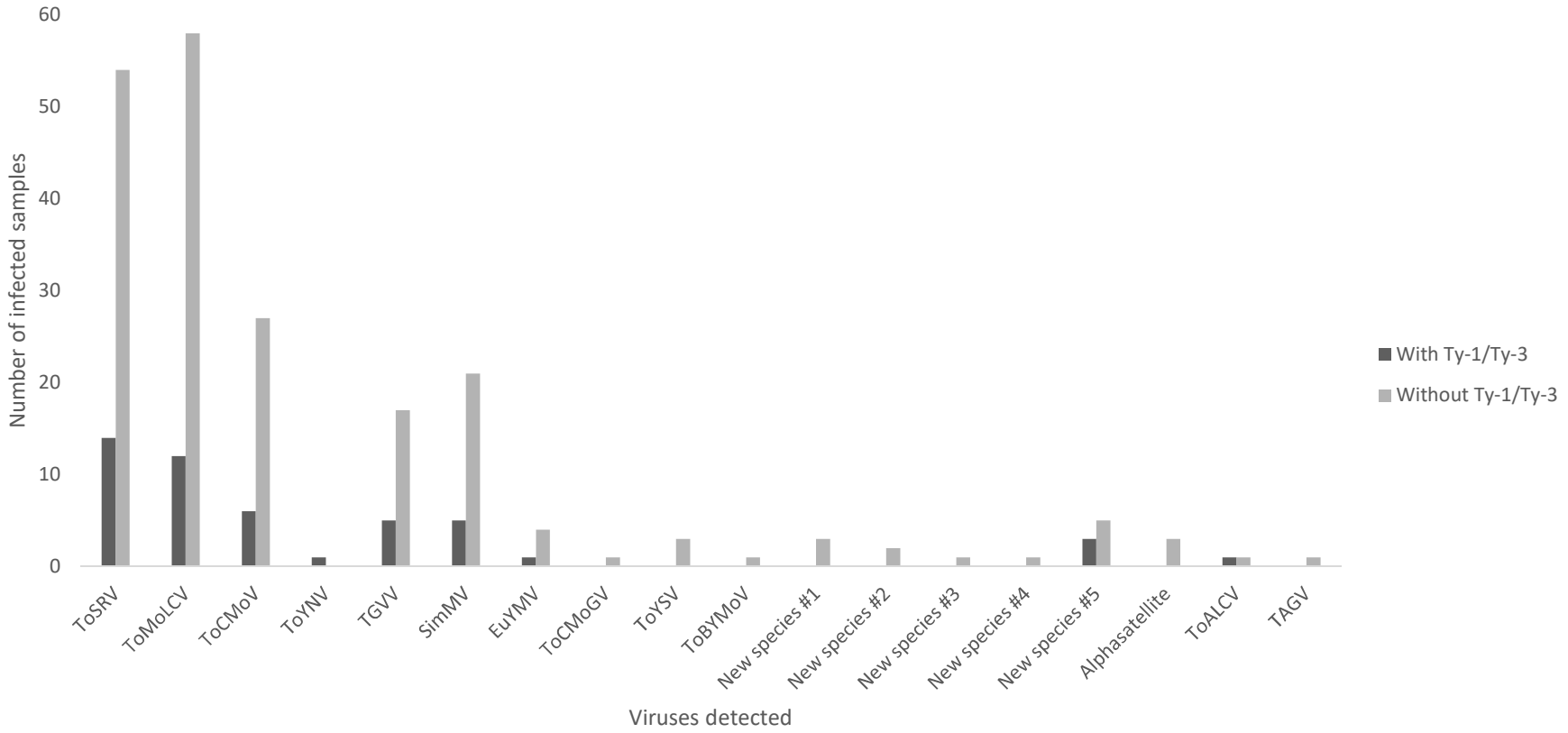

Figure 1. *Begomovirus*, *Topilevirus*, and Alphasatellite DNAs detected (X axis) versus (Y axis) the number of tomato samples (with the presence and absence of resistance/tolerance factors *Ty*–1/*Ty*–3) with infection of tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato chlorotic mottle virus (ToCMoV), tomato yellow net virus (ToYNV), tomato golden vein virus (TGVV), Sida micrantha mosaic virus (SimMV), Euphorbia yellow mosaic virus (EuYMV), tomato chlorotic mottle Guyane virus (ToCMoGV), tomato yellow spot virus (ToYSV), tomato bright yellow mottle virus (ToBYMoV), tomato apical leaf curl virus (ToALCV), and tomato associated geminivirus (TAGV).

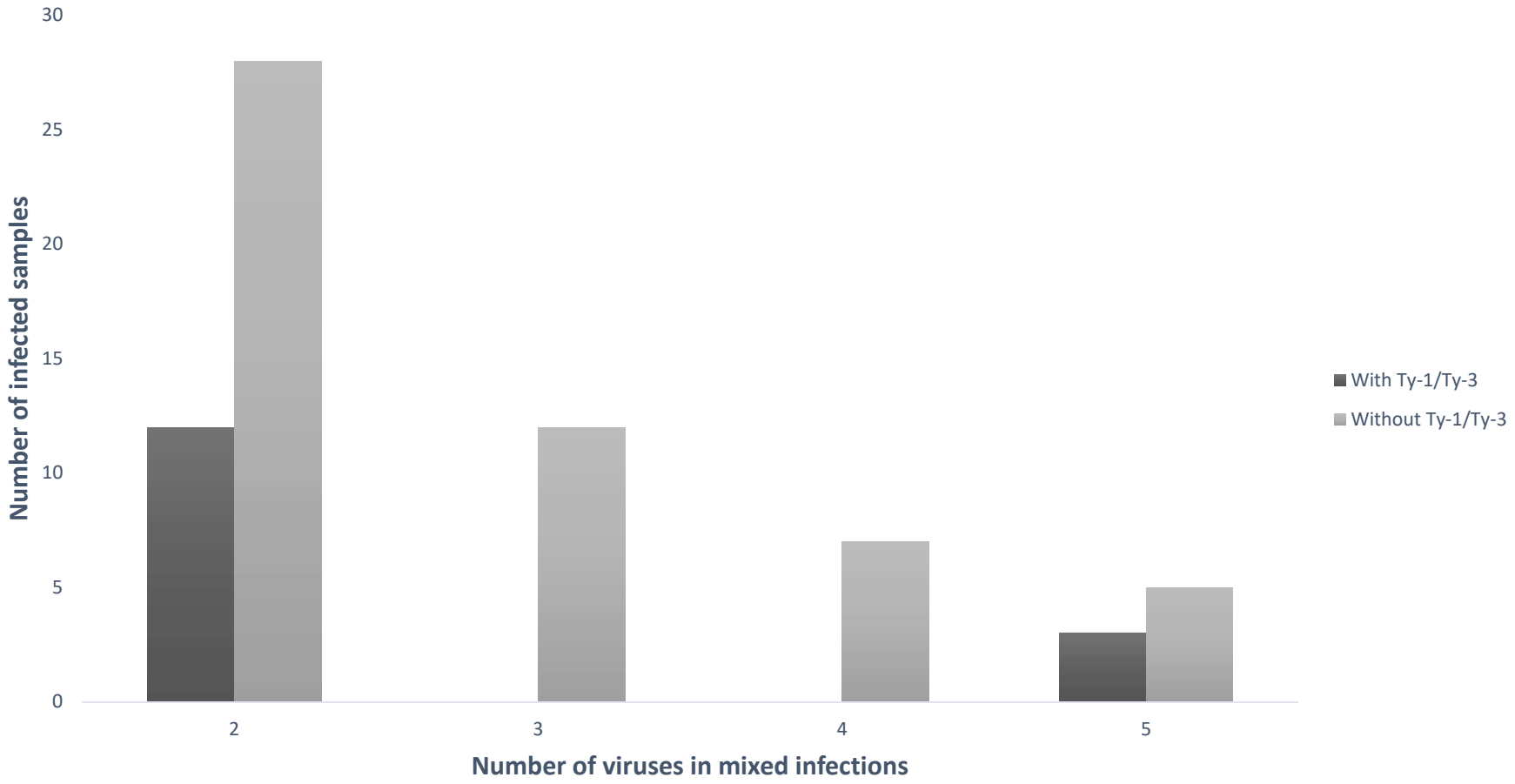

Figure 2. Number of viruses found in mixed infections (X axis) in tomato samples with the presence and absence of tolerance factors *Ty*–1/*Ty*–3 (Y axis).

4. Discussion

The most recent worldwide surveys revealed more than 300 viral species infecting the tomato crop (GenBank 2022; Host Data Base 2022, Kitajima 2020, see also chapter 1). The largest number of tomato-infecting viruses (221) are classified as *Begomovirus* species (family *Geminiviridae*), that is, this genus comprises 66.97% of the viral pathogens of this vegetable crop (GenBank 2022; Host Data Base 2022 and Kitajima (2020).

The current situation of tomato-infecting begomoviruses in Brazil points to a complex of more than 26 species (for recent review see Reis et al., 2023). And this scenario of ample diversity is more likely to expand due to genetic plasticity of this group of pathogens which is generated via mutation, recombination and pseudorecombination events (Roossinck 1997; Seal et al. 2006). Herein, we carried out a very extensive HTS-based virome of ssDNA viruses (*Begomovirus* and *Topilevirus* species) as well as genomes of subviral agents. With this sequencing platform, we recovered viral genomes and subviral agents infecting tomato plants from the five regions of Brazil grouped into two regional pools.

The discovery of a new set of begomovirus species through HTS reinforces the high degree of species diversity of this genus, currently 445 species (ICTV 2024), and reinforces the notion that the species diversity of this genus is more likely yet underestimated in the Neotropical region. These results corroborate studies of the efficiency of HTS for studying the diversity of viruses in different environments (Guajardo-Leiva et al. 2020; Schneider et al. 2021) and hosts (Luria et al. 2019; Bejnerman et al. 2020; Quintanilha-Peixoto et al. 2021). In tomato, the use of this technology has effectively allowed the detection of new viral species and subviral agents (Reis et al. 2020; Reis et al., 2023; Souza et al. 2020).

Reis et al. (2020) described the impact of the *Ty*–1 factor on the viral diversity of tomato plants in the central region of Brazil. Likewise, a "filtering" effect was observed herein by the factors *Ty*–1 and *Ty*–3. In some cases, species-specific "filtering" was observed for SimMV in the Central-West/Central region, ToSRV in the South, ToMoLCV in the Northeast and Southeast, as well as ToCMoV and TGVV in the Southeast. Viruses present in samples that carry the *Ty*–1 and *Ty*–3 resistance factors, that is, that pass through the "filter" of the *Ty*–1 and *Ty*–3 factors, may be undergoing a differential evolutionary/adaptive process mediated by these genes (Reis et al. 2020), which could result in potential viral isolates with the superior capacity to overcome resistance mediated by these genes, especially for those with high adaptability, that is, those with greater predominance (e.g. ToSRV and ToMoLCV). The results obtained in this study demonstrate this adaptive capacity of some viral isolates to the tolerance effects

provided through the *Ty*–1 and *Ty*–3 genes and to the different tomato-producing macroregions of Brazil.

High-performance sequencing platforms have been allowing the discovery of new species through virome studies, thus making it possible to monitor the increase in viral diversity in different biomes and over time (Reis et al., 2020). **New species #1**, detected in the states of Ceará (CE–001) and Pernambuco (PE–011 and PE–012), displayed a DNA–A segment (2604 nts) with 90.40% identity with isolated tomato interveinal chlorosis virus (NC_038469). **New species #2** (2631 nts) shared 90.17% identity with tomato mottle leaf curl virus (MT215005) and detected in the state of Paraná (PR–173 and PR–174). For **New species #1 and New species #2**, their cognate DNA–B was not found, indicating that they are two putative monopartite species. **New species #3** (2657 nts), presented 87.1% with tomato bright mottle virus (NC_038468.1), detected in the state of Tocantins (TO–167). **New species #4** displayed 80.2% identity with tomato golden leaf distortion virus (HM357456) and was detected in a single sample in the state of Paraná (PR–144). It has a typical bipartite begomovirus DNA–A segment of 2,612 nucleotides (nts), with cognate DNA–B of 2,565 nts. And finally, **New species #5**, detected in the state of Goiás and the Federal District, presented a DNA–A segment of 2561 nts and 89.3% identity with tomato golden vein virus (MN928612.1), its DNA–B segment cognate with 2527 nts. All five new begomovirus species meet the species demarcation criterion of less than 91% identity with other species in the genus (Brown et al., 2015). The greater number of new species outside the Southeast and Mid-West/Central regions (pool BP2) can be explained by the fact that these regions have been subjected, over the last few decades, to a greater number of prospecting works and surveys of begomovirus diversity either via conventional PCR strategies or via HTS (Reis et al. 2020; Souza et al. 2020). It is interesting to point out that all these five novel begomoviruses were detected in plants without either *Ty*–1 or *Ty*–3 genes, suggesting again a diversity filtering effect of both resistance/tolerance factors.

PCR assays with of species-specific primer pairs, allowed us to verify the presence of novel viruses as well as the geographical dispersion across distinct Brazilian regions of previously described tomato-infecting begomoviruses. Thus far, only four begomoviruses associated with tomato plants were reported in the **North (Amazonic) region of Brazil** (Fonseca et al. 2010; Fonseca et al. 2011; Fonseca et al. 2013; Quadros et al. 2019). Herein, a new virus was detected in the state of Amazonas which was previously considered as a begomovirus-free area. We detected ToCMoGV in the AM–035 sample originating from Iranduba (AM) collected in 2016. This virus was already reported in French Guiana (Lett et al.,

2015). Our result further expands the number of begomovirus species that infect tomato plants in Brazil. Also in the North region, ToYSV (= Leonurus mosaic virus; see Chapter 04) was reported for the first infecting tomato plants in the states of Tocantins and Roraima. This ToYSV was detected in the samples TO–046 (collected in 2008 in the municipality of Gurupi), RR–003 and RR–004 (both collected in Boa Vista in 2013). Until now, reports of ToYSV infecting tomato plants in Brazil were restricted to the Southeast region, in the state of Minas Gerais (Calegario et al., 2007). Novel reports of tomato plants infected by ToMoLCV and SimMV in the North of Brazil. These relatively few reports of begomoviruses indicates that the North region is little explored in the study of viromes in tomato crops, but more extensive surveys may reveal a peculiar set of species in the tropical rain forest (Amazon) biome.

Thus far, ToMoLCV is the prevalent tomato-infecting begomivirus **in the warm and sem-arid Northeast region of Brazil** (Fernandes et al. 2008; Albuquerque et al. 2012; Ferro et al. 2017; Mituti et al. 2019), corroborating the results of the present study. However, before our results, there were no reports in the literature of infections in tomato plants by SimMV in the Brazilian Northeast region. SimMV infection, reported here for the first time, can be explained by the great transmission efficiency and the polyphagous habit of the vector *B. tabaci* (Gilbertson et al. 2015) as well as by the frequent presence of weeds of the genus *Sida* which are often in association with commercial tomato cultivation. This observation reinforces the epidemiological importance of as a repository and source of inoculum for tomato-infecting viruses (Barreto et al. 2013).

There is an overall lack of information about the panorama of begomovirus on tomatoes in the **South region of Brazil**. Only ToSRV infection was reported in tomatoes in Santa Catarina (Lima et al. 2007) and Paraná (Fernandes-Acioli et al. 2014) states. Here, we report by the first time the presence of ToSRV in Rio Grande do Sul and SimMV infecting tomato plants in Paraná State. We also provide the first confirmation of tomato plants infected by ToMoLCV in the across all states of the South regions (Paraná, Rio Grande do Sul and Santa Catarina). Our study conducted with yet a relative low number of samples (24) suggest that the viral diversity associated with tomatoes is likely to be underestimated in this region.

The number of begomoviruses in **Mid-West/Central region** is very high, corroborating previous studies in this geographic area (Ribeiro et al. 2007; Fernandes et al. 2008; Albuquerque et al. 2012; Macedo et al. 2018; Mituti et al. 2019; Reis et al. 2020; Souza et al. 2020). This is

the most important region for processing tomato production in the country. Our results confirm that ToSRV the most prevalent begomovirus in tomato this area (Cotrim et al. 2007; Fernandes et al. 2008; Reis et al. 2020). In fact, ToSRV shows the high adaptability, infecting a large number of hosts (Pereira-Silva et al. 2022) and being present across different regions of the country. These attributes of ToSRV may also explain its high prevalence in the Mid-West/Central region.

ToSRV, ToMoLCV, ToCMoV, and TGVV were found to be the most prevalent and with wider geographical distribution across the **temperate South-East region**. This region is the most important tomato-producing area for fresh-market consumption of the country and outbreaks of begomoviruses are very often detected across all states (Reis et al., 2020). The species ToSRV and ToMoLCV are the most relevant from the tomato breeding standpoint since they were often detected in association with tomato samples with and without the *Ty*–1/*Ty*–3 resistance factors, showing the high adaptive and dissemination capacity of the virus.

The alphasatellite was detected only in the Federal District, in the close cities of Gama (DF–024 and DF–027) and Ponte Alta (DF–057), revealing that, to date, this agent is endemic to the central region of Brazil. Satellite DNAs are subviral agents that can modulate viral pathogenesis depending on the interaction between the helper virus and the host plant (Fiallo-Olivé et al. 2016; Ferro et al. 2021; Kumar et al. 2021). The presence of alphasatellites associated with tomato crops was previously reported in Brazil by Reis et al. (2020) who found a subviral agent in tomato samples also from the Midwest, corroborating the results reported here. A closely related alphasatellite was formally detected in the weeds *Euphorbia heterophylla* (KY559640.1), *Sida* spp. (KX348227.1) and *Cleome affinis*, with either EuYMV or Cleome leaf crumple virus (ClLCrV) as helper viruses (Paprotka et al. 2010).

The TAGV (genus *Topilevirus*) was detected in a single sample in Central Brazil (GO–495), collected in 2001 in the city of Planaltina de Goiás (GO). The first report of this topilevirus in tomato plants was done also in Central Brazil (Fontenele et al., 2017). However, we detected the presence of the topilevirus ToALCV in Sao Paulo State (South-East region) in samples SP–172 and SP–173, both originating from Santo Antônio da Posse (SP) in 2015. As far as we know, the presence of ToALCV infecting tomato plants was restricted to the central region of Brazil (Batista et al., 2019; Souza et al. 2020). The first reports of topileviruses associated with tomato crops were made by Fontenele et al. (2017) in Brazil and Vaghi-Medina et al. (2018) in

Argentina. Currently, only two species are reported: tomato associated geminivirus (Fontenele et al. 2017) and tomato apical leaf curl virus (Vaghi-Media et al. 2018). One year after the publication of Vaghi-Medina et al. (2018), Batista et al. (2019) reported for the first time the occurrence of tomato apical leaf curl virus (ToALCV) infecting tomato plants in Central Brazil (Brasília-DF) and since then other studies have reported ToALCV associated with the crop in the Brazilian Mid-West/Central area (Souza et al .2020). Analyzes based on the amino acids of the CP protein were used to propose that ToALCV can be transmitted by the planthopper *Micrutalis maleifera* (family Membracidae). However, transmission trials have not yet been carried out to confirm this hypothesis (Vaghi-Medina et al. 2018). These successive reports of these viruses show the rapid distribution capacity of topileviruses. In fact, the result obtained here represents an expansion in the geographic distribution of the genus since it is the first report outside Central Brazil.

The use of the HTS sequencing platform associated with molecular detection via species-specific PCR primer was once again attested as a powerful strategy to study the dynamics of the tomato–begomovirus pathosystem. These same tools were applied by Reis et al. (2020), who found that the *Ty*–1 gene plays a role a "diversity filter" for begomoviruses. These results imply that viruses that infect tomato plants with these genes may be carrying that carry peculiar evolutionary/adaptive process. We also could observe that the number of tomato plants carrying either *Ty*–1 or *Ty*–3 genes displayed lower frequencies of both simple and mixed viral infections. Our study is the first to assess the impact of the *Ty*–3 gene/allele on the dynamics of the tomato/begomovirus pathosystem.

Herein, we increased the geographical amplitude of this pathosystem, encompassing different Brazilian ecosystems. Similar to what was observed by Reis et al. (2020), ToSRV was the prevalent one with higher levels of adaptation to the *Ty*–1/*Ty*–3 factors, followed by ToMoLCV, TGVV, and ToCMoV **(Figure 1**). It is important to highlight that ToMoLCV (a monopartite species) is the most widely distributed across the five Brazilian regions, not only in the Northeast, but reaching regions where ToSRV was not able to establish (**Table 7**). In addition, when comparing ToSRV and ToMoLCV regarding their ability to infect tomato plants with the presence of *Ty*–1/*Ty*–3 factors, both viruses displayed very similar frequency in these samples with 14 and 12 detections, respectively. The adaptation to the *Ty*–1/*Ty*–3 tolerance factors may be related to the diversity of viral RNA present in tomato plants that carry this gene, since it has already been found that the presence of the crinivirus tomato chlorosis virus (ToCV)

may reduce the efficiency of the *Ty*–1-mediated resistance/tolerance to tomato yellow leaf curl virus (ToYLCV) (Fortes et al., 2023). In this scenario, the use of gene pyramiding multiple virus resistance to crinivirus (Gonzales-Arcos et al. 2018) and begomoviruses would be a promising strategy for generate phenotypical stable sources of resistance (Prabhandakavi et al. 2021).

5. Conclusion

In our study, we demonstrated the efficiency of sequencing platforms via HTS combined with molecular detections by species-specific PCR were successfully employed as tools for studying viral diversity in different regions over different time intervals. Novel species and novel tomato-begomovirus interactions were detected. Herein, we provide a more accurate overview of the current situation of begomoviruses in tomato plants in Brazil that could help the understanding of the population dynamics of these viruses and their behaviour in relation to the main tolerance genes used to control these viruses in the country (*Ty*–1/*Ty*–3). The present study also provided new insights on the begomovirus distribution across the five producing macroregions in Brazil and the verification of the ToSRV and ToMoLCV as the most prevalent, most disseminated, and the best adapted to the *Ty*–1/*Ty*–3 factors. All this data contributes to guide tomato breeding programs regarding the most effective control strategies and to updating the current status of tomato begomoviruses in Brazil.

References

Adams IP, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene, M., Samuitiene M, Boonham, N (2009) Next-generation sequencing and metagenomic analysis: A universal diagnostic tool in plant virology. Molecular Plant Pathology 10:537–545

Albuquerque LC, Varsani A, Fernandes FR, Pinheiro B, Martin DP, Ferreira PTO, Lemos TO, Inoue–Nagata AK (2012) Further characterization of tomato-infecting begomoviruses in Brazil. Archives of Virology 157:747–752

Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, Chen L, Lapidot M, Levin I (2009) Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from *Solanum peruvianum*. Theoretical and Applied Genetics 119:519–530

Arguello-Astorga G, Lopez-Ochoa L, Kong LJ, Orozco BM, Settlage SB, Hanley-Bowdoin L (2004) A novel motif in geminivirus replication proteins interacts with the plant retinoblastoma-related protein. Journal of Virology 78:4817–4826

Arguello-Astorga G, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminiviruses: identification of potential interacting amino acid-base pairs by a comparative approach. Archives of Virology 146:1465–1485

Barreto S, Hallwass M, Aquino O, Inoue-Nagata A. 2013. A study of weeds as potential inoculum sources for a tomato-infecting begomovirus in central Brazil. Phytopathology 103: 436–444.

Batista JG, Melo FL, Pereira-Carvalho RC, Alves-Freitas DMT, Ribeiro SG (2019) First report of tomato apical leaf curl virus infecting tomato in Brazil. Plant Disease 103:1 Disponível em: https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-09-18-1636-PDN Acessado em: 15 de julho de 2022.

Bejerman N, Roumagnac P, Nemchinov LG (2020) High-throughput sequencing for deciphering the virome of alfalfa (*Medicago sativa* L.). Frontiers in Microbiology 11:1–17

Bian XY, Thomas MR, Rasheed MS, Saeed M, Hanson P, De Barro PJ, Rezaian MA (2007) A recessive allele (tgr-1) conditioning tomato resistance to geminivirus infection is associated with impaired viral movement. Phytopathology 97:930–937

Boiteux LS, Fonseca MEN, Giordano LB, Melo PCT (2012) Melhoramento genético. Produção de tomate para processamento industrial. Embrapa p. 31–50

Boiteux LS, Fonseca MEN, Simon PW (1999) Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. Journal American Society for Horticultural Science 124:32–38

Briddon RW, Patil BL, Bagewadi B, Nawaz-ul-Rehman MS, Fauquet CM (2010) Distinct evolutionary histories of the DNA–A and DNA–B components of bipartite begomoviruses. BMC Evolutionary Biology 10:1–17

Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JCF, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of begomovirus taxonomy based on pairwise sequence comparisons. Archives of Virology 160:1593–1619

Butterbach P, Verlaan MG, Dullemans A, Lohuis D, Visser RG, Bai Y, Kormelink R (2014) Tomato yellow leaf curl virus resistance by *Ty–1* involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. Proceedings of the National Academy of Sciences 35:12942–12947

Calegario RF, Ferreira SS, Andrade EC, Zerbini FM (2007) Characterization of Tomato yellow spot virus, a novel tomato-infecting begomovirus in Brazil. Pesquisa Agropecuária Brasileira 42:1335–1343

Calegario, R. F. (2004). Caracterização de um isolado do begomovírus Sida micrantha mosaic virus (SimMV). (Dissertação de Mestrado)

Cantú-Iris M, Pastor-Palacios G, Mauricio-Castillo JA, Bañuelos-Hernández B, Avalos-Calleros JA, Juárez-Reyes A, Rivera-Bustamante R, Argüello-Astorga GR (2019) Analysis of a new begomovirus unveils a composite element conserved in the CP gene promoters of

several Geminiviridae genera: Clues to comprehend the complex regulation of late genes. PloS One 14:1–24

Castillo-Urquiza GP, Beserra JR JEA, Bruckner FP, Lima ATM, Varsani A, Alfenas-Zerbini P, Zerbini FM (2008) Six novel begomoviruses infecting tomato and associated weeds in Southeastern Brazil. Archives of Virology 153:1985–1989

Colariccio A, Eiras M, Chaves ALR, Bergmann JC, Zerbini FM, Harakava R, Chagas CM (2007) Tomato yellow vein streak virus, a new begomovirus on tomato from Brazil: complete DNA–A sequence and some molecular and biological features. Journal of Plant Pathology 89:385–390

Costa AS, Oliveira AR, Silva DM (1975) Transmissão mecânica do mosaico dourado do tomatiro. Revista da Sociedade Brasileira de Fitopatologia 6:147

Cotrim AA, Krause-Sakate R, Narita N, Zerbini FM, Pavan MA (2007) Diversidade genética de begomovírus em cultivos de tomateiro no Centro-Oeste Paulista. Summa Phytopathologica 33:300–303

Cooper JI, Jones AT (1983) Responses of plants to viruses: proposals for the use of terms. Phytopathology 73:127

De Barro PJ, Liu S-S, Boykin LM, Dinsdale AB (2011) *Bemisia tabaci*: A statement of species status. Annual Review of Entomology 56:1–19

Duarte MF, Fonseca ME, Costa H, Fernandes NA, Reis A, Boiteux LS, Pereira-Carvalho RC (2021a). Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. Virus Genes 57:83–93

Duarte MF, Pereira-Carvalho RC, Reis LNA, Rojas MR, Gilbertson RL, Costa H, Boiteux LS, Fonseca MEN (2021b) Natural infection of tomatoes (*Solanum lycopersicum*) by Euphorbia yellow mosaic virus isolates across four Brazilian states. Plant Disease 105:517-518

Faria JC, Souza-Dias J, Slack S, Maxwell D (1997) A new geminivirus associated with tomato in the state of São Paulo, Brazil. Plant Disease 81:423–423.

Fernandes-Acioli, NAN, Boiteux LS, Fonseca MDN, Segnana LRG, Kitajima EW (2014) Report of Tomato yellow spot virus infecting *Leonurus sibiricus* in Paraguay and within tomato fields in Brazil. Plant Disease 98:1445–1445

Fernandes DS, Okuma D, Pantoja-Gomez LM, Cuenca A, Corrêa AS (2022) *Bemisia tabaci* MEAM1 still remains the dominant species in open field crops in Brazil. Brazilian Journal of Biology 84:1–8

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

Fernandes NAN (2015) Begomoviroses no cultivo do tomateiro no Brasil: Variabilidade e caracterização de novas espécies virais e diversidade do vetor *Bemisia tabaci*. (Tese de Doutorado)

Ferro MM, Ramos-Sobrinho R, Silva JT, Assunção IP, Lima GS (2017) Genetic structure of populations of the begomoviruses *Tomato mottle leaf curl virus* and *Sida mottle Alagoas virus* infecting tomato (*Solanum lycopersicum*) and *Sida* spp., respectively. Tropical Plant Pathology 42:39-45

Ferro CG, Zerbini FM, Navas-Castillo J, Fiallo-Olivé E (2021) Revealing the complexity of sweepovirus-deltasatellite–plant host interactions: expanded natural and experimental helper virus range and effect dependence on virus-host combination. Microorganisms 9:1–19

Fiallo-Olivé E, Lett JM, Martin DP, Roumagnac P, Varsani A, Zerbini FM, Navas-Castillo J, Consortium IR (2021) ICTV virus taxonomy profile: *Geminiviridae* 2021. The Journal of General Virology 102:1–2.

Fiallo-Olivé E, Navas-Castillo J (2020) Molecular and biological characterization of a New World mono-/bipartite begomovirus/deltasatellite complex infecting *Corchorus siliquosus*. Frontiers in Microbiology 11:1–14

Fiallo-Olivé, E, Navas-Castillo, J. (2023). The role of extensive recombination in the evolution of geminiviruses. Viral Fitness and Evolution: Population Dynamics and Adaptive Mechanisms 439: 139–166

Fiallo-Olivé E, Tovar R, Navas-Castillo J (2016) Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. New Phytologist 212:680–692

Fontes EP, Eagle PA, Sipe PS, Luckow VA, Hanley-Bowdoin L (1994) Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. Journal of Biological Chemistry 269:8459-8465

Fortes IM, Fernández-Muñoz R, Moriones E (2023). The crinivirus tomato chlorosis virus compromises the control of tomato yellow leaf curl virus in tomato plants by the *Ty*–1 gene. Phytopathology 2:54

Flores E, Silberschmidt K, Kramer M (1960) Observações de "clorose infecciosa" das malváceas em tomateiros do campo. O Biológico 26:65–69

Fonseca M, Boiteux LS, Fernandes N (2011) Genomic organization of Chino del tomate Amazonas virus a bipartite begomovirus endemic to the Amazon region of Brazil. [Genbank]. Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/NC_038443.1 Acessado em: 10 de julho de 2022

Fonseca M, Boiteux LS, Fernandes N (2013) tomato bright yellow mosaic virus and Tomato bright yellow mottle virus: two new tomato-infecting begomoviruses in Brazil. [Genbank] Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/KC791690.1 Acessado em 10 de julho de 2022

Fonseca M, Boiteux LS, Fernandes N (2010) tomato golden leaf distortion virus, a new begomovirus infecting tomato in Tocantins state, Brazil. [Genbank] Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/NC_043122.1 Acessado em: 10 julho de 2022

Fontenele RS, Lamas NS, Lacorte C, Lacerda ALM, Varsani A, Ribeiro SG (2017) A novel geminivirus identified in tomato and cleome plants sampled in Brazil. Virus Research 240:175–179

Gaafar YZ, Herz K, Hartrick J, Fletcher J, Blouin AG, MacDiarmid R, Ziebell H (2020) Investigating the pea virome in Germany–Old friends and new players in the field (s). Frontiers in Microbiology 11:1–15

Galvao RM, Mariano AC, Luz DF, Alfenas PF, Andrade EC, Zerbini FM, Fontes EP (2003) A naturally occurring recombinant DNA–A of a typical bipartite begomovirus does not require the cognate DNA–B to infect *Nicotiana benthamiana* systemically. Journal of General Virology 84:715–726

González-Arcos, M., Fonseca, MEN, Arruabarrena A, Lima, MF, Michereff-Filho M, Moriones E., Fernandez-Munhoz R, Boiteux, LS (2018). Identification of genetic sources with attenuated Tomato chlorosis virus-induced symptoms in *Solanum* (section *Lycopersicon*) germplasm. Euphytica, 214: 178.

García-Cano E, Resende RO, Boiteux LS, Giordano LB, Fernández-Muñoz R, Moriones E (2008) Phenotypic expression, stability, and inheritance of a recessive resistance to monopartite begomoviruses associated with tomato yellow leaf curl disease in tomato. Phytopathology 98:618–627

Gilbertson RL, Batuman O, Webster CG, Adkins S (2015) Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annual Review of Virology 2:67–93

Gill U, Scott JW, Shekasteband R, Ogundiwin E, Schuit C, Francis DM, Sim SC, Smith H, Hutton SF (2019) Ty-6, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus. Theoretical and Applied Genetics 132:1543–1554

Guajardo-Leiva S, Chnaiderman J, Gaggero A, Díez B (2020) Metagenomic insights into the sewage RNA virosphere of a large city. Viruses 12:1–15

Hanson P, Green S, Kuo G (2006) Ty-2, a gene on chromosome 11 conditioning geminivirus resistance in tomato. Report of the Tomato Genetics Cooperative 56:17–18

Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S (2013) Geminiviruses: masters at redirecting and reprogramming plant processes. Nature Reviews Microbiology 11:777–788

Host Data Base (2022) Disponível em: https://www.genome.jp/virushostdb/view/?VirusHostLineage=ssDNA|Viruses. Acessado em: 15 de junho de 2022.

Hu T, Chitnis N, Monos D, Dinh A (2021) Next-generation sequencing technologies: An overview. Human Immunology 82:801–811

Hu T, Song Y, Wang Y, Zhou X (2020) Functional analysis of a novel βV1 gene identified in a geminivirus betasatellite. Science China Life Sciences 63:688–696

International Committee on Taxonomy of Viruses (ICTV). Disponível em: https://ictv.global/taxonomy. Acessado em: 09 de janeiro de 2024

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. Journal of Virological Methods 116:209–211

Ji Y, Schuster DJ, Scott JW (2007) *Ty–3*, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus *Ty–1* on chromosome 6 of tomato. Molecular Breeding 20:271–284

Ji Y, Scott JW, Schuster DJ (2009a) Toward fine mapping of the tomato yellow leaf curl virus resistance gene *Ty*–2 on chromosome 11 of tomato. HortScience 44:614–618

Ji Y, Scott JW, Schuster DJ, Maxwell DP (2009b) Molecular mapping of Ty-4, a new tomato yellow leaf curl virus resistance locus on chromosome 3 of tomato. Journal of the American Society for Horticultural Science 134:281–288

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

Kitajima EW (2020). An annotated list of plant viruses and viroids described in Brazil (1926-2018). Biota Neotropica, 20: 2 – 21

Kumar M, Zarreen F, Chakraborty S (2021) Roles of two distinct alphasatellites modulating geminivirus pathogenesis. Virology Journal 18:1–11

Li F, Xu X, Huang C, Gu Z, Cao L, Hu T, Zhou X (2015) The AC 5 protein encoded by Mungbean yellow mosaic India virus is a pathogenicity determinant that suppresses RNA silencing-based antiviral defenses. New Phytologist 208:555–569

Li P, Su F, Meng Q, Yu H, Wu G, Li M, Qing L (2021) The C5 protein encoded by Ageratum leaf curl Sichuan virus is a virulence factor and contributes to the virus infection. Molecular Plant Pathology 22:1149–1158.

Lima ATM, Pereira CDO, Alfenas PF, Paula MB, Mello RN, Zerbini FM (2006). Primeiro relato de infecção pelo geminivírus Tomato severe rugose virus (ToSRV) em tomateiro no estado de Santa Catarina. Fitopatologia Brasileira 31:1

Luria N, Smith E, Sela N, Koren A, Lachman O, Dombrovsky A (2019) Insights sobre um viroma de melancia contribuem para monitorar a distribuição de vírus transmitidos por mosca-branca. Phytobiomes Journal 3:61–70

Macedo MA, Costa TM, Barbosa JC, Pereira JL, Michereff-Filho M, Gilbertson R L, Inoue-Nagata AK, Bergamin Filho A (2018) Temporal and spatial dynamics of begomovirus disease in tomatoes in central Brazil. Plant Pathology 66:529–538

Maclot F, Candresse T, Filloux D, Malmstrom CM, Roumagnac P, Van-der-Vlugt R, Massart, S (2020) Illuminating an ecological blackbox: using high throughput sequencing to characterize the plant virome across scales. Frontiers in Microbiology 11:1–16

Machado M, Fonseca MEN, Ferro DDX, Boiteux LS (2013). Disponível em: https://www.alice.cnptia.embrapa.br/handle/doc/1001734. Acessado em: 10 de outubro de 2021

Martins TP, Souza TA, da Silva PS, Nakasu EY, Melo FL, Inoue-Nagata AK, Nagata T (2021) Nanopore sequencing of tomato mottle leaf distortion virus, a new bipartite begomovirus infecting tomato in Brazil. Archives of Virology 166:3217–3220

Matyis JC, Silva DM, Oliveira AAR, Costa AS (1975) Purificação e morfologia do vírus do mosaico dourado do tomateiro. Summa Phytopathologica 1:267–274

Maxwell DP, Martin C, Sallust MS, Montes L, Mejía L (2006) Tagging begomovirus resistance genes. Disponível em: www.plantpath.wisc.edu/. Acessado em: 10 de outubro de 2021

Melgarejo TA, Rojas MR, Gilbertson RL (2019). A bipartite begomovirus infecting *Boerhavia erecta* (family Nyctaginaceae) in the Dominican Republic represents a distinct phylogenetic lineage and has a high degree of host specificity. Phytopathology 109:1464–1474

Menzel P, Ng K, Krogh A (2016) Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nature Communications 7:1–9

Mituti T, Moura MF, Macedo MA, Silva TN, Pinto LR, Costa H, Krause-Sakate R, Inoue-Nagata A, Nunes GG, Lima MF, Rezende JA (2019) Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil. Tropical Plant Pathology 44:468–472

Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE 9:1–8

Noueiry AO, Lucas WJ, Gilbertson RL (1994) Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. Cell 76:925–932

Paprotka T, Metzler V, Jeske H (2010) The first DNA 1-like α satellites in association with New World begomoviruses in natural infections. Virology 404:148–157

Pereira-Carvalho RC, Díaz-Pendón JA, Fonseca MEN, Boiteux LS, Fernández-Muñoz R, Moriones E, Resende RO (2015) Recessive resistance derived from Tomato cv. Tyking-limits drastically the spread of Tomato yellow leaf curl virus. Viruses 7: 2518–2533

Pereira-Carvalho RC, Reis-GoncalvesVPM, Boiteux LS, Fonseca MEN, Naito FY, Rojas MR, Gilbertson RL [GenBank] Disponível em: https://www.ncbi.nlm.nih.gov/nuccore/MN145943.1 Acessado em: 10 de junho de 2020.

Pereira-Silva J, Boiteux LS, Fonseca MEN, Reis LNA, Souza AS, Nery FMB, Madeira NR, Pereira-Carvalho RC (2022) Novel natural hosts of tomato severe rugose virus (ToSRV) in the Fabaceae, Solanaceae, and Oxalidaceae families. Journal of Plant Diseases and Protection. 129:425–431.

Prabha K, Baranwal VK, Jain RK (2013) Applications of next generation high throughput sequencing technologies in characterization, discovery and molecular interaction of plant viruses. Indian Journal of Virology 24:157–165

Prabhandakavi P, Pogiri R, Kumar R, Acharya S, Esakky R, Chakraborty M, Pinnamaneni R, Palicherla SR (2021) Pyramiding *Ty*-1/*Ty–3*, *Ty*-2, *ty*-5 and *ty*-6 genes into tomato hybrid to develop resistance against tomato leaf curl viruses and recurrent parent genome recovery by ddRAD sequencing method. Journal of Plant Biochemistry and Biotechnology 30:462–476

Quadros AF, Silva JP, Xavier CA, Zerbini FM, Boari AJ (2019) Two new begomoviruses infecting tomato and *Hibiscus* sp. in the Amazon region of Brazil. Archives of Virology 164:1897–1901

Quintanilha-Peixoto G, Fonseca PLC, Raya FT, Marone MP, Bortolini DE, Mieczkowski P, Olmo RP, Carazzolle MF, Voigt CA, Soares ACF, Pereira GAG, Góes-Neto A, Aguiar ERGR (2021) The sisal virome: uncovering the viral diversity of agave varieties reveals new and organ-specific viruses. Microorganisms 9:1–21

Rêgo-Machado CM, Nakasu EY, Blawid R, Nagata T, Inoue-Nagata AK (2019) Complete genome sequence of a new bipartite begomovirus infecting tomato in Brazil. Archives of Virology 164: 2873–2875

Reis, LNA (2020) Metagenomic analysis of the begomovirus diversity in tomatoes in Central Brazil and impact of the *Ty–1* tolerance gene on viral evolutionary dynamics. (Tese de Doutorado). Universidade de Brasília (UnB).

Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RDC (2020) Metagenomics of neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty–1* gene. Viruses *12*:1–22

Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RDC (2021) Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome-wide pairwise identity of multiple isolates. Virus Genes 57:127–131

Reis LNA, Boiteux LS, Fonseca MEN, Rojas MR, Gilbertson RL, Pereira-Carvalho RC (2023) Tomato golden net virus and tomato yellow net virus: two novel New World begomoviruses with monopartite genomes. Archives of Virology 168:1-6

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 begomoviruses in Brazil. Archives of Virology 148: 281–295

Ribeiro SG, Martin DP, Lacorte C, Simões IC, Orlandini DRS, Inoue-Nagata AK (2007) Molecular and biological characterization of tomato chlorotic mottle virus suggests that recombination underlies the evolution and diversity of Brazilian tomato begomoviruses. Phytopathology 97:702–711

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

Rojas MR, Macedo M, Maliano M, Soto-Aguilar M, Souza J, Briddon R, Kenyon L, Rivera Bustamante R, Zerbini F, Adkins S (2018) World management of geminiviruses. Annual Review of Phytopathology 56: 637–677

Rosen R, Kanakala S, Kliot A, Pakkianathan BC, Farich BA, Santana-Magal N, Elimelech M, Kontsedalov S, Lebedev G, Cilia M (2015) Persistent, circulative transmission of begomoviruses by whitefly vectors. Current Opinion in Virology 15:1–8

Roossinck MJ (1997) Mechanisms of plant virus evolution. Annual Review of Phytopathology, 35:191–209

Scott JW, Hutton SF, Freeman JH (2015) Fla. 8638B and Fla. 8624 tomato breeding lines with begomovirus resistance genes *ty*-5 plus *Ty*-6 and *Ty*-6, respectively. HortScience 50:1405–1407

Schneider J, Engler M, Hofmann J, Selinka HC, Jones TC, Drosten C, Diedrich S, Corman VM, Böttcher S (2021) Molecular detection of cosaviruses in a patient with acute flaccid paralysis and in sewage samples in Germany. Virus Research 297:1–5

Seal S, VandenBosch F, Jeger M (2006) Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Critical Reviews in Plant Sciences, 25:23–46

Silva SJC, Castillo-Urquiza GP, Hora-Júnior BT, Assunção IP, Lima GSA, Pio-Ribeiro G, Mizubuti ESG, Zerbini FM (2012) Species diversity, phylogeny and genetic variability of begomovirus populations infecting leguminous weeds in northeastern Brazil. Plant Pathology 61:457–467

Silva FN, Lima AT, Rocha CS, Castillo-Urquiza GP, Alves-Júnior, M, Zerbini FM (2014) Recombination and pseudorecombination driving the evolution of the begomoviruses tomato severe rugose virus (ToSRV) and tomato rugose mosaic virus (ToRMV): two recombinant DNA–A components sharing the same DNA–B . Virology Journal 11:1–66

Souza TA, Silva JMF, Nagata T, Martins TP, Nakasu EYT, Inoue-Nagata AK (2020) A temporal diversity analysis of brazilian begomoviruses in tomato reveals a decrease in species richness between 2003 and 2016. Frontiers in Plant Science 11:1–13

Sunter G, Hartitz MD, Hormuzdi SG, Brough CL, Bisaro DM (1990) Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for efficient DNA replication. Virology 179:69–77

Tavares SS, Ramos-Sobrinho R, González-Aguilera J, Lima GSA, Assunção IP, Zerbini FM (2012) Further molecular characterization of weed-associated begomoviruses in Brazil with an emphasis on Sida spp. Planta Daninha 30:305–315

Vaghi-Medina CG, Teppa E, Bornancini VA, Flores CR, Marino-Buslje C, López Lambertini PM (2018) Tomato apical leaf curl virus: a novel, monopartite geminivirus detected in tomatoes in Argentina. Frontiers in Microbiology 8:1–11

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RG, Scott JW, Edwards JD, Bai Y (2013) The tomato yellow leaf curl virus resistance genes *Ty*–1 and *Ty*–3 are allelic and code for DFDGD-class RNA–dependent RNA polymerases. PLoS Genetics 9:1–11

Villamor DEV, Ho T, Rwahnih MA,Martin RR, Tzanetakis IE (2019) High throughput sequencing for plant virus detection and Discovery. Phytopathology 109:716–725

Watanabe LFM, Bello VH, De Marchi BR, Da Silva FB, Fusco LM, Sartori MM, Pavan MA, Krause-Sakate R (2019) Performance and competitive displacement of *Bemisia tabaci* MEAM1 and MED cryptic species on different host plants. Crop Protection 124:1–6

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleban T, Van-Oss H. 1994. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, *Ty*-1. Theoretical and Applied Genetics 88: 141–146

Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J, Rivera-Bustamante R, Roumagnac P, Varsani A, Consortium, IR (2017) ICTV virus taxonomy profile: *Geminiviridae*. The Journal of General Virology 98:131–133

CAPÍTULO 03

Genomic characterization of a highly divergent tomato chlorotic mottle Guyane virus strain in the Brazilian Amazon River Basin.

Genomic characterization of a highly divergent tomato chlorotic mottle Guyane virus strain in the Brazilian Amazon River Basin.

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Abstract

Foliar samples were collected from the tomato (*Solanum lycopersicum*) cultivar 'IPA-6' exhibiting begomovirus-like symptoms (apical leaf curling and chlorosis) in Iranduba (Brazilian Amazon). PCR tests with degenerate primers targeting DNA–A and DNA–B components of a sample designated as AM–035 were positive for both genomic regions. Full-length cognate DNA–A and DNA–B components of AM–035 were cloned and sequenced via Sanger dideoxy termination reaction. The complete sequence of the DNA–A component (MK878452) comprised 2,630 nucleotides (organized in five open reading frames – ORFs), and the DNA–B component (MK878451) displayed 2,593 nucleotides (two ORFs). The highest identities ranged from 90 to 92% with tomato chlorotic mottle Guyane virus (ToCMoGV) isolates from French Guiana, indicating a highly divergent Brazilian strain of this virus. The present work reinforces the scenario of the high diversity of tomato-infecting begomoviruses in Brazil. Further studies are needed to determine the distribution and prevalence of ToCMoGV across tomato-producing areas in the Brazilian Amazon River Basin.

Keywords: Begomoviruses, tomato, HTS, virome, Brazilian biomes.

Introduction

The tomato (*Solanum lycopersicum* L.) crop is severely affected by diseases caused by a complex of more than 20 begomoviruses occurring under Brazilian conditions (Reis et al., 2023). These pathogens are distributed across the main production areas in the Northeast, Central, and Southeast regions (Duarte et al., 2021; Reis et al., 2021). Nonetheless, information on tomato-infecting begomoviruses occurring in the Brazilian Amazon is yet very limited, as is the knowledge of viral diversity in this geographic area.

The metagenomic analyses have allowed the discovery of new members of the family *Geminiviridae* associated with the tomato crop in Neotropical areas (Reis et al., 2020). In the present work, we report a highly divergent strain of tomato chlorotic mottle Guyane virus (ToCMoGV) infecting tomatoes in production areas encompassing the Brazilian Amazon River Basin.

Material and Methods

In 2016, in Iranduba district (State of Amazonas–AM), ten samples of tomato leaves (AM–032 to AM–042 isolates) were collected from individual plants displaying begomovirus-like symptoms (apical leaf curl and chlorosis) in addition to persistent association with whitefly (*Bemisia tabaci*) infestation. A total of three commercial fields were sampled with all of them displaying ≈ 5% of plants with conspicuous viral symptoms. Foliar DNA extraction, PCR assays with degenerate primer pairs 'PAL1978/PAR496' and 'PBL1/ CRC-1', and enrichment of circular DNAs by Rolling Circle Amplification (RCA) were performed according to Reis et al. (2020). The PCR-amplified fragments were sequenced via Sanger dideoxy termination reaction. A sample with positive PCR tests for begomovirus (designated as AM–035) was enriched via RCA and subsequently digested with ApaI and EcoRV restriction enzymes. The putative full-length DNA–A and DNA–B monomeric components present in the sample AM–035 were cloned into vectors pSL1180/AM–035DNA–A/ApaI and pSL1180/AM–035DNA–

B/EcoRV, respectively (Melgarejo et al., 2015). AM–035 and other samples with positive PCR tests for begomoviruses from Iranduba–AM (*n*=5) were also submitted to High Throughput Sequencing (HTS) technology in an Illumina platform with the NovaSeq-6000 system (Macrogen Inc., South Korea). The HTS-derived begomovirus sequences were analyzed according to Reis et al. (2020). The viral contigs were annotated and the reads were trimmed using the Geneious® 11.0 program (Kearse et al., 2012). Sequences were initially analyzed with the BLASTn algorithm, and sequence identity to the closest begomoviruses was determined with Species Demarcation Tool v.1.2 (SDT). Full-length DNA–A genomes were aligned with the MUSCLE multiple alignment program. Phylogenetic trees were generated by Maximum likelihood phylogenies – PhyML with HKY + I + G nucleotide substitution model selected by jModelTest with 1,000 bootstrap replications in the Geneious® 11.0 program (Kearse et al., 2012). Recombination events were also evaluated using the RDP5 program (Martin et al., 2021).

Results

Amplicons of the expected-size (1.1 kb and 0.5 kb) were obtained after PCR tests with the degenerate primer pairs, indicating the infection by a bipartite begomovirus in the foliar tomato sample designated as AM–035. The complete sequence of the DNA–A component (MK878452) comprised 2,630 nucleotides, and the DNA–B component (MK878451) displayed 2,593 nucleotides. Sanger dideoxy termination sequencing of the AM–035 isolate showed a typical genomic organization of New World (NW) bipartite begomoviruses (Fig.1). The sequence comparisons of the DNA–A component of AM–035 indicated 92% identity with ToCMoGV isolates (KR263179 to KR263181) from French Guiana (Lett et al., 2015). The DNA–B component showed 90% identity with the DNA–B component of ToCMoGV (KR263172-KR263178). The complete and nearly identical sequences of the DNA–A and

DNA–B components of the isolate AM–035 were recovered by both HTS and Sanger dideoxy termination sequencing.

The DNA–A of the AM–035 isolate was found to be organized into five ORFs, whereas the DNA–B genome was composed of two ORFs (Fig. 1). A common region (CR) of 210 nucleotides was identified for the components of AM–035 and their CR sequences were 91% identical. Both components exhibited a nonanucleotide (TAATATTAC) and the iteron GGTGA – Rep IRD: MPPPKRFRIN (Fig. 1). The CP gene promoter region of the DNA–A displayed the quasi-palindromic motif ACTT-AGTCCCC-AAGT, identical to the ToCMoGV isolate reported in French Guiana (KR263179 to KR263181). The amino acid sequence of the Helix 4 motif was identified from the CP gene region via *in silico* translation of its amino acids. The following Helix 4 motif sequence was annotated for the isolate AM–035: MDF GQV FNM FDN EPS TAT VKN DLR DRF QVM HRF YGK VTG GQY ASN EQA LVK RFW KVN N. Only four amino acid differences were detected in comparison to the sequences of the ToCMoGV isolates from French Guiana (KR263179 to KR263181). The phylogenetic analysis performed with the complete DNA–A sequence (Fig. 2) assigned the AM–035 isolate into a clade with the ToCMoGV isolates from French Guiana (KR263180). SDT alignments of the DNA–A sequence indicated 92 % identity with ToCMoGV isolates, which confirmed that AM–035 is a highly divergent viral strain (Fig. 2). In comparison to other bipartite begomoviruses from the NW, ToCMoGV from French Guiana, and AM–035 showed phylogenetic relationships to bean golden yellow mosaic virus (BGYMV) (GenBank D00201 = the validated REFSEQ accession NC_001439), sharing nucleotide identity of 76% (Fig. 2). No significant recombination events were detected.

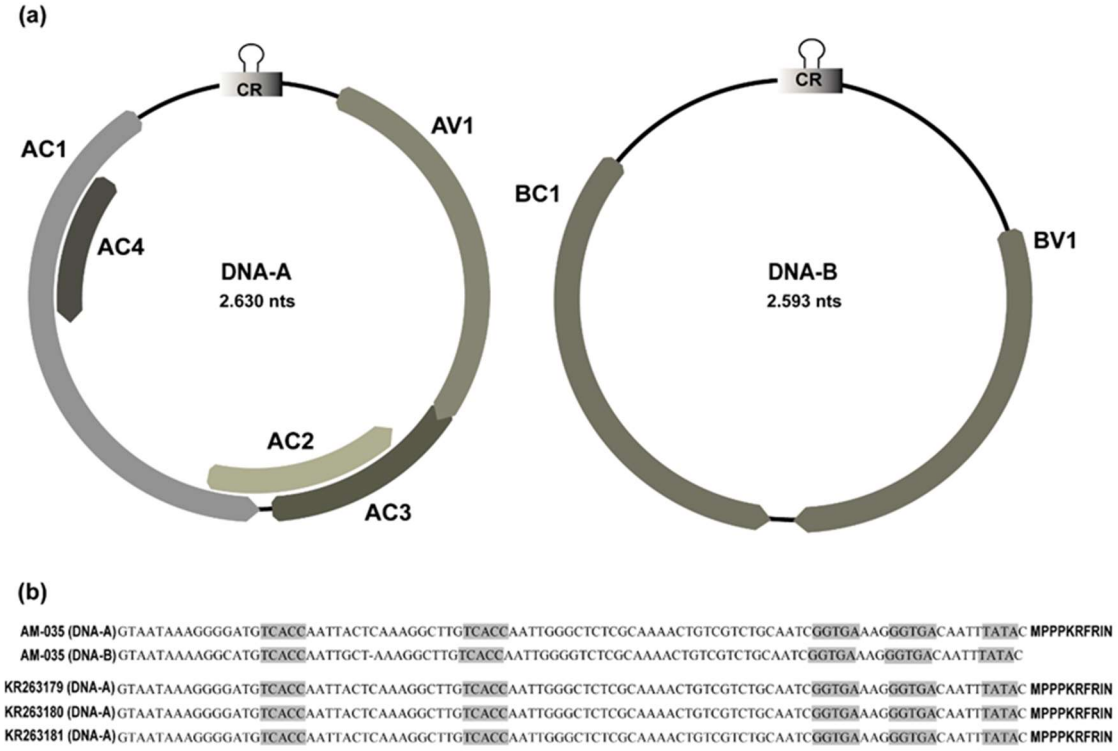

Fig. 1 Diagrammatic representation of the genome organization of a highly divergent strain (AM–035) of tomato chlorotic mottle Guyane virus (ToCMoGV). (a) The following open reading frames (ORFs) were annotated in the DNA–A component according to their presumed function of the coding proteins: AV1 = capsid protein; AC1 = replication-associated protein; AC2 = transactivator protein; AC3 = replication enhancer; AC4 = symptom determinant protein. The following ORFs were annotated in the DNA–B component: BV1 = Nuclear shuttle protein; BC1 = protein involved in the cell-to-cell viral movement, and CR = common region, encompassing the hairpin (in both components). (b) Iteron GGTGA, complementary CCACT, TATAbox, and Rep-IRD MPPPKRFRIN were highlighted in the CR sequence alignment of both DNA components of AM–035 with the DNA–A segment of ToCMoGV isolates from French Guiana (KR263179 to KR263181).

Discussion

Herein, we report the first detection of ToCMoGV infecting tomatoes in Brazil. Lett et al., (2015) first described this virus in French Guiana and isolates of both countries are distantly related to other begomoviruses occurring in Brazil as indicated by a comprehensive phylogenetic analysis with representative members of the genus *Begomovirus* occurring in the NW (Fig. 2). The close phylogenetic relationship of Brazilian and French Guiana isolates suggest that ToCMoGV belongs to a recently emerged lineage of NW bipartite begomoviruses

(Lett et al., 2015). Likewise, the close relationship of the French Guiana ToCMoGV isolates and the AM–035 isolate to the BGYMV bipartite lineage reinforces the phylogenetic cohesion that exists among the NW bipartite begomoviruses. The present work underpins the scenario of high (and not yet fully characterized) diversity of tomato-infecting begomoviruses in Brazil (Reis et al., 2020, 2023). Additional studies are needed to determine the distribution, prevalence, and economic importance of this virus for tomato crops in the Amazon region, and whether available resistance sources (Giordano et al., 2005; Pereira-Carvalho et al., 2010) are also effective against ToCMoGV isolates.

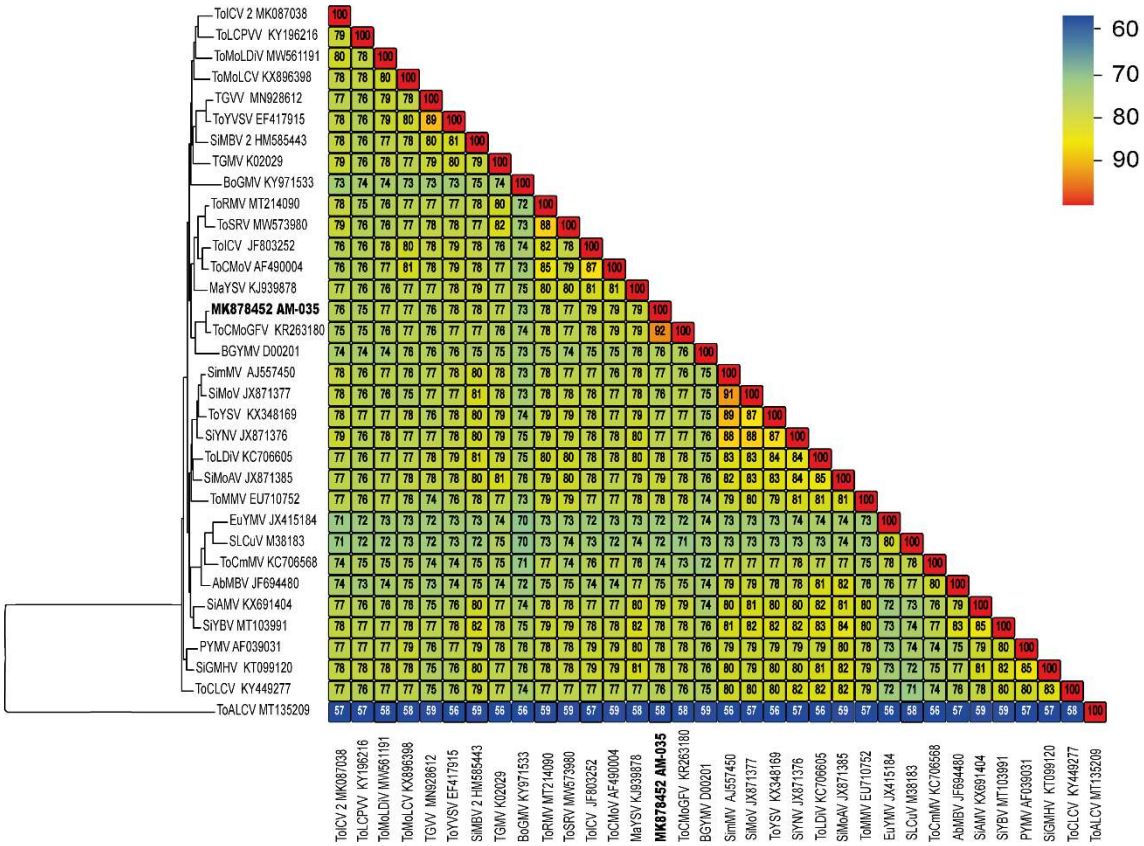

Fig. 2 Phylogenetic trees and Sequence Demarcation tool (SDT) of tomato chlorotic mottle Guyane virus DNA–A. Isolate AM–035 grouped in the same clade as ToCMoGV from French Guiana (KR263180). The nucleotide identity of the DNA–A segment between isolates AM-35 and KR263180 was 92%. 1. ToICV-2 – Tomato interveinal chlorosis virus-2 (MK087038); 2. ToLCPVV – Tomato leaf curl purple vein virus (KY196216); 3. ToMoLDiV – Tomato mottle leaf distortion virus (MW561191); 4. ToMoLCV – Tomato mottle leaf curl virus (KX896398); 5. TGVV – Tomato golden vein virus isolate (MN928612); 6. ToYVSV – Tomato yellow vein streak virus (EF417915); 7. SiMBV-2 – Sida mosaic Bolivia virus 2 (HM585443); 8. TGMV – Tomato golden mosaic virus (K02029); 9. BoGMV – Boerhavia golden mosaic virus

(KY971533); 10. ToRMV – Tomato rugose mosaic virus isolate (MT214090); 11. ToSRV – Tomato severe rugose virus isolate (MW573980); 12. ToICV – Tomato interveinal chlorosis virus (JF803252); 13. ToCMoV – Tomato chlorotic mottle virus (AF490004); 14. MaYSV – Macroptilium yellow spot virus (KJ939878); 15. AM–035 – Tomato chlorotic mottle Guyane virus (MK878452); 16. ToCMoGFV – Tomato chlorotic mottle Guyane virus (KR263180); 17. BGYMV – Bean golden yellow mosaic virus (D00201); 18. SimMV – Sida micrantha mosaic virus (AJ557450); 19. SiMoV – Sida mottle virus (JX871377); 20. ToYSV – Tomato yellow spot virus isolate (KX348169); 21. SiYNV – Sida yellow net virus (JX871376); 22. ToLDiV – Tomato leaf distortion virus (KC706605); 23. SiMoAV – Sida mottle Alagoas virus (JX871385); 24. ToMMV – Tomato mild mosaic virus (EU710752); 25. EuYMV – Euphorbia yellow mosaic virus (JX415184); 26. SLCuV – Squash leaf curl virus (M38183); 27. ToCmMV – Tomato common mosaic virus (KC706568); 28. AbMBV – Abutilon mosaic Brazil virus (JF694480); 29. SiAMV – Sida angular mosaic virus isolate (KX691404); 30. SiYBV – Sida yellow blotch virus (MT103991); 31. PYMV – Potato yellow mosaic virus (AF039031); 32. SGMHV – Sida golden mosaic Honduras virus (KT099120); 33. ToCLCV – Tomato chlorotic leaf curl virus (KY449277); 34. ToALCV – Tomato apical leaf curl virus (MT135209)

Author contributions: Conceptualization, I.A.O., M.E.N.F.B., L.S.B. and R.C.P.C.; methodology, I.A.O., M.R.R., A.R., G.R.L., V.P.M.R.V., and R.C.P.C.; software, L.N.A.R., I.A.O. and R.C.P.C.; validation, R.C.P.C.; formal analysis, I.AO., R.C.P.C.; and L.N.A.R.; investigation, I.A.O., N.A.N.F., R.C.P.C., and V.P.M.R.G., resources, R.C.P.C., data curation, R.C.P.C., M.E.N.F.B., L.S.B., writing—original draft, I.A.O., L.S.B. and R.C.P.C.; preparation, I.A.O., L.R.G., L.S.B. and R.C.P.C.; writing—review and editing, M.R.R., R.L.G., I.A.O., L.S.B. and R.C.P.C.; visualization, L.S.B. and R.C.P.C.; supervision, L.S.B. and R.C.P.C.; project administration, R.L.G., L.S.B. and R.C.P.C.; funding acquisition, R.L.G., M.E.N.F.B., L.S.B. and R.C.P.C. All authors have read and agreed to the published version of the manuscript.

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References:

Duarte M.F., Fonseca M.E.N., Costa H., Fernandes N.A., Reis A., Boiteux L.S., Pereira-Carvalho R.C. (2021). Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. *Virus Genes* 57, 83–93.

Giordano, L.B., Silva-Lobo, V.L., Santana, F.M., Fonseca, M.E.N., Boiteux, L.S. (2005). Inheritance of resistance to the bipartite tomato chlorotic mottle begomovirus derived from *Lycopersicon esculentum* cv. 'Tyking'. *Euphytica* 143, 27–33. https://doi.org/10.1007/s10681-005-1685-1

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Lett, J.M., De Bruyn, A., Hoareau, M., Ouattara, A., Claverie, S., Dalmon, A., Laplace, D., Lefeuvre, P., Hostachy, B. (2015). Tomato chlorotic mottle Guyane virus: A novel tomato-infecting bipartite begomovirus from French Guiana. *Archives of Virology* 160, 2887–2890. https://doi.org/10.1093/bioinformatics/bts199

Martin, D.P., Varsani, A., Roumagnac, P., Botha G., Maslamoney, S., Schwab, T., Kelz, Z., Kumar, V., Murrell, B. (2021). RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. *Virus Evolution* 7, 1–3. https://doi.org/10.1093/ve/veaa087

Melgarejo, T.A., Kon, T., Gilbertson, R.L. (2015). Molecular and biological characterization of distinct strains of Jatropha mosaic virus from the Dominican Republic reveal a potential to infect crop plants. *Phytopathology* 105, 141–153. https://doi.org/10.1094/PHYTO-05-14-0135-R

Pereira-Carvalho, R.C., Boiteux, L.S., Fonseca, M.E.N., Díaz-Pendón, J.A., Moriones, E., Fernández-Muñoz, R., Charchar, J.M., Resende, R.O. (2010). Multiple resistance to *Meloidogyne* spp. and to bipartite and monopartite *Begomovirus* spp. in wild *Solanum*

(*Lycopersicon*) accessions. *Plant Disease* 94, 179–185. https://doi.org/10.1094/PDIS-94-2-0179

Reis, L.N.A., Fonseca, M.E.N., Ribeiro, S.G., Naito, F.Y.B., Boiteux, L.S., Pereira-Carvalho, R.C. (2020). Metagenomics of Neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty–1* gene. *Viruses* 12, 819. https://doi.org/10.3390/v12080819

Reis, L.N.A., Boiteux, L.S., Fonseca, M.E.N., Pereira-Carvalho, R.C. (2021). Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome-wide pairwise identity of multiple isolates. *Virus Genes* 57, 127–131. https://doi.org/10.1007/s11262-020-01810-z

Reis, L.N.A., Boiteux, L.S., Fonseca, M.E.N., Rojas, M.R., Gilbertson, R., Pereira-Carvalho, R.C. (2023). Tomato golden net virus and tomato yellow net virus: Two novel New World begomoviruses with monopartite genomes. *Archives of Virology*, 168, 235. https://doi.org/10.1007/s00705-023-05836-0

CAPÍTULO 04

A proposal of merging Leonurus mosaic virus and tomato yellow spot virus into a single species based upon prevalent host interactions, phylogenetic relationships, and conserved genomic motif analyses.

A proposal of merging Leonurus mosaic virus and tomato yellow spot virus into a single species based upon prevalent host interactions, phylogenetic relationships, and conserved genomic motif analyses.

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Work submitted to Virus Genes

Abstract

More than 26 begomoviruses were detected in tomatoes in Brazil and some of which have been described originally infecting weeds. Likewise, weeds are reservoirs of tomato-infecting begomoviruses. Leonurus mosaic virus (LeMV) was characterized in 1992 in the weed *Leonurus sibiricus* L. (Lamiaceae). In 2007, a putative new tomato-infecting virus was described and validated by the ICTV as tomato yellow spot virus (ToYSV). Afterwards, ToYSV was reported as the only *Leonurus*-infecting begomovirus, with no novel report of LeMV. However, our pairwise comparisons employing the partial DNA–A sequence (1193 bp) of the original LeMV isolate (LMU92532) with ToYSV isolates in GenBank indicated identities above 92%, suggesting that they might be the same virus. These discrepancies prompted us to carry out a reassessment of their taxonomic status employing all available ToYSV/LeMV isolates plus our novel tomato and *L. sibiricus* isolates characterized via High-Throughput Sequencing. ToYSV was only sporadically detected in tomatoes (eight samples with ToYSV in 1340 samples), whereas it was the sole begomovirus detected in 47 samples of *L. sibiricus*, suggesting this weed as the primary viral host and not tomatoes. Iterons and conserved Rep motifs in the common region of tomato and *L. sibiricus* isolates displayed very low levels of divergence, reinforcing the hypothesis that they are strains of a single, large-host range begomovirus. Hence, we propose ToYSV/LeMV should be merged into a single virus in accordance to all biological and molecular evidences presented herein.

Keywords: *Leonurus sibiricus* L., *Solanum lycopersicum* L., *Begomovirus*.

Begomovirus (family *Geminiviridae*) is the largest genus of plant viruses [1]. In Brazil, begomovirus outbreaks in tomatoes had a dramatic increase after the invasion of *B. tabaci* Middle East–Asia Minor 1 (MEAM 1 = biotype B) in the early 1990s [2]. Field surveys conducted afterwards revealed a complex of >26 *Begomovirus* species, occurring across all biomes and major tomato-producing regions of Brazil [2, 3]. Weeds can often serve as reservoirs of tomato-infecting begomoviruses under Brazilian conditions [4].

The first report a begomovirus infecting the weed *L. sibiricus* (Lamiaceae) was done in samples collected within bean (*Phaseolus vulgaris* L.) fields in 1992 in Dourados (MS), Brazil (Farias and Maxwell, 1999). In 2007, tomato yellow spot virus – ToYSV was described in field surveys carried out in South-East Brazil [5, 6]. Afterwards, only ToYSV was reported infecting *Leonurus sibiricus*, with no novel reports of LeMV in this weed [7, 8]. In 2015, a new set of taxonomic criteria was established for the demarcation of species of the genus *Begomovirus* [1]. Given the new taxonomic scenario, we propose a reassessment of the taxonomic status of ToYSV and LeMV, using all available isolates of both viruses available in GenBank, added to those obtained in the present work.

Foliar samples of tomato plants with symptoms of begomovirus infection were collected in the main producing regions of the North (13 samples), Northeast (36) and South (24) of Brazil. Likewise, 31 samples of *L. sibiricus* displaying begomovirus-like symptoms (**Figure 1B**) were collected by [8] in the South Region of Brazil and they were also analyzed in the present study. The DNA of the samples was extracted [9] and stored in a freezer at -20°C. The total DNA was submitted to enrichment by means of rolling circle amplification–RCA [10]. Tomato samples from all regions were pooled and submitted to High-Throughput Sequencing (HTS) on the *Illumina* NovaSeq-6000 platform. The contigs were assembled using the Genomics Workbench 7.5 software (Qiagen). The contigs were analyzed using the BLASTn tool and compared with ssDNA virus databases. Subsequently, the contigs were analyzed in the Geneious program [11] aiming to assemble the genomes. The full DNA–A and DNA–B genomes of ToYSV-related isolates from three tomato samples collected in North Brazilian States of Roraima (RR–003 and RR–004) and Tocantins (TO–046) were recovered via PCR assays with the following species-specific primers: Primer DNA–A Forward: 5'–ACG AAA TCT TTT AGG AGC TAA TGG–3' and Primer DNA–A Reverse: 5'–CGT ATT TCT GCA AAA AAC TAC TTC CT–3' and Primer DNA–B Forward: 5'–AAT AAG GCG AAA GGT TAA AAG AAT ATG GCG–3' and Primer DNA–B Reverse: 5'–GCC TTA TTC ACT TCA CCT TCT TCG ATT CAC–3'. These primers obtained using the primer design tool of the

Geneious program ([11]. ToYSV-specific amplicons of the begomovirus samples from *L. sibiricus* and tomato were Sanger-sequenced.

Phylogenetic analyzes were carried out with alignments made with MUSCLE in the software Sequence Demarcation Tool (SDT) [12]. All available isolates identified as ToYSV in our surveys as well as the ones available at the GenBank database (https://www.ncbi.nlm.nih.gov/) were employed in this set of analyses. Phylogenetic reconstructions were carried [13]. Evaluations of iterons and conserved motifs in the common region (CR) among LeMV and ToYSV isolates were performed [14]. The quasi-palindromic segment [ACTT–(N7)–AAGT] of DNA–A [15], divergence of the helix 4 structural motif [16] and recombination events among the isolates were also evaluated using the RDP5 program [17].

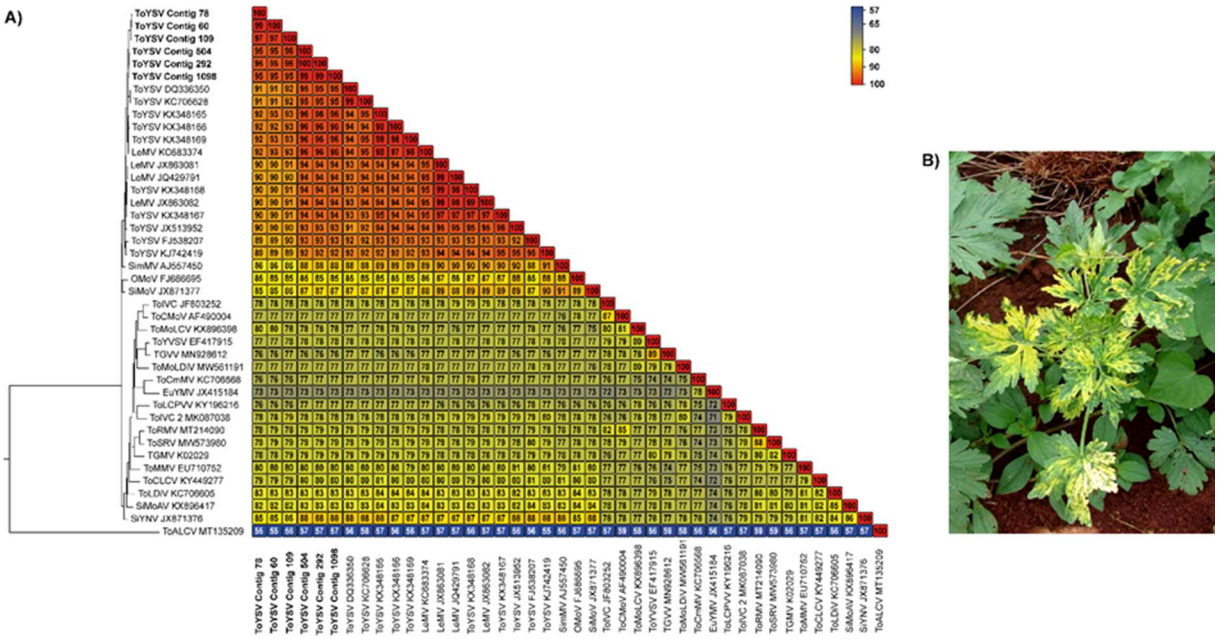


Fig. 1 Panel (A) Phylogenetic tree and SDT (Sequence Demarcation Tool) of complete DNA–A sequences with identities and distances between isolates described as either tomato yellow spot virus (ToYSV) or Leonurus mosaic virus (LeMV). The ToYSV sequences recovered in the present study are highlighted in bold, corresponding to contigs 60, 78, 109, 292, 504, and 1098. Isolates named LeMV (JX863082) shared 99% identity with ToYSV isolate (KX348168). The ToYSV sequences recovered by HTS in this study share 92 to 96% identity with the LeMV isolate (KC683374). Panel (B) ToYSV-infected Leonurus sibiricus plant within a tomato crop field in Marilândia do Sul (Paraná State, Brazil) displaying golden mosaic symptoms

We carried out a preliminary pairwise nucleotide comparison employing only the partial DNA–A genomic sequence (1193 bp) of the original LeMV isolate (LMU92532) with our novel isolates as well as all 32 isolates named as ToYSV/LeMV at the GenBank displayed identities ranging from 92 to 99%, reinforcing the hypothesis that they might be strains of a

single virus (**Figure 1A**). These results prompted us to carry out a reassessment of the taxonomic status of both viruses employing isolates from tomato, beans, passion fruit, *Capsicum*, *Salvia*, and *Leonurus*. The reassessment of the taxonomic status of LeMV and ToYSV was then carried out based upon phylogenetic analyses of multiple isolates. The results consistently indicated nucleotide identity levels above 91% in the whole DNA–A genomes (**Figure 1A**). A subset of isolates identified as ToYSV (KJ742419, FJ538207, JX513952, and KX348167) shared between 92 to 98% identity with the LeMV isolate (JX863082) (**Figure 1A**). In addition to these high levels of nucleotide identities observed here, all surveys carry out thus far are indicating ToYSV as the sole virus detected in *L. sibiricus* [8, 18]. However, in our collection of tomato-infecting begomoviruses we were able to detect ToYSV only sporadically in this host. Altogether, a total sample of 1340 isolates from tomato were sequence-characterized and only eight were found to be infected by ToYSV (the present survey). These field observations provided further evidence that ToYSV and LeMV might be strains of a single virus, indicating *L. sibiricus* as the primary viral host.

Thus far no complete LeMV DNA–B sequence is available in the GenBank database. Sequence comparisons of iterons as well as motifs conserved in the common region [14] were carried out with *Leonurus* and tomato ToYSV/LeMV isolates available at the GenBank, including the novel isolates discovered in our survey (Supplementary Figure 1). In addition, we also included ToYSV isolates from beans, *Capsicum*, and *Salvia* in these analyses. Evaluations of iterons and iteron-related domains (IRD) separated the isolates into two groups displaying either the iteron **GGAGT** or the iteron **GGTGA**. The **group #1** comprised three LeMV isolates (JX963081, JX863082, and JQ429791) and ten ToYSV isolates (MN508243, MN508245, MN508241, KJ742419, MN518741, JX513952, KX348179, FJ538207, and MN518733). The **group #2** included one LeMV isolate (KC683374), and 22 ToYSV isolates (DQ336350, KC706628, KX348172, KX348175, KX348176, KX348178, KX348169, KX348171, KX348173, KX348166, KX348170, KX348174, KX48177, KX348165, KX348168, KX348167, MT103978, MT103993, MT103994, MT103995, MT103992, and MN508247). ToYSV isolates corresponding to our HTS contigs 60, 78, 109, 292, 504, and 1098 were also placed into the **group #2**. Isolates of the **group #1** had the same IRD (MP**S**K**P**RRFRVQ) except for the LeMV isolate (JQ429791), which did not show a consistent IRD [14]. In the **group #2**, the ToYSV isolates obtained in the present study displayed the IRD MP**L**A**P**KRFRIS, whereas LeMV and ToYSV isolates from GenBank displayed MP**S**A**H**KRFRIS, indicating only two divergences in their amino acid sequences.

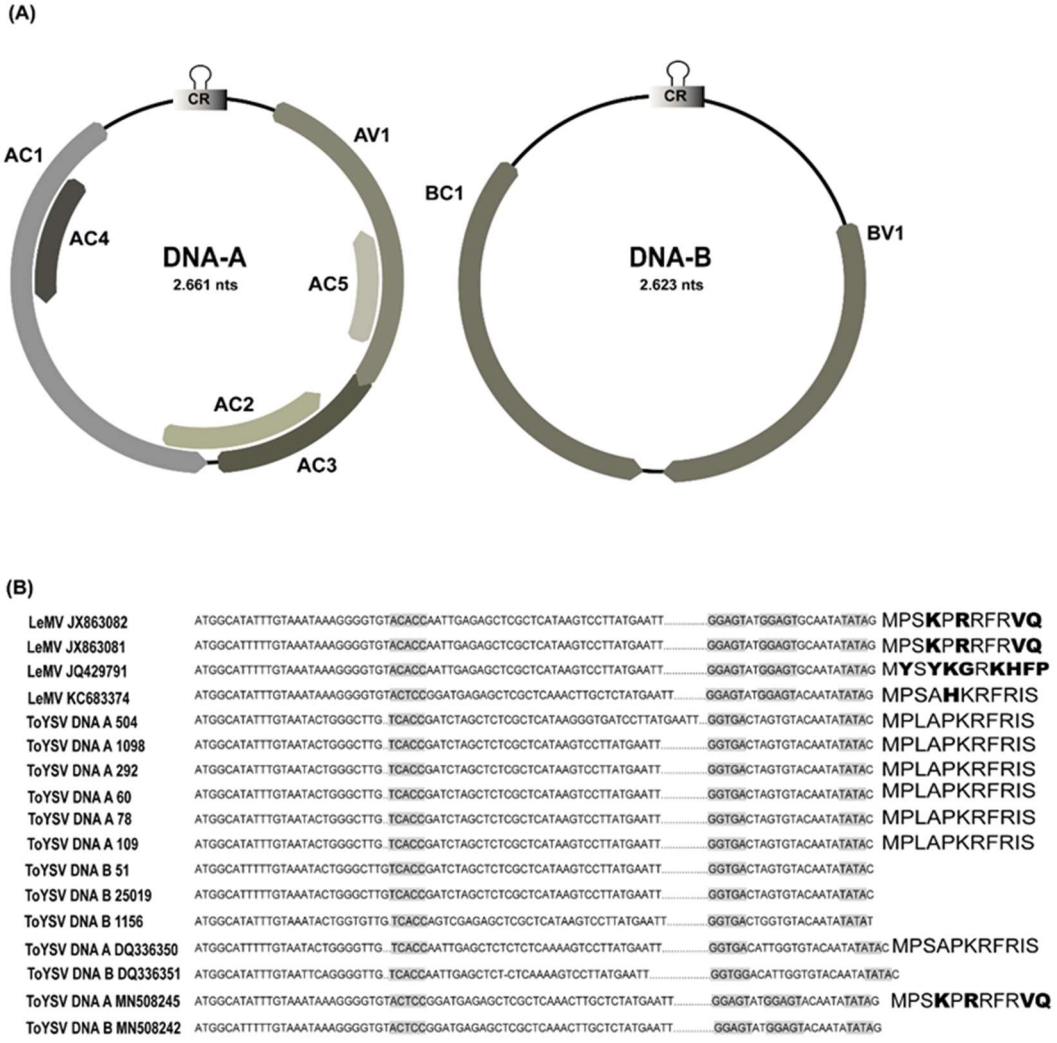
The quasi-palindromic DNA–A segments **[ACTT–(N7)–AAGT]** of LeMV and ToYSV isolates were also analyzed. Five ToYSV isolates from the GenBank (MT103978, MT103992, MT103993, MT103994, and MT103995) shared the sequence ACGT-G**T**TCCCT-AAGT. On the other hand, all the ToYSV isolates described in the present survey as well as a subset of ToYSV isolates from the GenBank (KX348172, KX348178, KX348176, KX348175, KX348177, KX348166, KX348165, KX348173, KX348171, KX348174, KX348170, KX348169, MN508247, KX348167, KX348168, KX348179, JX513952, MN518741, KJ742419, MN508241, MN508245, MN508243, MN518733, FJ538207, KC706628, and DQ336350) shared the sequence ACGT-G**G**TCCCT-AAGT. This sequence was also annotated in a subset of LeMV isolates (KC683374, JX863081, JX863082, and JQ429791). Therefore, the quasi-palindromic segment of DNA–A **[ACTT–(N7)–AAGT]** of LeMV and ToYSV isolates displayed only one base divergence among them.

The Helix 4 motif was also included for in-depth analysis because it is highly conserved across geminivirus replication proteins [16]. We annotated that a single LeMV (JX863082) isolate had the conserved Rep motif: DKR TAL QII KEK LPE **R**YL FQF HNL NSN LDR IF**S** KAP EPW VPP FPL SSF TNV PDE MQE W which is identical to the isolates identified as ToYSV (KX348167; KX348168; MT103978; MT103992; MT103993; MT103994 and MT103995). The LeMV isolate KC683374 and ToYSV isolates KX348165; KX348169; KX348173; KX348175; KX348176, and KX348178 had the same conserved Rep motif: DKR TAL QII KEK LPE **K**YL FQF HNL NSN LDR IF**K** KAP EPW VPP FPL SSF TNV PDE MQE W. The ToYSV isolates obtained in the present study (Contigs 60, 78, 109, 292, 504, and 1098) had the conserved motif DKR TAL QII KEK LPE **K**YL FQF HNL NSN LDR IF**S** KAP EPW VPP FPL SSF TNV PDE MQE W identical to other GenBank isolates identified as ToYSV (MN508241; MN508243; MN508245; MN508247; MN518733; MN518741; DQ336350; FJ538207; JX513952; KC706628, and KJ742419).

Analyses with RDP5 found a recombination event in the ToYSV isolate corresponding to our contig 109. This recombinant isolate displayed the tomato interveinal chlorosis virus-2 (MK087038) as a major parent and okra mottle virus (FJ686695) as the minor parent. This recombination event of 481 nucleotides encompassed the CP/AC5/Ren/TrAP ORFs.

In summary, our analyses consistently indicated identity levels above 91% among the majority of the ToYSV and LeMV isolates, reinforcing the hypothesis that they are strains of a single virus. Similar study was reported with two South American begomoviruses (tomato golden vein virus – TGVV and tomato yellow veins streak virus – ToYVSV) in which a reappraisal of the classification status was proposed [13]. Hence, we propose ToYSV/LeMV

should be merged into a single virus in accordance to all biological and molecular evidences presented here.



Supplementary Figure 1. Diagrammatic representation of the genomic organization of an isolate named tomato yellow spot virus (ToYSV) recovered by High-Throughput Sequencing (HTS) in association with tomato plants (*Solanum lycopersicum* L.). **Panel (A)** The following open reading frames (ORFs) were annotated in the DNA–A component according to their presumed function of the coding proteins: AV1 = capsid protein; AC1 = replication-associated protein; AC2 = transactivator protein; AC3 = replication enhancer; AC4 = symptom determinant protein; AC5 = gene silencing suppressor protein. The following ORFs were annotated in the DNA–B component: BV1 = Nuclear shuttle protein; and BC1= Movement protein involved in cell-to-cell viral movement, and CR = common region, encompassing the hairpin (in both components). **Panel (B)** Iteron GGAGT, TATAbox and Rep-IRD identified in the CR of both DNA components in a set of isolates identified as either Leonurus mosaic virus (LeMV) or ToYSV from either the GenBank or from the present study (retrieved by HTS): DNA–A (contigs 60, 78, 109, 292, 504, and 1098) and DNA–B (contigs 51, 1156, and 25019)

Author contributions: Conceptualization, I.A.O., M.E.N.F., L.S.B. and R.C.P.C.; methodology, I.A.O., R.C. P.C and L.S.B..; software, L.N.A.R., I.A.O. and R.C.P.C.; validation, R.C.P.C.; formal analysis, I.AO., R.C.P.C.; and L.N.A.R.; investigation, I.A.O., L.S.B., M.E.N.F and R.C.P.C., resources, R.C.P.C., data curation, R.C.P.C., M.E.N.F., L.S.B., writing—original draft, I.A.O., L.S.B. and R.C.P.C.; preparation, I.A.O., L.N.A.R., L.S.B. and R.C.P.C.; writing—review and editing, I.A.O., L.N.A.R., L.S.B. and R.C.P.C.; visualization, L.S.B. and R.C.P.C.; supervision, L.S.B. and R.C.P.C.; project administration, L.S.B. and R.C.P.C.; funding acquisition, M.E.N.F., L.S.B. and R.C.P.C. All authors have read and agreed to the published version of the manuscript.

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Statements and Declarations:

Competing interests: The authors declare no competing interests.

References

1. Brown SJK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Archives of Virology. 160:1593–1619. https://doi.org/10.1007/s00705-015-2398-y

2. Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RDC (2020) Metagenomics of Neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty–1* gene. Viruses 8:1–22. https://doi.org/10.3390/v12080819

3. Duarte MF, Fonseca MEN, Costa H, Fernandes NA, Reis A, Boiteux LS, Pereira-Carvalho RC (2021b) Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. Virus Genes 57:83–93 https://doi.org/10.1007/s11262-020-01812-x

4. Pereira-Silva J, Boiteux LS, Fonseca MEN, Reis LNA, Souza AS, Nery FMB, Madeira NR, Pereira-Carvalho RC (2022) Novel natural hosts of tomato severe rugose virus (ToSRV) in the Fabaceae, Solanaceae, and Oxalidaceae families. Journal of Plant Diseases and Protection. 129: 425–431. https://doi.org/10.1007/s41348-021-00533-z

5. Calegario RF, Ferreira SDS, Andrade ECD, Zerbini FM (2007) Characterization of Tomato yellow spot virus, a novel tomato-infecting begomovirus in Brazil. Pesquisa Agropecuária Brasileira. 42:1335–1343. https://doi.org/10.1590/S0100-204X2007000900016

6. International Committee on Taxonomy of Viruses (ICTV). Available in: https://ictv.global/report/chapter/geminiviridae/geminiviridae/begomovirus Accessed in: 25 August of 2022.

7. Barbosa JC, Eckstein B, Filho AB, Rezende JAM, Dallagnol LJ (2013) First report of tomato yellow spot virus infecting *Leonurus sibiricus* in Brazil. Plant Disease 97:289–289. https://doi.org/10.1094/PDIS-07-12-0692-PDN

8. Fernandes-Acioli NAN, Boiteux LS, Fonseca MEN, Segnana LRG, Kitajima EW (2014) Report of tomato yellow spot virus infecting *Leonurus sibiricus* in Paraguay and within tomato fields in Brazil. Plant Disease. 98:1445–1445. https://doi.org/10.1094/PDIS-11-12-1016-PDN

9. Boiteux LS, Fonseca MEN, Simon PW (1999) Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. Journal American Society Horticulture Science. 124:32–38. https://doi.org/10.21273/JASHS.124.1.32

10. 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. Journal of Virological Methods. 116:209–211. https://doi.org/10.1016/j.jviromet.2003.11.015

11. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

12. Muhire BM, Varsani A, Martin DP (2014) SDT: A Virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE. 9:1–8. https://doi.org/10.1371/journal.pone.0108277

13. Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RDC (2021) Tomato yellow vein streak virus and Tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome–wide pairwise identity of multiple isolates. Virus Genes 57:127–131. https://doi.org/10.1007/s11262-020-01810-z

14. Argüello-Astorga G, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminiviruses: identification of potential interacting amino

acid-base pairs by a comparative approach. Archives of Virology. 146:1465–1485. https://doi.org/10.1007/s007050170072

15. Cantú-Iris M, Pastor-Palacios G, Mauricio-Castillo J, Bañuelos-Hernández B, Avalos-Calleros J, Juárez-Reyes A, Rivera-Bustamante R, Argüello-Astorga G (2019) Analysis of a new begomovirus unveils a composite element conserved in the CP gene promoters of several geminiviridae genera: Clues to comprehend the complex regulation of late genes. PLoS ONE. 14:1–24. https://doi.org/10.1371/journal.pone.0210485

16. Arguello-Astorga G, Lopez-Ochoa L, Kong LJ, Orozco BM, Settlage SB, Hanley-Bowdoin L (2004) A Novel motif in Geminivirus replication proteins interacts with the plant retinoblastoma-related protein. Journal of Virology. 78:4817–4826. https://doi.org/10.1128/jvi.78.9.4817-4826.2004

17. Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B (2021) RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evolution. 7:1 – 3. https://doi.org/10.1093/ve/veaa087

18. Ferro CG, Silva JP, Xavier CAD, Godinho MT, Lima ATM, Mar TB, Mar TB, Zerbini FM (2017) The ever-increasing diversity of begomoviruses infecting non-cultivated hosts: New species from *Sida* spp. and *Leonurus sibiricus*, plus two New World alphasatellites. Annals of Applied Biology. 170:204–218. https://doi.org/10.1111/aab.12329

CAPÍTULO 05

Full-genome sequencing and construction of monomeric infectious clones of tomato golden leaf spot virus: A novel bipartite tomato-infecting begomovirus from subequatorial Brazil

Full-genome sequencing and construction of monomeric infectious clones of tomato golden leaf spot virus: A novel bipartite tomato-infecting begomovirus from subequatorial Brazil

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Work to be submitted to Archives of Virology

Abstract

Herein, we describe a new tomato-infecting bipartite begomovirus identified in Araguaína County (State of Tocantins), subequatorial Brazil. Total DNA from foliar samples from a symptomatic plant (exhibiting dwarfism and yellow leaf mottle) was used as template in PCR assays and High-Throughput Sequencing (HTS). The complete sequences of the DNA–A and DNA–B components of one isolate (named as TO–083) displayed genomic organization of the New World bipartite begomoviruses, exhibiting common iterons and the nonanucleotide (TAATATTAC) sequences. The DNA–A component displayed the highest identity level (89.49%) with tomato bright yellow mottle virus (TBYMoV), confirming that TO–083 is a novel viral species and reinforcing the notion that the diversity of begomoviruses in Neotropical regions is yet largely undervalued. No significant recombination signal was detected in TO–083 genome. Mosaic and apical leaf distortion were observed in tomato seedlings 45 days after biolistic inoculation with monomeric infectious clones obtained for the DNA–A and DNA–B components. We tentatively named this novel pathogen as tomato golden leaf spot virus (ToGLSV). The present work presents a more complex panorama in terms of tomato-infecting begomovirus diversity and indicates the emergence of endemic species in different agroecological regions of Brazil. This scenario of extreme viral diversity is an indication that genetic control strategies for begomoviruses may prove unstable using varieties identified as resistant in different producing regions.Therefore, the availability of infectious ToGLSV clones can be a very useful as a breeding tool for screening tomato germplasm in search of resistance factors against this emergent virus.

Keywords: *Tomato golden leaf spot virus*, viral diversity, tomato breeding.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops across Neotropical areas of Brazil [1]. Tomato production is concentrated in tropical and subtropical areas of the country, but with the development of novel cultivar it is expanding to warm and humid areas [2]. Diseases caused by members of the *Geminiviridae* family are major yield-limiting factors in these regions [3]. *Geminiviridae* is so far the largest group of plant viruses, comprising members with small circular single-stranded DNA genomes encapsidated into twinned quasi-icosahedral virions [4], being transmitted by insects classified into *Hemiptera* order [5]. Currently, the *Geminiviridae* is composed of ≈ 520 species assigned into 14 genera [6].

Begomovirus is the most important genus of the *Geminiviridae* family with members presenting twinned particles (with 18 to 30 nanometers) with single-strand DNA genomes that could be either monopartite (only DNA–A component) or bipartite (with DNA–A and DNA–B). Viruses of this group are characterized by their ability to remodulate biochemical and physiological processes of the host cell aiming to impair plant defenses, allowing their replication and systemic movement [7]. The viruses with bipartite genomes have two components of ≈ 2600 nucleotides of size, with the DNA–A component displaying five open reading frames (ORFs) in the New World, and six ORFs in the Old World [8]. The monopartite viruses have only one component homologous to the DNA–A component of the bipartite viruses [8]. All components have a common region (CR) of ≈ 200 nucleotides (nts) that allows for the identification of cognate genomic components of the same bipartite species. In the CR is possible to find iterons and the nonanucleotide (TAATATTAC) sequence, which corresponds to the initial site of virus replication [9, 10].

ORFs in DNA–A component of the New World begomoviruses code for four proteins in a complementary sense: AC1, which is the replication polymerase with function of duplicating the viral genome [11]. The ORF AC2, which is the transcriptional activator with function of initiating the replication of other ORFs [12]. In turn, the ORF AC3 is the Replication enhancer protein, which is a protein able of increasing the replication of the viral genome [13]. On the other hand, the ORF AC4 codes for the protein C4, which function is the suppression of post-transcriptional regulation [14]. The ORF AC5 is found in a more restricted subgroup of *Begomovirus* species, which function was identified as a pathogenicity determinant, involved in the intensification of symptoms, and suppressing post-transcriptional gene silencing [15]. In the viral sense, AV1 code for the coat protein that is responsible for the protection of the viral genome, presenting also a crucial role in the vector transmission [16]. On the other hand, the

DNA–B component encompasses the ORF BC1 coding for the cell-to-cell viral movement via interaction with the plasmodesma apparatus [17], whereas the ORF BV1 (nuclear shuttle protein) controls the movement of the viral genome into the cell nucleus [17].

Begomovirus species also have a conserved promoter region of the capsid protein, a region of six nucleotides precisely before the beginning of the AV1 ORF [18]. The current set of criteria for the demarcation of a new *Begomovirus* species is based upon MUSCLE alignment and DNA–A sequence analysis via Sequence Demarcation Tool (SDT) program [19]. In this new taxonomic context, a new species must display nucleotide identity levels of the complete DNA–A genome below 91% with previously described viruses [19].

In Brazil, the tomato crop has been heavily affected by a wide array of diseases caused by a complex *Begomovirus* species transmitted by *Bemisia tabaci* Middle East Asian Minor – MEAM 1 [20]. Severe economic losses due to diseases caused by a complex of more than 20 *Begomovirus* species have been described across all major tomato-producing areas, mainly in tropical and subtropical Brazil [20]. However, biomes representing the transition between equatorial and subequatorial humid climates have been neglected in terms of tomato-infecting begomovirus surveys with few studies dealing with the viral diversity in these geographic regions. Recently, novel begomoviruses have reported infecting tomato crops across the North region of Brazil [21, 22]. Nonetheless, the overall information on tomato-infecting begomoviruses occurring in the Subequatorial areas of Brazil is yet scarce. In the present work, we describe the complete DNA–A and DNA–B genomic sequences and the construction of infectious clones of a new tomato-infecting bipartite *Begomovirus* species found in the State of Tocantins located in the North region of Brazil.

Material and Methods

Plant sample collection, DNA extraction, Rolling Circle Amplification, and recovery of sequences using Sanger and High-Throughput Sequencing (HTS)

Twenty-six (26) foliar samples were collected from tomato plants cultivated under open-field conditions and exhibiting begomovirus-like symptoms of mosaic, mottle, leaf deformation and dwarfism and symptom across from distinct counties within the State of Tocantins. The samples were submitted to RCA – Rolling Circle Amplification, using Φ-29 DNA polymerase (TempliPhi, GE Healthcare, Massachusetts, USA) [6]. Circular DNA-enriched samples were subsequently used as template in PCR reactions with universal *Begomovirus* primers for the DNA–A component (PAL/PAR1978-496) and for the DNA–B component (PBL1 and CRC-1) [23]. The infectious clones were obtained using restriction enzymes and cloning in psL1180 [8,

24] as described below. Beside this, a subgroup of these six samples exhibiting begomovirus-like symptoms in the State of Tocantins were grouped into a pool of samples (named as BP1 pool), containing symptomatic leaf tissues of tomato collected across the North (13 samples), Northeast (36 samples), and South (24 samples) regions. After the preparation of the pools [20], samples were submitted to HTS on the Illumina NovaSeq-6000 platform.

Construction of monomer infectious clones of the TO–083

Restriction enzymes targeting the restriction site of psL1180 were evaluated. Two enzymes with single cuts (with ≈ 2600 bp) were selected: *Apa*I (GGGCC/C) and with *Nde*I (CA/TATG) for the DNA–A and DNA–B components, respectively. These fragments were eluted from 0.8% agarose gels with Zymoclean Band Purification Kit. The cloning was carried out as described in a previous work [8, 24]. The vector psL1180 was prepared by digestion with *Apa*I and alkaline phosphatase for the DNA–A component and *Nde*I and phosphatase for the DNA–B component. The rate of 5:1 (insert: vector) was used for the ligation complex using 20 µL per tube of ligase. The solution was incubated overnight (16 ºC). The transformation was carried out via thermal shock using 10 µL of the solution for each tube of competent cells *Escherichia coli* DH5 α (50 µL). In the next step, 500 µL of Luria Bertani (LB) medium (without ampicillin) was added to the tube and incubated for 1.5 hours at 37 °C. After this, 250 µL of the transformation solution was plated per Petri dish (with LB medium supplemented with ampicillin) with also IPTG and X-Gal which was maintained overnight at 37 °C. White colonies were selected and transferred to LB (with ampicillin) and again maintained overnight. Then, the plasmid DNA was extracted using a standard protocol [25]. The confirmation of the clones was made by digestion with *Apa*I and *Nde*I. Two clones of each isolate were selected, and their sequences were obtained via Sanger dideoxy sequencing. Internal primers were designed to obtain the complete genome.

Bioassays to determine the infectivity of clones for DNA–A and DNA–B

Tomato seedlings were employed in the infectivity bioassays via inoculation with particle bombardment method. The DNA was coated in gold sheets and bombarded in seedlings 155 PSI with PDS-1000 (Bio-Rad) [8, 24]. Eight tomato plants were used. Control plants were bombarded with only gold particles. PCR assays with species-specific primers were performed to confirm plants inoculated [8, 24].

Analysis of sequenced samples High-Throughput Sequencing (HTS)

The results obtained were initially analyzed using the CLC Genomics Workbench 7.5 program (Qiagen) and Geneious program [26] following previous workflow methodology [20]. The reads obtained were mapped to the contig of the putative virus to obtain the final contig. With the help of the Geneious program and the Map to reference tool, using the parameters 90 to 99% of minimum overlap identity, the genomes of each contig were increased with mapping in the reads file provided by HTS. All contigs were subjected to comparisons with viral sequences present in local or GenBank libraries using the BLASTn algorithm. The contig selected correspondent to virus studied here was analyzed using MUSCLE alignments performed in the Geneious program to annotate ORFs based on the reference genome. In addition to molecular characterization with ORFs annotation, the common region present in bipartite viruses were analyzed looking for the nonanucleotide and iterons and REP-IRD (Rep Iteron-Related Domains). Subsequently, for the comparison among isolates and viral species, the sequences were subjected to multiple pairwise alignment MUSCLE with the aid of the SDT program [27].

Phylogenetic analyses and recombination analyses

The cognate region and iterons were analyzed via sequence alignments using MUSCLE parameter in the Geneious 8.2 software [26]. Sequences of the DNA–A and DNA–B clones were compared with all the genomes available for DNA–A and DNA–B in GenBank database to build up the phylogenetic tree. A preliminary tree was constructed using the fast tree parameter in the Geneious R8 with all begomovirus described in ICTV. The closest species from this, composed by 47 genomes sequences were used to phylogenetic analyses. After this process it was selected the cluster within the virus we constructed. The cluster was analyzed using Bayesian inference, with the MrBayes [28] within the Geneious R11 using Mr Model Test. The RDP5 was used to detect recombinant events. The nucleotide sequences were compared first in BLASTn, to identify other genomic sequences similar to ours and make a first investigation of a possible new species. After this activity, it was downloaded similar sequences of begomovirus and they were compared using SDT v.2 [27].

Results

PCR reactions with universal *Begomovirus* primers for the DNA–A and DNA–B components using as template DNA extracted from one tomato leaf sample (named as TO-083) exhibiting begomovirus-like symptoms in Araguaína (State of Tocantins, North Brazil), and

generated amplicons of 1220 bp and 550 bp, respectively. These TO-083-derived amplicons were fully characterized via Sanger dideoxy sequencing and preliminary results indicated the presence in this sample of a potentially new begomovirus. The complete sequences of the DNA–A (2659 nucleotides) and DNA–B (2609 nucleotides) components of TO-083 were recovered by Sanger dideoxy sequencing from the very same tomato sample collected in Araguaína county (**Fig. 1**). The genomic organization of DNA–A and DNA–B components are illustrated in **Fig. 2.** In the BLASTn alignments, the DNA–A component of TO-083 displayed the highest identity level (89.49%) with a novel viral species (not yet fully characterized) named as tomato bright yellow mottle virus (TBYMoV). Five ORFs were annotated in the DNA–A component with three of them in the complementary sense, encoding for Rep (replication-associated protein), TrAP (transcriptional activator protein) and Ren (Replication enhancer protein) with 365, 130, 132 amino acids, respectively. In viral sense the CP gene codifies coat protein with 251 amino acids. In comparison with other begomoviruses, the Rep, TrAp, and Ren genes showed the highest nucleotide identities to the genes from tomato golden net virus, with 87.3%, 87.8% and 86.7% identity, respectively. The coat protein (CP) showed 90.7 % of identity with TBYMoV.

Fig.1 Satellite map view of a subset of Brazilian States, displaying Araguaína County (red symbol) in the State of Tocantins (North Brazil) where tomato (*Solanum lycopersicum* L.) leaf samples exhibiting begomovirus-like symptoms were collected and characterized in the present study

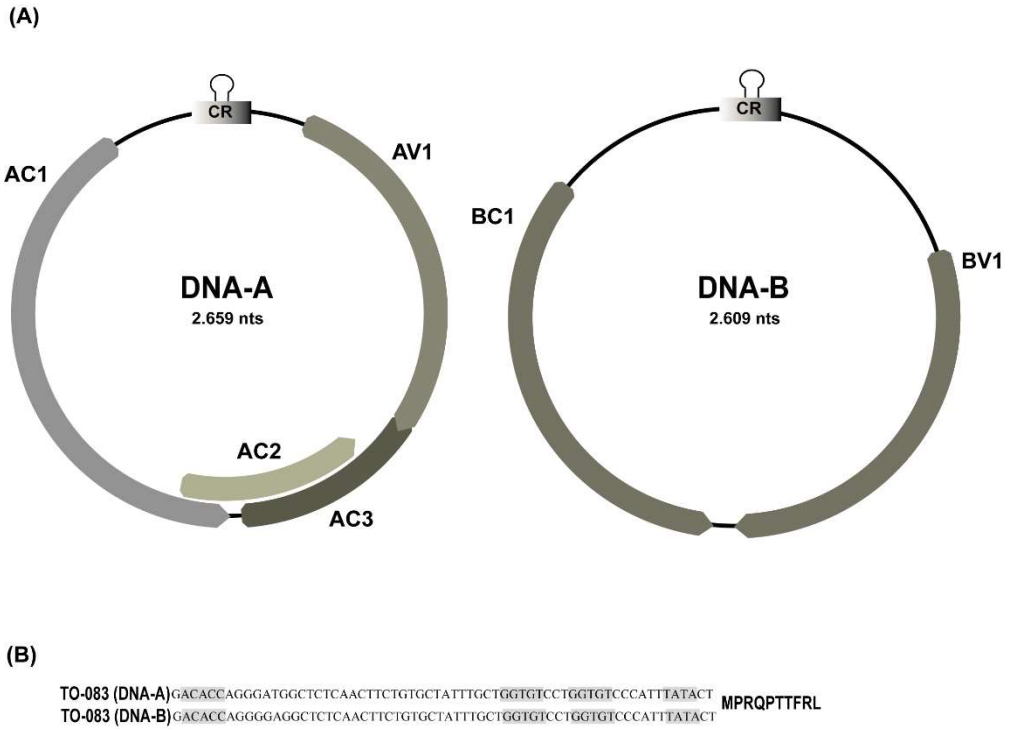

Fig. 2 The DNA–A and DNA–B components of the isolate TO-083, representing a new *Begomovirus* species tentatively named as tomato golden leaf spot virus (ToGLSV). **Panel (A):** Four Open reading frames (ORFs) were observed in the DNA–A component: Gene AV1 (Coat Protein – CP) in the virion sense strand and three in complementary sense: AC1 (Replicase Polymerase – Rep), AC2 (Transcriptional Activator Protein – TrAP) and AC3 (Replication enhancer – Ren). The DNA–B encodes two proteins related to viral movement. Gene in viral sense BV1 (Nuclear Shuttle Protein – NSP) and complementary sense BC1 (Movement Protein – MP). **Panel (B):** The duplicated GGTGT item and its complementary ACACC, TATA box located in the common region (CR) of both segments of ToGLSV (isolate TO–083) and Rep – IRD MPRQPTTFRL

The REP and REN are widely studied proteins, being involved in viral replication. The multifunctional REP protein acts as a helicase [29], in viral DNA synthesis process [30, 31, 32] and can mediate recognition of cognate origin of replication in DNA–A and DNA–Bcomponents [33]. On the other hand, REN (replication enhancer) acts increasing viral DNA accumulation on infect host cells [33]. The protein TrAP is expressed at the early stage of the infection process, being responsible to regulate (at transcriptional level) the expression of the late genes such as CP and the two genes encoded in the DNA–B [32]. However, unlike other well-characterized transactivators, TrAP does not bind dsDNA in a sequence-specific manner [34, 35]. In viral sense, the coat protein is crucial for vector specificity and viral movement. In the DNA–B component, the ORF MP codes for the movement protein (in complementary sense), whereas the ORF NSP codes for the nuclear shuttle protein (in the viral sense). MP

displayed 882 nts (293 aa) and showed 79.9% of identity with tomato crinkle leaf yellow virus. NSP displayed 771 nts (256 aa) and showed 77.7% of identity with tomato interveinal chlorosis virus-2.

The common regions (182 nts) of DNA–A and DNA–B components displayed more than 90% of identity to each other, implying that they are cognate components from a single bipartite species. It was also found the TATA box and the iterons GGTGT twice and was found its complementary reverse once. We also identified in the isolate TO-083 the quasi–palindromic DNA–A segment ACTT-GGGCGCT-AAGT [18] as well as the conserved motif Rep-IRD MPRQP**T**TFR. Compared to the phylogenetically closest species (TBYMoV), the Rep-IRD showed a divergence in a single amino acid sequence (MPRQP**N**TFRL). The analysis of the conserved structural motif of Helix 4 of TO-083 revealed the following amino acid sequence: EAL AII R**T**G DPK AFI VQH HNI **S**AN I**H**K IFA **Q**SP EPW TPP F**Q**L SSF TNV PDE MQE WAD D. This sequence displayed five (underlined) divergences in the amino acid sequence in relation to the phylogenetically closest isolate (KC791691), which displayed the following sequence: EAL AII R**A**G DPK AFI VQH HNI **N**AN I**Q**K IFA **K**SP EPW TPP F**P**L SSF TNV PDE MQE WAD D. These comparative results of the conserved motifs reinforce the result shown in **Fig. 3**, that although displaying close phylogenetic relationship, TBYMoV and TO-83 are distinct viral species.

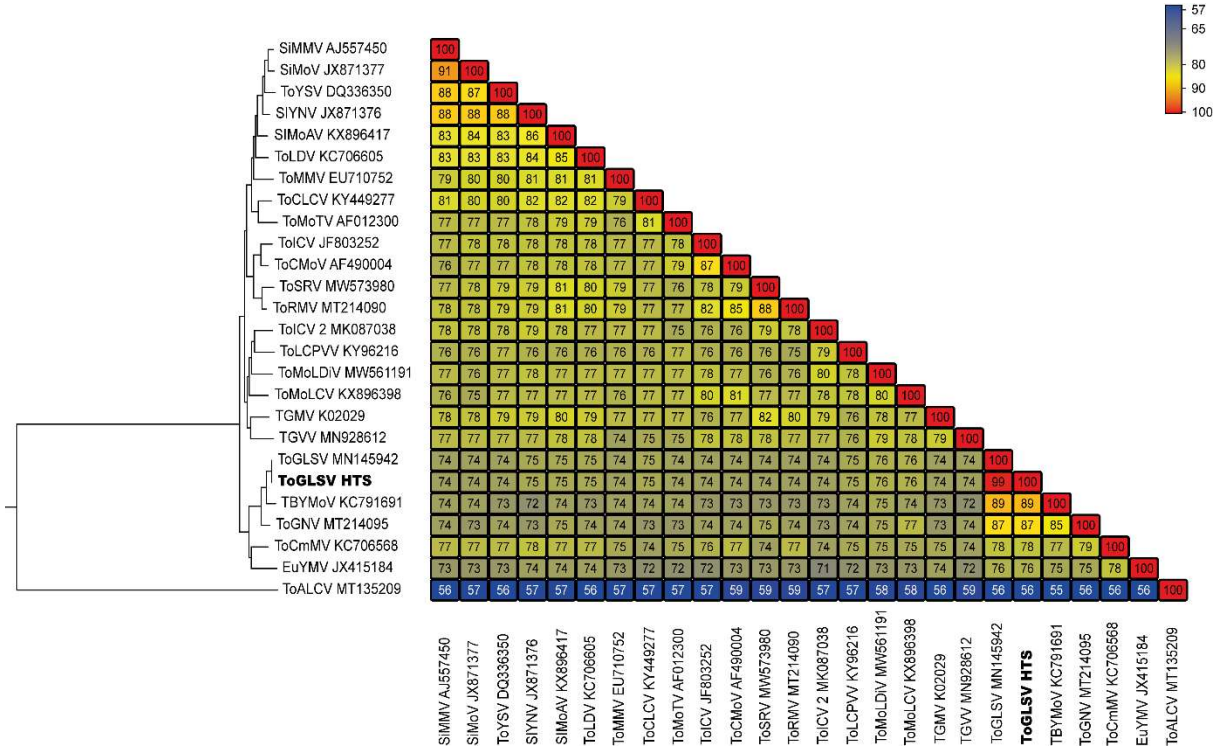

Fig. 3 Phylogenetic tree and Sequence Demarcation Tool (SDT) containing distance between the nucleotide sequences of the DNA–A segment of other begomoviruses in relation to the new begomovirus provisionally named tomato golden leaf spot virus (ToGLSV) recovered by

Sanger (ToGLSV MN145942) and High-Throughput Sequencing (ToGLSV HTS). Our ToGLSV isolate recovered by Sanger and HTS achieved 89% nucleotide identity with ToBYMoV (KC791691). 1. SimMV – Sida micrantha mosaic virus (AJ557450); 2. SiMoV – Sida mottle virus (JX871377); 3. ToYSV – Tomato yellow spot virus (DQ336350); 4. SiYNV – Sida yellow net virus (JX871376); 5. SiMoAV – Sida mottle Alagoas virus 1 (KX896417) 6. ToLDV – Tomato leaf distortion virus (KC706605); 7. ToMMV – Tomato mild mosaic virus (EU710752); 8. ToCLCV – Tomato chlorotic leaf curl virus (KY449277); 9. ToMoTV – Tomato mottle Taino virus (AF012300); 10. ToICV – Tomato interveinal chlorosis virus (JF803252); 11. ToCMoV – Tomato chlorotic mottle virus (AF490004); 12. ToSRV – Tomato severe rugose virus (MW573980); 13. ToRMV - Tomato rugose mosaic virus (MT214090); 14. ToICV-2 – Tomato interveinal chlorosis virus-2 (MK087038); 15. ToLCPVV – Tomato leaf curl purple vein virus (KY196216); 16. ToMolDiV – Tomato mottle leaf distortion virus (MW561191); 17. ToMoLCV – Tomato mottle leaf curl virus (KX896398); 18. TGMV – Tomato golden mosaic virus (K02029); 19. TGVV – Tomato golden vein virus (MN928612); 20. ToGLSV (GenBank MN145942); 21. ToGLSV recovered after HTS; 22. TBYMoV – Tomato bright yellow mottle virus (KC791691); 23. ToGNV – Tomato golden net virus (MT214095); 24. ToCmMV – Tomato common mosaic virus (KC706568); 25. EuYMV – Euphorbia yellow mosaic virus (JX415184); 26. ToALCV – Tomato apical leaf curl virus (MT135209)

Illumina NovaSeq-6000 sequencing resulted in the following raw readings for the BP1 pool of tomato samples from the North, Northeast and Southern regions: 7230366 reads, 38575 contigs, with 137 of them corresponding to viruses according to our BLASTn analyses. Two of the pool samples were correspondent to the DNA–A and DNA–B of TO–083. Comparison between TO–083 sequences recovered by Sanger and HTS showed 99.86% of identity for DNA–A component and 99% for DNA–B component. Pairwise analyses were carried out on all begomovirus sequences identified in GenBank using BLASTn (results not shown). A large set of related species was used to generate the phylogenetic and SDT v1.2. analyses (**Fig. 3**).

The demarcation criterion for the classification of a new *Begomovirus* species is less than 91% identity for the complete DNA–A sequence in comparison with the same genomic component of any other begomovirus species [36]. The highest degree of identity (89.8%) was observed with TBYMoV (a distinct begomovirus not yet fully characterized). Moreover, the iterons of TO-083 and TBYMoV were distinct. The RDP5 program [37] was employed to verify the presence of recombination events in the TO–083 genome. However, no recombination event could be detected.

Mosaic and apical leaf distortion symptoms were observed in tomato seedlings 45 days after biolistic inoculation with monomeric infectious clones obtained for the DNA–A and DNA–B components of TO–083 (**Fig. 4**). To confirm if this putative novel virus was present in the inoculated samples, PCR reactions with universal *Begomovirus* primers for the component

DNA–A (PAL/PAR1978-496) were performed and viral presence was verified via Sanger dideoxy sequencing.

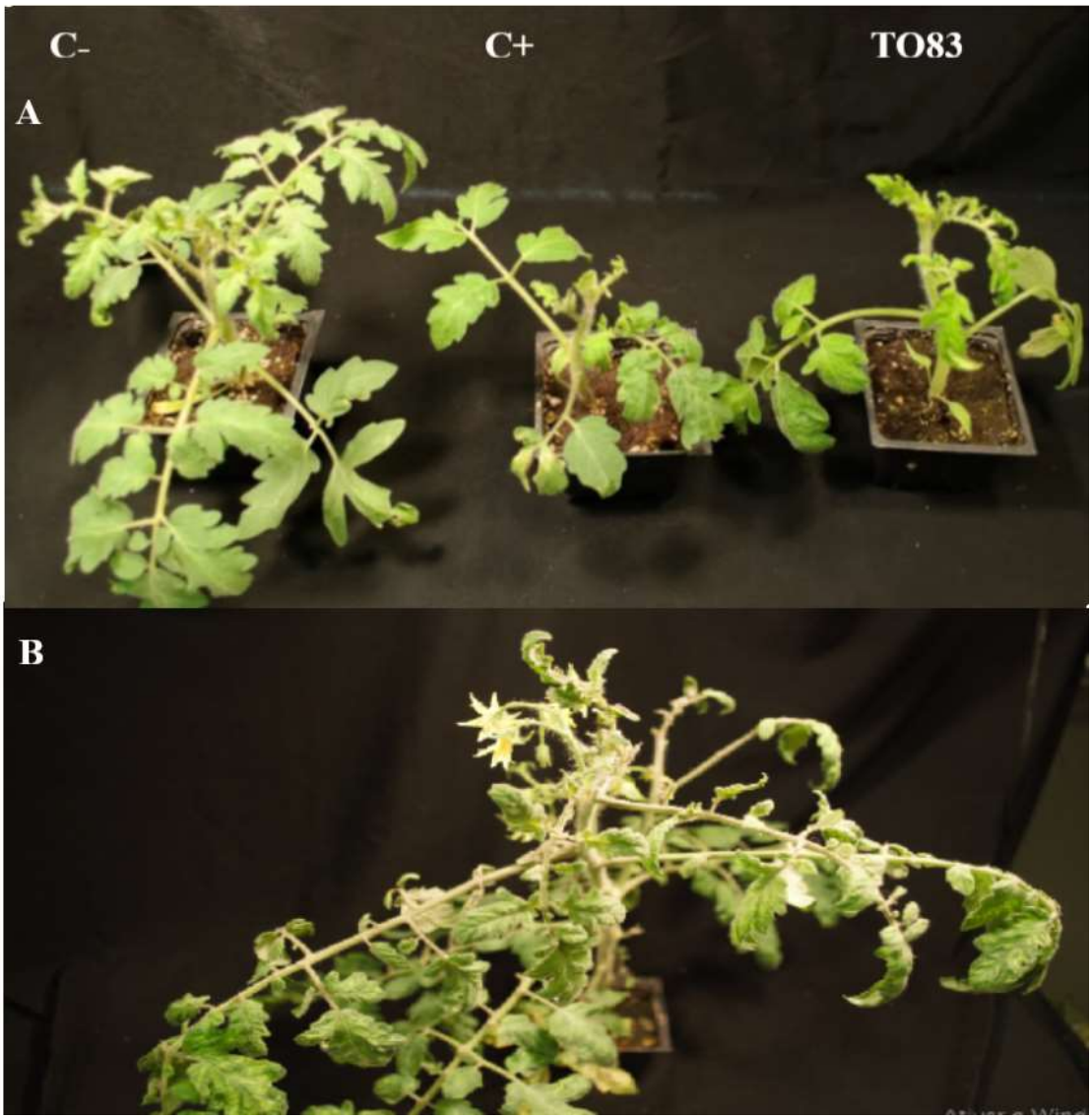

Fig. 4 Panel (A): Mosaic and apical leaf distortion symptoms induced 45 days after biolistic inoculation of the tomato (*Solanum lycopersicum* L.) with the infectious clone of tomato golden leaf spot virus (ToGLSV) (isolate TO-083). Negative control (in the left), empty vector control and the biolistic-inoculated tomato (*Solanum lycopersicum*) seedling with an infectious clone of ToGLSV isolate TO–083 (in the right). Panel (B): Details of the symptoms induced in tomato plant by biolistic-inoculated ToGLSV isolate TO–083

Discussion

Our analyses allowed us to unequivocally conclude that the TO–083 isolate represents a new viral species. We tentatively named this novel viral pathogen as tomato golden leaf spot virus – ToGLSV**.** In Brazil, the tomato crop has been heavily affected by diseases caused by a complex of at least 26 *Begomovirus* species transmitted by *B. tabaci* MEAM 1 [20, 38, 39].

Nationwide surveys have indicated *Tomato severe rugose virus* as most important species in the South and Central regions, whereas *Tomato mottle leaf curl virus* in the predominant species in Northeast region of the country [20, 38, 39]. However, the warm subequatorial Brazilian region has been neglected in terms of tomato-infecting begomovirus surveys, having few studies of the diversity in the region. Here, we describe a new bipartite begomovirus found infecting tomatoes in the warm, subequatorial region of Brazil, reinforcing the notion that the diversity of tomato-infecting begomoviruses in Neotropical areas is yet largely underestimated. In conclusion, the present report expands the ever-increasing number of *Begomovirus* species in which is the most important genus of the *Geminiviridae* family. In the present work, we also developed infectious viral clones. The availability of infectious clones can be a very useful breeding tool for screening tomato germplasm in search of resistance factors against this emergent virus.

References

1.FAOSTAT (2020) Statistics Division of the Food and Agricultural Organization of the United Nations. https://www.fao.org/faostat/en/#data/QCL/visualize. Acessado 05 de junho de 2022.

2.IBGE (2021) Instituto Brasileiro de Geografia e Estatística. Levantamento Sistemático da Produção Agrícola: Estatítica da produção agrícola 94 p.

3.Rojas MR, Macedo MA, Maliano MR, Soto-Aguilar M, Souza JO, Briddon RW, Bridon RW, Kenyon L, Bustamante RFR, Zerbini FM, Adkins S, Legg JP, Kvarnheden A, Wintermantel WM, Sudarsha MR, Peterschmitt M, Lapidot M, Martin DP, Moriones E, Inoue-Nagata AK, Gilbertson RL (2018) World management of geminiviruses. Annual Review Phytopathology 56:637–677.

4. Paprotka T, Deuschle K, Metzler V, Jeske H (2011) Conformation-selective methylation of geminivirus DNA. Journal of Virology 85: 12001–12012.

5.Whitfield AE, Falk BW, Rotenberg D (2015) Insect vector-mediated transmission of plant viruses. Virology 479:278–289.

6.Fiallo-Olivé E, Lett JM, Martin DP, Roumagnac P, Varsani A, Zerbini FM, Navas-Castillo J, Consortium IR (2021) ICTV virus taxonomy profile: Geminiviridae 2021. The Journal of General Virology 102: 1-2.

7.Wilson VG (2017) Viral interplay with the host sumoylation system. In SUMO Regulation of Cellular Processes 963: 359–388.

8.Macedo MA, Albuquerque LC, Maliano MR, Souza JO, Rojas MR, Inoue-Nagata AK, Gilbertson RL (2018) Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. Archives of Virology 163:737–743.

9.Argüello-Astorga GR, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminiviruses: Identification of potential interacting amino acid-base pairs by a comparative approach. Archive Virology146:1465–1485.

10.King AMQ, Lefkowitz E, Adams MJ, Carstens EB (2011) Virus taxonomy: Ninth report of the International Committee on Taxonomy of Viruses; Elsevier, 2011; Vol. 9

11.Faria JC, Zerbini FM (2000) Família Geminiviridae: Taxonomia, replicação e movimento. Revisão Anual de Patologia de Plantas 8:27–57.

12.Sunter G, Bisaro DM (1991) Transactivation in a geminivirus: AL2 gene product is needed for coat protein expression. Virology 180:416–419.

13.Sunter G, Hartitz MD, Hormuzdi SG, Brough CL, Bisaro DM (1990) Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for efficient DNA replication. Virology 179:69–77.

14.Briddon RW, Pinner MS, Stanley J, Markham PG (1990) Geminivirus coat protein gene replacement alters insect specificity. Virology 177:85–94.

15.Li P, Su F, Meng Q, Yu H, Wu, G, Li M, Qing L (2021) The C5 protein encoded by Ageratum leaf curl Sichuan virus is a virulence factor and contributes to the virus infection. Molecular Plant Pathology 22:1149–1158

16.Pan LL, Chi Y, Liu C, Fan,YY, Liu SS (2020) Mutations in the coat protein of a begomovirus result in altered transmission by different species of whitefly vectors. Virus Evolution 6:1–12

17.Noueiry AO, Lucas WJ, Gilbertson RL (1994) Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. Cell 76: 925–932.

18.Cantú-Iris M, Pastor-Palacios G, Mauricio-Castillo JA, Bañuelos-Hernández B, Avalos-Calleros JA, Juárez-Reyes A, Rivera-Bustamante R, Argüello-Astorga GR (2019) Analysis of a new begomovirus unveils a composite element conserved in the CP gene promoters of several *Geminiviridae* genera: Clues to comprehend the complex regulation of late genes. PLoS One 14:1-24.

19.Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JCF, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Archives of Virology160:1593–1619.

20.Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RC (2020) Metagenomics of neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty–1* gene. Viruses 12:1–22.

21.Quadros AFF, Silva JP, Xavier CAD, Zerbini FM, Boari AJ (2019) Two new begomoviruses infecting tomato and *Hibiscus* sp. in the Amazon region of Brazil. Archives of Virology 164:1897–1901.

22. Oliveira IA, Reis LNA, Nery FMB, Fernandes NAN, Gonçalves VPMR, Rojas MR, Gilbertson RL, Reis A, Boiteux LS, Fonseca-Boiteux MEN, Pereira-Carvalho RC (2024) Genomic characterization of a highly divergent tomato chlorotic mottle Guyane virus strain in the Brazilian Amazon River Basin. Phytoparasitica 52:1–5

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

24. Melgarejo TA, Kon T, Gilbertson RL (2015) Molecular and biological characterization of distinct strains of Jatropha mosaic virus from the Dominican Republic reveal a potential to infect crop plants. Phytopathology 105:141–153.

25. Sambrook J, Russell DW, Sambrook J (2006) The condensed protocols from molecular cloning: A laboratory manual. NY Cold Spring Harb. Lab. Press v 2006

26. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

27. Muhire BM, Varsani A, Martin DP (2014) SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9:1–8.

28. Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

29. Choudhury NR, Malik PS, Singh DK, Lam MN, Kaliappan K, Mukherjee SK (2006) The oligomeric Rep protein of Mungbean yellow mosaic India virus (MYMIV) is a likely replicative helicase. Nucleic Acids Research 34:6362–6377.

30. Fontes EP, Eagle PA, Sipe PS, Luckow VA, Hanley-Bowdoin L (1994) Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. Journal Biological Chemistry 269:8459–8465.

31. Laufs J, Jupin I, David C, Schumacher S, Heyraud-Nitschke F, Gronenborn B (1995) Geminivirus replication: Genetic and biochemical characterization of Rep protein function, a review. Biochimie 77:765–773.

32. Orozco BM, Hanley-Bowdoin L (1996) A DNA structure is required for geminivirus replication origin function. Journal Virology 70:48–158.

33. Fontes EP, Gladfelter HJ, Schaffer RL, Petty IT, Hanley-Bowdoin L (1994) Geminivirus replication origins have a modular organization. Plant Cell 6:405–416.

34. Hartitz MD, Sunter G, Bisaro DM (1999) The tomato golden mosaic virus transactivator (TrAP) is a single-stranded DNA and zinc-binding phosphoprotein with an acidic activation domain. Virology 263: 1–14.

35. Sung YK, Coutts RHA (1996) Potato yellow mosaic geminivirus AC2 protein is a sequence non-specific DNA binding protein. FEBS Letters 383: 51–54.

36. Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S (2013) Geminiviruses: Masters at redirecting and reprogramming plant processes. Nature Review of Microbiology 11:777–788.

37. Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B (2021) RDP5: a computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evolution. 7:1 – 3.

38. Duarte MF, Fonseca MEN; Costa H, Fernandes NA, Reis A, Boiteux LS, Pereira-Carvalho RC (2021) Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. Virus Genes 57:83–93.

39. Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RDC (2021) Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American Begomovirus species based upon genome–wide pairwise identity of multiple isolates. Virus Genes 57:127–131.

CAPÍTULO 06

Tomato iridescent mottle virus and tomato iridescent apical mosaic virus: Two new monopartite begomoviruses infecting tomato plants in the Northeast and South of Brazil

Tomato iridescent mottle virus and tomato iridescent apical mosaic virus: Two new monopartite begomoviruses infecting tomato plants in the Northeast and South of Brazil

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Abstract

The DNA–A of two new begomoviruses was recovered via High-throughput sequencing (HTS) from symptomatic tomato samples. Using PCR with species-specific primers, the new species were detected in samples from the Northeast and South regions of Brazil. One of the new begomoviruses (named as iridescent mottle virus – ToIMoV), was detected in the states of Ceará (CE–001) and Pernambuco (PE–011 and PE–012) it is phylogenetically most closely related to tomato interveinal chlorosis virus (ToICV; JF803252), with the complete DNA–A segment sharing 90% nucleotide identity. The other novel begomovirus (tentatively named as tomato iridescent apical mosaic virus – ToIAMV) was detected in two foliar samples (PR–173 and PR–174) in Paraná state, Southern Brazil. ToIAMV was found to be phylogenetically closer to tomato mottle leaf curl virus (ToMoLCV; MT215005) sharing 90% identity of nucleotides across their DNA–A segments. The cognate DNA–B segments of ToIMoV were not found *in silico* analyses and also in exhaustive PCR assays with the degenerate primer pairs 'PBL1v2040/PCRc1' in all positive samples where this virus was detected. In the ToIAMV samples, only tomato severe rugose virus (ToSRV)-derived DNA–B segments were detected due to the presence of mixed infections with this pathogen. ToIMoV displayed a 473-nucleotide recombination event overlapping the Rep, TrAp, and REn ORFs. For ToIAMV, the recombination event occurs involving Rep and TrAp (of 889 nucleotides). Both species presented recombination events with other begomoviruses that infect tomato in Brazil. Our results point out to the discovery of two new species of monopartite begomoviruses in Brazil, expanding the list of tomato-infecting begomoviruses in the selected group of Brazilian monopartites. Our results contribute to the recognition of the current scenario of tomato begomoviruses in Brazil, as well as to maintaining the diversity of species in the establishment phase in tomato producing regions.

Begomoviruses (family *Geminiviridae*) are circular single-stranded DNA viruses of approximately 2,600 nucleotides that can have either one (monopartite) or two (bipartite) DNA segments [1]. The genus *Begomovirus* is the most important within the family displaying a wide range of hosts, comprising more than 440 species, whose isolates are efficiently transmitted by whiteflies (*Bemisia tabaci*) [2]. Tomatoes (*Solanum lycopersicum* L.) was a minor host of begomoviruses during the 1950s and 1970s with only sporadic outbreaks [3–5]. This situation changed dramatically after the invasion of *B. tabaci Middle East-Asia Minor 1* (MEAM1) during the 1990s. Currently, a complex of 30 tomato-infecting begomoviruses occurs in Brazil, occurring across different biomes of the country. This complex is mostly formed by bipartite begomoviruses including tomato golden mosaic virus – TGMV [5], tomato severe rugose virus – ToSRV [6], tomato rugose mosaic virus – ToRMV [7], tomato chlorotic mottle virus – ToCMoV [7], tomato yellow vein streak virus – ToYVSV [8], tomato golden vein virus –TGVV [9], tomato interveinal chlorosis virus –ToICV [9], tomato interveinal chlorosis virus 2 – ToICV2 [10], tomato yellow spot virus – ToYSV [11], tomato common mosaic virus – ToCmMV [12], tomato leaf distortion virus – ToLDV [12], tomato mild mosaic virus –ToMMV [12], tomato chlorotic leaf curl virus – ToCLVV [13], tomato mottle leaf distortion virus – ToMoLDV [14], tomato chlorotic mottle Guyane virus –ToCMoGV [15]. Four putative monopartite species were also: tomato mottle leaf curl virus – ToMoLCV [16], tomato leaf curl purple vein virus – ToLCPVV [17], tomato golden net virus – ToGNV [18], and tomato yellow net virus – ToYNV [18]. In addition, five species initially detected in weeds, were also detected in tomato: Euphorbia yellow mosaic virus – EuYMV [19], Sida micrantha mosaic virus – SimMV [20]. Sida mottle virus – SiMoV [6], Sida yellow net virus – SiYNV [21] and Sida common mosaic virus – SiCmMV [21].

Begomoviruses with a monopartite genome in Brazil have been reported in the Northeast regions [16; 17] and Central-West Brazil [18]. Two were confirmed as monopartite species: tomato mottle leaf curl virus – ToMoLCV [16] and tomato leaf curl purple vein virus – ToLCPVV [17]. Tomato golden net virus – ToGNV [18], and tomato yellow net virus – ToYNV [18] are yet considered as putative monoparatite species since inoculation bioassays are still lacking for both [18]. Herein, two new putative monopartite species were described displaying typical genomic organizations of New World begomoviruses. These begomoviruses were detected in symptomatic tomato samples from two ecologically distinct Brazilian regions: Northeast and South.

In the present work, 73 foliar samples were collected from tomato plants exhibiting typical symptoms of begomovirus infection (mosaic, dwarfism, leaf deformations, and shrinking) in the North (13 samples), Northeast (36), South (24) regions of Brazil in the years 2002 and 2017. The samples were subjected to total DNA extraction according to the modified CTAB protocol [22] and stored at –20 °C until use. An initial characterization was carried out using PCR with the degenerate primer pair 'PAR1c496' / 'PAL1v1978' [23]. Circular DNA enrichment was performed by RCA (Rolling Circle Amplification) [24]. The enriched DNA was subjected to High-throughput sequencing (HTS) on the Illumina NovaSeq-6000 platform. The data generated by HTS were processed in the following steps: **(*i*)** elimination of low-quality reads; **(*ii*)** assembly of contigs using the CLC Genomics program, and **(*iii*)** alignment with the BLASTn algorithm with sequences available in the GenBank database [18]. Contigs were annotated and mapped using the 'Map to reference' tool available in the Geneious® 11 program [25]. The complete DNA–A segments were aligned with the multiple alignment algorithm MUSCLE. Phylogenetic trees were created based on the alignment of the complete DNA–A sequences of the selected begomoviruses by Maximum-likelihood, using the GTR+F+R3 substitution model with bootstrap support of 100 repetitions. Figures were assembled using the Adobe Illustrator CC and EvolView [18] programs. Recombination events were analyzed using the RDP5 program [26].

High-Throughput Sequencing (HTS) provided 7,230,366 reads, with the help of the CLC Genomics program, it allowed the assembly of 137 contigs of viral genomes, of which two contigs displayed nucleotide identity below 91% (according to previous analysis by BLASTn). These identity values correspond to putative new species of the genus *Begomovirus* according to the current species demarcation criteria [27]. Specific primer pairs were designed for both species: new species #1: **F1A** 5'–AAG TAA GGA AAA AAT TCT TGG CTT GG –3' / **R1A** 5'–ATC CCA AGT GTT CCC TGA CGA AAG AG –3' and new species #2 **F2A** 5'–TAT CAA TTC GTC GTC TCC TGA TTC CT–3' / **R2A** 5'–AAC TTT ACC AAA CTT AGT GAC CAA G–3'. PCR assays with specific primers were performed, from which the new species #1 was detected, in mixed infections with ToMoLCV, in samples from the Northeast, in the states of Ceará (CE–001) and Pernambuco (PE–011 and PE–012). While the new species #2 was detected in two samples from Paraná State (PR–173 and PR–174). According to the symptoms observed in the field, for the new species #1, the name tomato iridescent mottle virus (ToIMoV) is proposed, while for the new species #2 the suggested name is tomato iridescent apical mosaic virus (ToIAMV). PCR assays with primers specific for eight begomoviruses, we found that ToIAMV is present mixed infection with tomato severe rugose virus (ToSRV). In

this way, PCR assays were conducted with primers for the DNA–B component [9; 14] and the amplicons (**Supplementary Figure 1**) were validated via Sanger dideoxy chain termination sequencing.

Both species presented the DNA–A segment typical of New World begomoviruses (**Fig. 1**), displaying the ORFs V1 (756 nucleotides), which encodes the viral protein coat protein (CP), C1 (1077 nts), which is responsible for the replicase (Rep); C2 (390 nts), which encodes the transcription transactivator protein (TrAp); C3 (399 nts), encoding the replication enhancer protein (REn). and C4 (294 nts), which is involved in gene silencing. For new species #2, only C1 diverged in size (1056 nts). The 5'–TAATATT/AC–3' nucleotide sequence (conserved across begomoviruses) was also located in the intergenic region (IR) of both species. We also carried out analyses of the iterons and iteron-related domains (Rep-IRD) in the sequences of the two new species [28].

ToIMoV, with a genome of 2,604 nucleotides (nts), presented the GGAGT iteron (Rep-IRD = MPLPRRFKIQ) (**Fig. 1A**), while ToIAMV (2,631 nts) presented the GGGGT iteron (Rep-IRD = MPLPRRFKIH) (**Fig. 1B**) [28]. We were unable to identify cognate sequences in silico analyses from the assembled HTS contigs (Supplementary Figure 2). However, DNA–B segments of ToSRV were detected in both samples with ToIAMV, indicating a mixed infections. The comparison of the Rep/CP intergenic regions of the DNA–A sequences with all the DNA–B sequences (13) obtained by HTS, the identities varied from 40.3 to 79.5% (**Supplementary Figure 2**), indicating that none of them could function as cognates the DNA–B segments are of ToIAMV.

Analysis by RDP5 revealed that both new species displayed significant recombination signals. For ToIMoV, a recombination event was detected by six methods, indicating tomato interveinal chlorosis virus (ToICV; JF803252) as the major parent and tomato leaf curl purple vein virus (ToLCPVV; KY196216) as the minor parent. The statistical values calculated for the six methods were: RDP (*p-value* = 7.039 *x* 10^{-13}), GENECONV (*p-value* = 1.123 *x* 10^{-7}), BootScan (*p-value* = 2.478 *x* 10^{-9}), MaxChi (*p-value* = 5.789 x 10^{-7}), Chimaera (*p-value* = 2.917 x 10^{-8}) and 3Seq (*p-value* = 2.271 x 10^{-10}). The recombination event encompasses 473 nucleotides, initiating at nucleotide 1268 and ending at nucleotide 1740, partially overlapping the Rep, TrAp and REn ORFs (**Fig. 1A**).

On the other hand, ToIAMV displayed significant recombination signals for methods: RDP (*p-value* = 9.009 *x* 10^{-12}), GENECONV (*p-value* = 1.992 *x* 10^{-9}), BootScan (*p-value* = 1.084 *x* 10^{-9}), MaxChi (*p-value* = 4.218 *x* 10^{-8}), Chimaera (*p-value* = 6.972 *x* 10^{-7}) and SiScan (*p-value* = 2.210 *x* 10^{-23}). These analyses showed that ToIAMV has TGMV (K02029) as the

major parent and ToICV (JF803252) as the minor parent. The recombination (of 889 nucleotides) was identified starting at nucleotide 1491 and ending at nucleotide 2379, comprising the majority of Rep and the beginning of TrAp (**Fig. 1B**).

Analysis by Sequence Demarcation Tool (SDT) (**Fig. 2**) showed that ToIMoV is also more phylogenetically related ToICV (isolate JF803252), sharing 90% DNA–A nucleotide identity. ToIAMV shares the same clade as the monopartite begomovirus isolate tomato mottle leaf curl virus – ToMoLCV (MT215005), sharing 90% identity (**Fig. 2**).

In Brazil, the begomoviruses that infect tomatoes are predominantly bipartite [32, 33]. However a yet restrict group of monopartite begomoviruses is being recently characterized [18]. Currently, there are four monopartite species that affect tomato crops: tomato mottle leaf curl virus (ToMoLCV) [16], tomato leaf curl purple vein virus (ToLCPVV) [17], tomato yellow net virus (ToYNV) and tomato golden net virus (ToGNV) [18]. ToMoLCV and ToLCPVV were prevalent in the semi-arid northeastern region of Brazil [16, 17]. Likewise, ToIMoV is also originate from semi-arid areas of Ceará and Pernambuco states in the Brazilian Northeast. Recently, however, TYNV and TGNV (two putative monopartite begomoviruses) were reported infecting tomato in central Brazil (outside the Northeast region) [18]. Herein, we also report a new putative monopartite begomovirus outside the Northeast region. ToIAMV was detected in subtropical state of Paraná, in Southern Brazil.

Although the monopartite populations are much smaller than the bipartite ones, the former remain successfully present in the field due to local population of their vectors and the susceptibility of their primary host (tomato) [30]. In addition, studies have demonstrated that the CP and C4 proteins of the monopartite Old World tomato yellow leaf curl virus (ToYLCV) can perform a homologous function to the BV1 protein of the bipartites [31], increasing monopartite maintenance strategies in the fields.

In our putative new monopartite species, we report the occurrence of recombination events. ToIMoV has recombination with ToICV (major parent) and ToLCPVV (minor parent), which are two begomoviruses thus far endemic to the Northeast region [9]. Interestingly, there is thus far no cognate DNA–B sequence of ToICV available in public databases. ToIAMV, on the other hand, displayed recombination events with TGMV (major parent) and ToICV (minor parent). It is interesting that although TGMV was the first begomovirus described in Brazil [3-5], extensive surveys since 1990s are not being able to recover this species [for review see 32].

The recombination events, detected in both new species described in the present work, corroborate the hypothesis that this genetic mechanism contributes to the emergence of new begomovirus strains and species associated with the tomato crop [18, 34]. Additional studies

using biological assays with infectious clones will be necessary to further strengthen the evidence of these two begomoviruses as bona-fide monopartite species.

References:

1. Hesketh EL, Saunders K, Fisher C, Potze J, Stanley J, Lomonossoff GP, Ranson NA (2018) The 3.3 Å structure of a plant geminivirus using cryo-EM. Nat Commun 9:1–10

2. Fiallo-Olivé E, Lett J-M, Martin DP, Roumagnac P, Varsani A, Zerbini FM, Navas-Castillo J, Consortium IR (2021) ICTV virus taxonomy profile: *Geminiviridae* 2021. Journal General Virology 102:001696

3. Flores E, Silberschmidt K, Kramer M (1960) Observações de "clorose infecciosa" das malváceas em tomateiros do campo. O Biológico 26:65–69

4. Costa AS (1974) Moléstias do tomateiro no Brasil transmitidas pela mosca-branca *Bemisia tabaci*. Fitopatologia 9:1–47

5. Matyis JC, Silva DM, Oliveira AAR, Costa AS (1975) Purificação e morfologia do vírus do mosaico dourado do tomateiro. Summa Phytopathologica 1:267–274

6. Cotrim AA, Krause-Sakate R, Narita N, Zerbini FM, Pavan MA (2007) Diversidade genética de begomovírus em cultivos de tomateiro no Centro-Oeste Paulista. Summa Phytopathologica 33:300–303

7. Ribeiro S, Ambrozevicius L, Avila A, Bezerra I, Calegario R, Fernandes J, Lima M, De Mello R, Rocha H, Zerbini F (2003) Distribution and genetic diversity of tomato-infecting begomoviroses in Brazil. Arch Virol 148:281–295

8. Albuquerque LC, Martin D, Avila A, Inoue-Nagata A (2010) Characterization of tomato yellow vein streak virus, a begomovirus from Brazil. Virus Genes 40:140–147

9. Albuquerque LC, Varsani A, Fernandes F, Pinheiro B, Martin D, Ferreira P, Lemos T, Inoue-Nagata A (2012) Further characterization of tomato-infecting begomoviruses in Brazil. Archives of Virology 157:747–752

10. Rêgo-Machado CM, Nakasu EY, Blawid R, Nagata T, Inoue-Nagata AK (2019) Complete genome sequence of a new bipartite begomovirus infecting tomato in Brazil. Arch Virol 164:2873–2875

11. Calegario R, Ferreira S, Andrade E, Zerbini F (2007) Characterization of tomato yellow spot virus, a novel tomato-infecting begomovirus in Brazil. PAB 42:1335–1343

12. Castillo-Urquiza G, Beserra J, Bruckner F, Lima A, Varsani A, Alfenas-Zerbini P, Zerbini F (2008) Six novel begomoviroses infecting tomato and associated weeds in Southeastern Brazil. Arch Virol 153:1985–1989

13. Quadros AF, Silva JP, Xavier CA, Zerbini FM, Boari AJ (2019) Two new begomoviroses infecting tomato and Hibiscus sp. in the Amazon region of Brazil. Arch Virol 164:1897–1901

14. Martins TP, Souza TA, da Silva PS, Nakasu EY, Melo FL, Inoue-Nagata AK, Nagata T (2021) Nanopore sequencing of tomato mottle leaf distortion virus, a new bipartite begomovirus infecting tomato in Brazil. Archives of Virology 166:3217–3220

15. De Oliveira IA, Reis LNA, Nery FMB, Fernandes NAN, Gonçalves VPMR, Rojas MR, Gilbertson RL, Reis A, Boiteux LS, Fonseca-Boiteux MEN, Pereira-Carvalho RC (2024) Genomic characterization of a highly divergent tomato chlorotic mottle Guyane virus strain in the Brazilian Amazon River Basin. Phytoparasitica 52:1–5

16. Vu S, Melgarejo TA, Chen L, Souza JO, Macedo MA, Inoue- Nagata AK, Gilbertson RL (2015) Evidence that tomato mottle leaf curl virus form Northeastern Brazil is an indigenous New World monopartite begomovirus. Phytopathology 105:S4143

17. Macedo M, Albuquerque L, Maliano M, Souza J, Rojas M, Inoue-Nagata A, Gilbertson R (2018) Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. Arch Virol 163:737–743

18. Reis LNA, Boiteux LS, Fonseca MEN, Rojas MR, Gilbertson RL, Pereira-Carvalho RC (2023) Tomato golden net virus and tomato yellow net virus: Two novel New World begomoviruses with monopartite genomes. Archives of Virology 168:1-6

19. Duarte MF, Pereira-Carvalho R, Reis L, Rojas M, Gilbertson R, Costa H, Boiteux LS, Fonseca MEN (2021) Natural infection of tomatoes (*Solanum lycopersicum*) by Euphorbia yellow mosaic virus isolates across four Brazilian states. Plant Dis 105:518–518

20. Calegario R, Ferreira S, Andrade C, Zerbini F (2004) Caracterização de um isolado do begomovírus Sida micrantha mosaic virus (SiMMV) obtido de tomateiro. Fitopatol Bras 29:150

21. Duarte MF, Fonseca ME, Costa H, Fernandes NA, Reis A, Boiteux LS, Pereira-Carvalho RC (2021) Diversity of tomato-infecting begomoviruses and spatiotemporal dynamics of an endemic viral species of the Brazilian Atlantic rain forest biome. Virus Genes 57:83–93

22. Boiteux L, Fonseca M, Simon P (1999) Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. Journal of the American Society for Horticultural Science 124:32–38

23. Rojas MR, Gilbertson R, Russell D, Maxwell D (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Disease77:340–347

24. Inoue-Nagata AK, Albuquerque L, Rocha W, Nagata T (2004) A simple method for cloning the complete begomovirus genome using the bacteriophage φ29 DNA polymerase. Journal of Virological Methods 116:209–211

25. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

26. Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B (2021) RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evolution 7:087

27. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo Olivé E, Briddon RW, Hernández-Zepeda C, Idris A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Archives of Virology 160:1593–1619

28. Argüello-Astorga G, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminiviruses: identification of potential interacting amino acid-base pairs by a comparative approach. Archives of Virology 146:1465–1485

29. Ferro MM, Ramos-Sobrinho R, Silva JT, Assunção IP, Lima GS (2017) Genetic structure of populations of the begomoviruses Tomato mottle leaf curl virus and Sida mottle Alagoas virus infecting tomato (Solanum lycopersicum) and Sida spp., respectively. Tropical Plant Pathology 42:39-45

30. Souza JO, Melgarejo TA, Vu S, Nakasu EY, Chen LF, Rojas MR, Zerbini FM, Inoue-Nagata AL, Gilbertson RL (2022) How to be a successful monopartite Begomovirus in a bipartite-dominated world: emergence and spread of Tomato mottle leaf curl virus in Brazil. Journal of Virology 96: 1 – 23

31. Rojas RM, Jiang H, Salati R, Xoconostle-Cázares B, Sudarshana M, Lucas WJ, Gilbertson RL (2001) Functional analysis of proteins involved in movement of the monopartite begomovirus, tomato yellow leaf curl virus. Virology 291:110–125

32. Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RC (2020) Metagenomics of Neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty*-1 gene. Viruses 8: 819

33. Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RC (2021) Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome–wide pairwise identity of multiple isolates. Virus Genes 57:127–131)

34. Inoue-Nagata AK, Martin DP, Boiteux LS, Giordano LDB, Bezerra IC, Ávila, ACD (2006) New species emergence via recombination among isolates of the Brazilian tomato infecting Begomovirus complex. Pesquisa Agropecuária Brasileira, 41:1329–1332

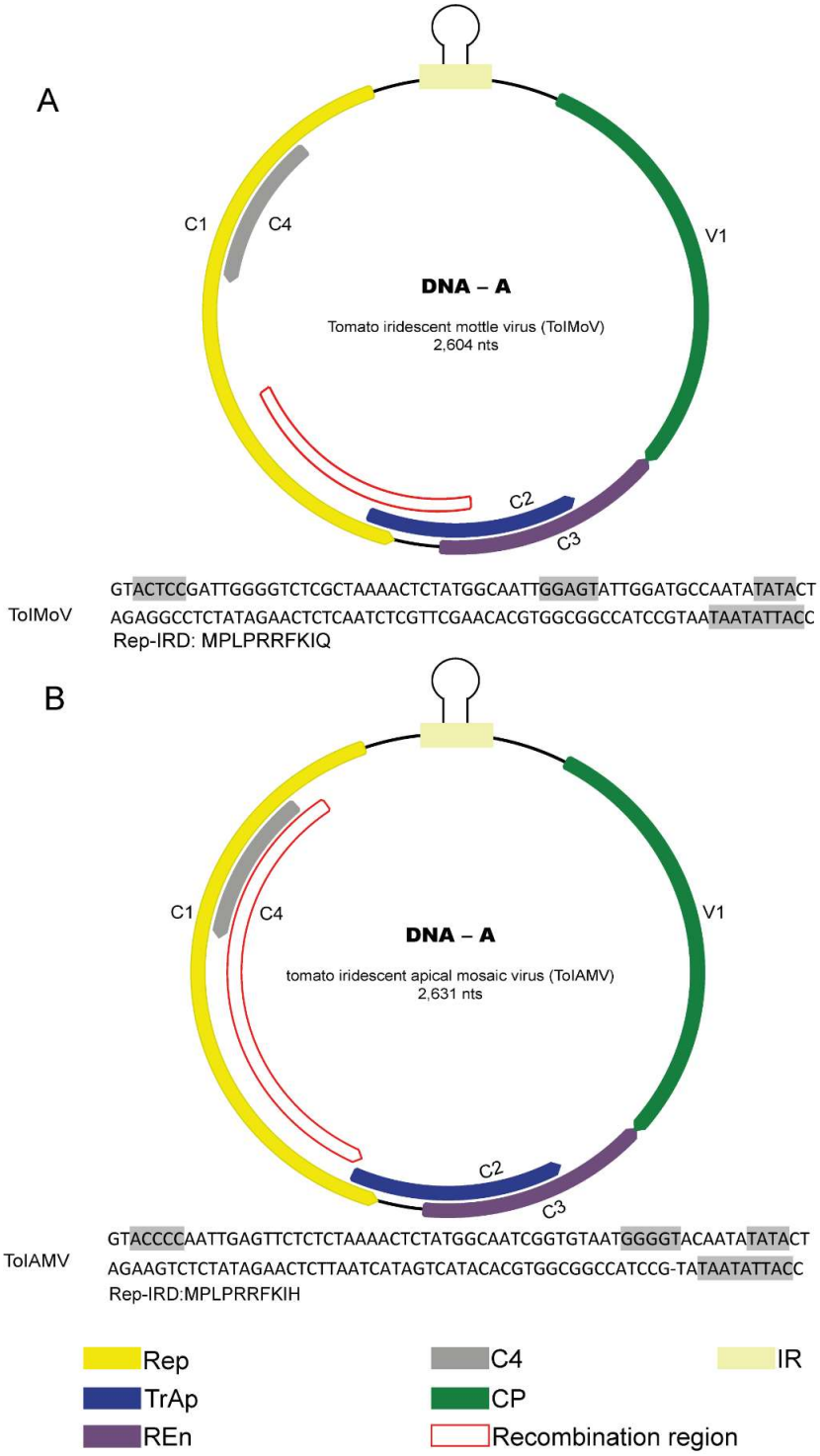


Fig. 1. Genomic organization, iterons, TATABOX, and Rep-IRD of the DNA–A segments of tomato iridescent mottle virus – ToIMoV **(Panel A)** and tomato iridescent apical mosaic virus – ToIAMV **(Panel B).** ORFs: C1 (coat protein – CP), V1 (viral replication-related protein – Rep), C2 (transcription transactivating protein – TrAp), C3 (replication-enhancing protein – REn), C4 (gene silencing-related protein – C4), intergenic region (IR), and regions encompassing recombination events.

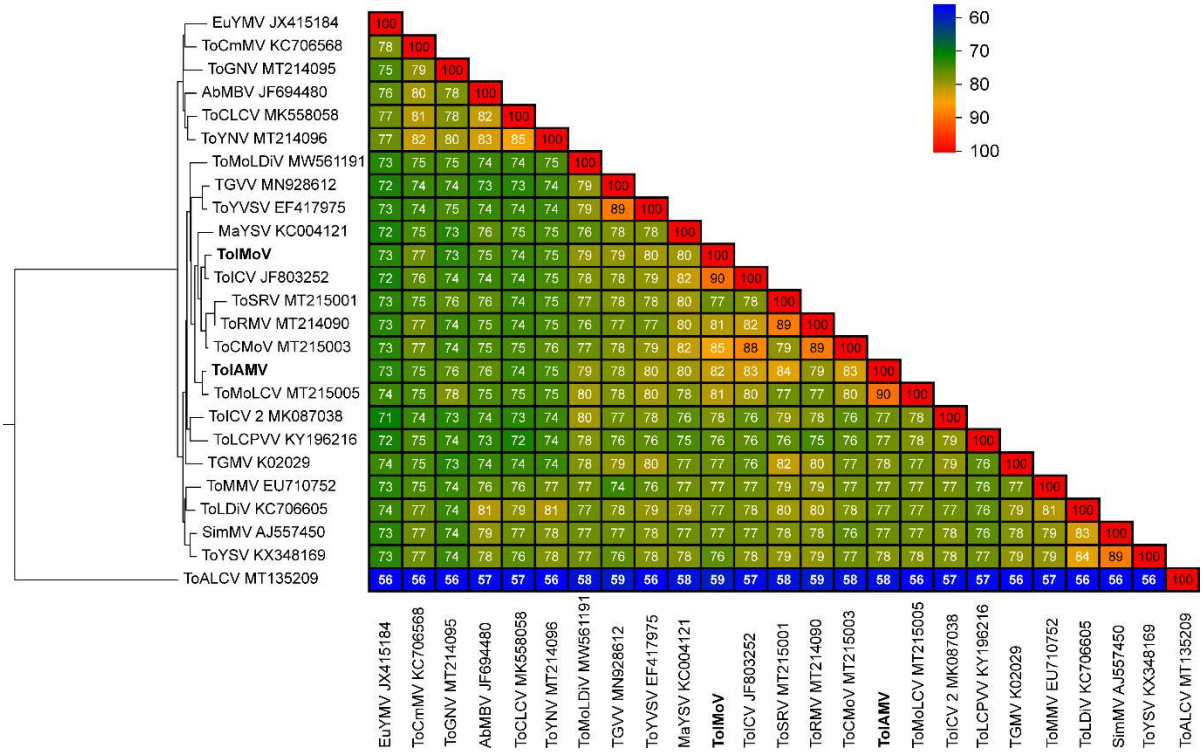
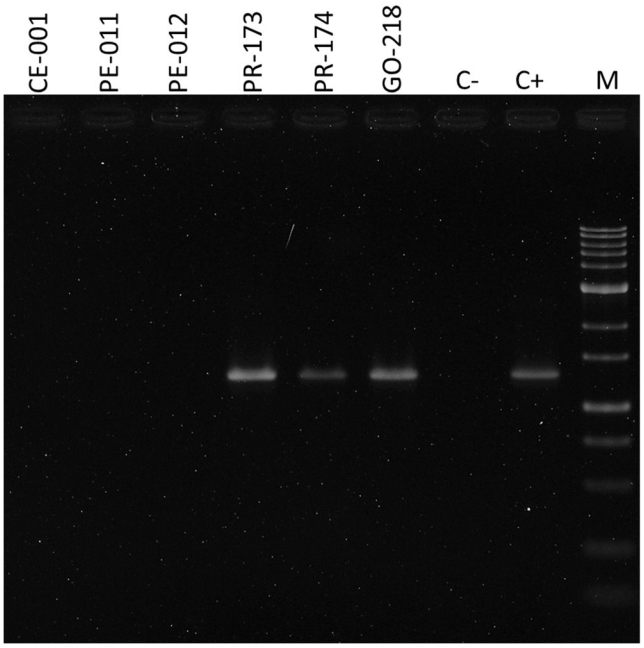
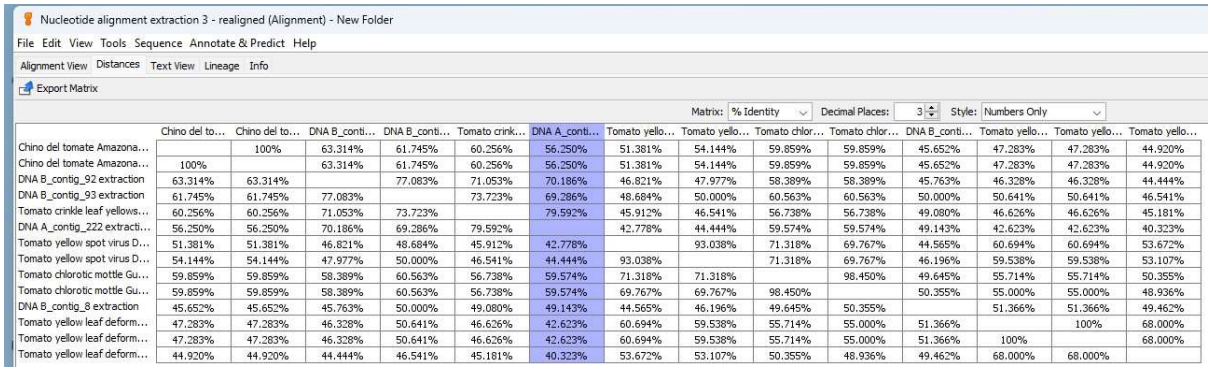


Fig. 2. Sequence Demarcation Tool (SDT) and phylogenetic reconstruction from sequences of the DNA–A segment of begomovirus isolates that infect tomato and weeds. Tomato iridescent mottle virus – ToIMoV is more phylogenetically related to the tomato interveinal chlorosis virus isolate – ToICV (JF803252), sharing 90% identity. Tomato iridescent apical mosaic virus ToIAMV has 90% identity with the tomato mottle leaf curl virus isolate – ToMoLCV (MT215005) to which it is phylogenetically closest. Euphorbia yellow mosaic virus – EuYMV (JX415184), tomato common mosaic virus – ToCmMV (KC706568), tomato golden net virus – ToGNV (MT214095), Abutilon mosaic Brazil virus – AbMBV (JF694480), tomato chlorotic leaf curl virus – ToCLCV (MK558058) , tomato yellow net virus – ToYNV (MT214096), tomato leaf distortion virus – ToLDiV (MW561191), tomato golden vein virus – TGVV (MN928612), tomato yellow vein streak virus – ToYVSV (EF417915), Macroptilium yellow spot virus – MaYSV (KC004121), tomato severe rugose virus – ToSRV (MT215001), tomato rugose mosaic virus – ToRMV (MT214090), tomato chlorotic mottle virus – ToCMoV (MT215003), tomato interveinal chlorosis virus 2 – ToICV2 (MK087038), tomato leaf curl purple vein virus – ToLCPVV (KY196216), tomato golden mosaic virus – TGMV (K02029), tomato mild mosaic virus – ToMMV (EU710752), tomato leaf distortion virus – ToLDiV (KC706605), Sida micrantha mosaic virus – SimMV (AJ557450), tomato yellow spot virus – ToYSV (KX348169). Outgroup, isolated from the genus Topilevirus, tomato apical leaf curl virus – ToALCV (MT135209).



Supplementary Figure 1. Agarose gel (1%) showing amplicons of approximately 1200 pb, generated via PCR for the DNA–B segment using total DNA extracted from tomato leaf samples designated as: lines 1 – 6: CE–001, PE–011, PE–012, PR–173, and PR–174. Positive control (C+): isolate GO–130, negative control (C-): Milli-Q water. Molecular marker (M) Kasvi 1 kb.



	Chino del to…	Chino del to…	DNA B_conti…	DNA B_conti…	Tomato crink…	DNA A_conti…	Tomato yello…	Tomato yello…	Tomato chlor…	Tomato chlor…	DNA B_conti…	Tomato yello…	Tomato yello…	Tomato yello…
Chino del tomate Amazona…		100%	63.314%	61.745%	60.256%	56.250%	51.381%	54.144%	59.859%	59.859%	45.652%	47.283%	47.283%	44.920%
Chino del tomate Amazona…	100%		63.314%	61.745%	60.256%	56.250%	51.381%	54.144%	59.859%	59.859%	45.652%	47.283%	47.283%	44.920%
DNA B_contig_92 extraction	63.314%	63.314%		77.083%	71.053%	70.186%	46.821%	47.977%	58.389%	58.389%	45.763%	46.328%	46.328%	44.444%
DNA B_contig_93 extraction	61.745%	61.745%	77.083%		73.723%	69.286%	48.684%	50.000%	60.563%	60.563%	50.000%	50.641%	50.641%	46.541%
Tomato crinkle leaf yellows…	60.256%	60.256%	71.053%	73.723%		79.592%	45.912%	46.541%	56.738%	56.738%	49.080%	46.626%	46.626%	45.181%
DNA A_contig_222 extracti…	56.250%	56.250%	70.186%	69.286%	79.592%		42.778%	44.444%	59.574%	59.574%	49.143%	42.623%	42.623%	40.323%
Tomato yellow spot virus D…	51.381%	51.381%	46.821%	48.684%	45.912%	42.778%		93.038%	71.318%	69.767%	44.565%	60.694%	60.694%	53.672%
Tomato yellow spot virus D…	54.144%	54.144%	47.977%	50.000%	46.541%	44.444%	93.038%		71.318%	69.767%	46.196%	59.538%	59.538%	53.107%
Tomato chlorotic mottle Gu…	59.859%	59.859%	58.389%	60.563%	56.738%	59.574%	71.318%	71.318%		98.450%	49.645%	55.714%	55.714%	50.355%
Tomato chlorotic mottle Gu…	59.859%	59.859%	58.389%	60.563%	56.738%	59.574%	69.767%	69.767%	98.450%		50.355%	55.000%	55.000%	48.936%
DNA B_contig_8 extraction	45.652%	45.652%	45.763%	50.000%	49.080%	49.143%	44.565%	46.196%	49.645%	50.355%		51.366%	51.366%	49.462%
Tomato yellow leaf deform…	47.283%	47.283%	46.328%	50.641%	46.626%	42.623%	60.694%	59.538%	55.714%	55.000%	51.366%		100%	68.000%
Tomato yellow leaf deform…	47.283%	47.283%	46.328%	50.641%	46.626%	42.623%	60.694%	59.538%	55.714%	55.000%	51.366%	100%		68.000%
Tomato yellow leaf deform…	44.920%	44.920%	44.444%	46.541%	45.181%	40.323%	53.672%	53.107%	50.355%	48.936%	49.462%	68.000%	68.000%	

Supplementary Figure 2. Alignment of the Rep–CP intergenic region of the DNA–A segment of tomato iridescent apical mosaic virus (ToIAMV) with DNA–B segments obtained by HTS showing an identity variation range of 40.3 to 79.5%.

CAPÍTULO 07

Tomato bright apical mosaic virus: A new bipartite begomovirus infecting tomatoes in Central Brazil

Tomato bright apical mosaic virus: A new tomato-infecting recombinant bipartite begomovirus endemic to Central Brazil

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Abstract

The complete bipartite genome of a new begomovirus from tomato (*Solanum lycopersicum* L.) was recovered via High-throughput sequencing (HTS) and validated via Sanger dideoxy chain termination sequencing of amplicons derived from virus-specific PCR primers. The highest nucleotide identity of the DNA–A component was 89% with tomato golden vein virus (TGVV), allowing its classification as a novel species in agreement with the current demarcation criteria of the genus *Begomovirus*. This novel tomato pathogen was tentatively named as tomato bright apical mosaic virus (ToBAMV). This virus displayed a DNA–A segment of 2,561 nucleotides with a typical genomic organization of the New World bipartite begomoviruses. *In silico* analyses allowed the identification of the cognate DNA–B segment of 2527 nts. The common region of both segments displayed 196 nucleotides in size with a 100% identity between them. Both segments displayed the iteron GGGTC/GACCC, TATA box, the nonanucleotide 5'–TAATATT/AC–3', and MPPPKRFTVN as the iteron-related Rep domain (Rep-IRD). ToBAMV displayed significant recombination signals with events involving two Neotropical species – tomato leaf curl purple vein virus (ToLCPVV) and tomato chlorotic mottle virus (ToCMoV). ToBAMV was detected with virus-specific PCR primers in eight out 81 symptomatic samples of our survey, being restricted to the state of Goiás (GO) and the Federal District (DF) in Central Brazil. The present reports confirms the uninterrupted emergence of endemic species across distinct agroecological regions of the country. This new discovery also reinforces the importance of continuous surveys to monitor the diversity of this viral genus with major importance for tomato breeding.

Begomovirus (family *Geminiviridae*) is a genus with over 440 species of single-stranded DNA viruses, which might have genomes with either one (monopartite) or two (bipartite) DNA segments [1]. The first reports of begomovirus infection in tomato (*Solanum lycopersicum* L.) plants in Brazil occurred between the 1950s and 1970s with negligible economic importance and only with sporadic occurrence [2, 3]. Begomoviruses became more relevant in Brazil only after the entry of the insect vector *Bemisia tabaci* Middle East Asia Minor 1 during the 1990s [4]. Tomato crop is one of the most affected by begomoviruses with the current report of up to 30 species, of which the prevalence of bipartite viruses [5]. More recent surveys in Brazil have shown that the Central-West region has a great diversity of begomovirus species infecting tomato [6, 7]. Among the tomato-infecting species reported in Central/Midwest are tomato severe rugose virus (ToSRV), tomato rugose mosaic virus (ToRMV), tomato golden vein virus (TGVV), tomato chlorotic mottle virus (ToCMoV), tomato mottle leaf curl virus (ToMoLCV), tomato yellow net virus (ToYNV), and Sida micrantha mosaic virus (SimMV) [5, 6]. Herein, we report the detection and molecular characterization of a new bipartite begomovirus present in tomato fields in Central Brazil.

Tomato leaf samples showing typical symptoms of begomovirus infection (mosaic, dwarfism, leaf deformations, and crumpling) were collected across the main tomato-producing areas of the Central-West (42 samples) and Southeast (39 samples) regions of Brazil. Total DNA extraction of the samples was carried out according to a modified CTAB protocol [8] and stored at –20 ºC up to use. By PCR, using degenerate primers for the *Begomovirus* genus PAR1c496/PAL1v1978, the isolates were previously characterized [9]. Circular DNA was enriched using RCA (Roling Circle Amplification) [10]. The enriched circular DNAs were pooled and subjected to sequencing via High-throughput sequencing (HTS) technology using the Nova-Seq-6000 illuminate platform. The data generated by the sequencing platform were processed: (*i*) elimination of low-quality reads; (*ii*) assembly of contigs using the CLC Genomics program, and (*iii*) alignment with the BLASTn algorithm with sequences available in the GenBank database [5]. Contigs were annotated and mapped using the 'Map to reference' tool available in the Geneious® 11 program [11]. HTS-derived DNA–A sequences were aligned using MUSCLE alignment and compared to other available genomes at the GenBank database using the Sequence Demarcation Tool (SDT) [12]. Based on the DNA–A sequences, species-specific primer pairs were designed and individual viral genomes were recovered from the samples by PCR assays. Phylogenetic reconstructions were performed based on Maximum-likelihood according to the GTR+F+R6 substitution model, with bootstrap support of 100

replications. Putative recombination events were also analyzed using the RDP5 program [13]. Figures were assembled using the Adobe Illustrator CC and EvolView tools [5].

Previous analysis via BLASTn of the assembled contigs allowed us to detect one DNA–A segment (named as contig 12) with the highest nucleotide identity of 89.03% with the TGVV isolate (MN928612), indicating that it could represent a new species according to the current demarcation criteria for species of the genus *Begomovirus* [12]. This novel tomato pathogen was tentatively named as tomato bright apical mosaic virus (ToBAMV). This virus displayed a DNA–A segment of 2,561 nucleotides with a typical genomic organization of the New World bipartite begomoviruses (**Fig. 1A**). *In silico* analyses allowed the identification of the cognate DNA–B segment of 2,527 nucleotides (**Fig. 1A**). The common region of both segments displayed 196 nucleotides in size with a 100% identity between them. Both segments displayed the iteron GGGTC/GACCC, TATA box, and the nonanucleotide 5'–TAATATT/AC–3' (**Fig. 1 B**). We also identified the iteron-related Rep domain (Rep-IRD): MPPPKRFTVN (**Fig. 1 B**) [14]. ToBAMV was detected with virus-specific PCR primers in eight out 81 symptomatic samples of our survey, being restricted to the state of Goiás (GO) (isolates GO–121, GO–124, GO–126, GO–127, and GO–218) and the Federal District (DF) (isolates DF–209, DF-216, and DF–235) in Central Brazil. Sanger dideoxy chain termination sequencing of the amplicons derived from virus-specific PCR primers were carried out at CNPH used to validate the presence of this novel begomovirus in these samples.

We identified a putative recombination event through RDP5 analyses. According to the analysis, two statistical methods indicated significant signals of recombination: GENECONV (*p-value* = 3,751 x 10^{-5}) e SiScan (*p-value* = 8,253 x 10^{-8}). The recombination region, of 416 nts displayed as beginning breakpoint the nucleotide 1221 and as ending breakpoint the nucleotide 1636, covering the ORFs AC1, AC2 and AC3 (**Fig. 1A**). The major and minor parents were, respectively, tomato leaf curl purple vein virus (ToLCPVV; KY196216) and ToCMoV (MT215003). Phylogenetic reconstructions and identity comparison by SDT (**Fig. 2**) demonstrated that ToBAMV is sharing a clade with isolates of two closely-related species TGVV (MN928612) and tomato yellow vein streak virus (ToYVSV; EF417915) [17], sharing identities of 89 and 85%, respectively (**Fig. 2**).

Our results highlight the detection efficiency of novel tomato-infecting begomoviruses via HTS in combination with species-specific PCR assays as previously described [6–7]. In addition to the begomoviruses already reported infecting tomatoes, these metagenomic studies allowed for the discovery of new species associated with this vegetable crop. These studies revealed a complex of over 26 species of begomoviruses are associated with tomato cultivation

in Brazil, the majority of which are bipartite [5]. In this context, our results further expand the number of bipartite species naturally infecting tomatoes.

This astonishing increase of begomoviruses may be related to polyphagous nature of the whitefly vector in association with recombination events that occur among members of this complex but also with naturally occurring begomoviruses from weeds and other Solanaceae hosts [5, 16]. In the case of ToBAMV, we detected a recombination event involving two tomato-infecting species (ToLCPVV and ToCMoV). This results corroborates the hypothesis already raised that recombination events among members of the tomato-infecting begomovirus complex may contribute to the emergence of new species with potentially novel pathogenic attributes [5, 16, 18]. Further studies will be used to evaluate the virulence profile of ToBAMV against the main resistance/tolerance factors to the begomoviruses detected in tomato germplasm in Brazil [6, 19–20] via infectious clones and biological assays.

References:

1. Fiallo-Olivé E, Lett J-M, Martin DP, Roumagnac P, Varsani A, Zerbini FM, Navas-Castillo J, Consortium IR (2021) ICTV virus taxonomy profile: *Geminiviridae* 2021. Journal General Virology 102:001696

2. Flores E, Silberschmidt K, Kramer M (1960) Observações de “clorose infecciosa” das malváceas em tomateiros do campo. O Biológico 26:65–69

3. Costa AS (1974) Moléstias do tomateiro no Brasil transmitidas pela mosca-branca *Bemisia tabaci*. Fitopatologia 9:1–47

4. 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 begomoviruses in Brazil. Archives of Virology 148:281–295

5. Reis LNA, Boiteux LS, Fonseca MEN, Rojas MR, Gilbertson RL, Pereira-Carvalho RC (2023) Tomato golden net virus and tomato yellow net virus: Two novel New World begomoviruses with monopartite genomes. Archives of Virology 168:235

6. Reis LNA, Fonseca MEN, Ribeiro SG, Naito FYB, Boiteux LS, Pereira-Carvalho RC (2020) Metagenomics of Neotropical single-stranded DNA viruses in tomato cultivars with and without the *Ty*-1 gene. Viruses 8: 819

7. Souza TA, Silva JMF, Nagata T, Martins TP, Nakasu EYT, Inoue-Nagata AK (2020) A temporal diversity analysis of Brazilian begomoviruses in tomato reveals a decrease in species richness between 2003 and 2016. Frontiers in Plant Science 11:1–13

8. Boiteux L, Fonseca M, Simon P (1999) Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. Journal of the American Society for Horticultural Science 124:32–38

9. Rojas MR, Gilbertson R, Russell D, Maxwell D (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Disease77:340–347

10. Inoue-Nagata AK, Albuquerque L, Rocha W, Nagata T (2004) A simple method for cloning the complete begomovirus genome using the bacteriophage φ29 DNA polymerase. Journal of Virological Methods 116:209–211

11. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649

12. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo Olivé E, Briddon RW, Hernández-Zepeda C, Idris A (2015) Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Archives of Virology 160:1593–1619

13. Martin DP, Varsani A, Roumagnac P, Botha G, Maslamoney S, Schwab T, Kelz Z, Kumar V, Murrell B (2021) RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. Virus Evolution 7:087

14. Argüello-Astorga G, Ruiz-Medrano R (2001) An iteron-related domain is associated to Motif 1 in the replication proteins of geminiviruses: Identification of potential interacting amino acid-base pairs by a comparative approach. Archives of Virology 146:1465–1485

15. Mituti T, Moura MF, Macedo MA, Silva TN, Pinto LR, Costa H, Krause-Sakate R, Inoue-Nagata A, Nunes GG, Lima MF, Rezende JA (2019) Survey of begomoviruses and the crinivirus, tomato chlorosis virus, in solanaceous in Southeast/Midwest of Brazil. Tropical Plant Pathology 44:468–472

16. Inoue-Nagata AK, Martin DP, Boiteux LS, Giordano LDB, Bezerra IC, Ávila, ACD (2006) New species emergence via recombination among isolates of the Brazilian tomato infecting Begomovirus complex. Pesquisa Agropecuária Brasileira, 41:1329–1332

17. Reis LNA, Boiteux LS, Fonseca MEN, Pereira–Carvalho RC (2021) Tomato yellow vein streak virus and tomato golden vein virus: A reappraisal of the classification status of two South American *Begomovirus* species based upon genome–wide pairwise identity of multiple isolates. Virus Genes 57:127–131.

18. Macedo M, Albuquerque L, Maliano M, Souza J, Rojas M, Inoue-Nagata A, Gilbertson R (2018) Characterization of tomato leaf curl purple vein virus, a new monopartite New World begomovirus infecting tomato in Northeast Brazil. Archives of Virology 163:737–743

19. Giordano LB, Silva-Lobo VL, Santana FM, Fonseca MEN, Boiteux LS (2005) Inheritance of resistance to the bipartite tomato chlorotic mottle begomovirus derived from *Lycopersicon esculentum* cv. 'Tyking'. Euphytica 143: 27–33.

20. Pereira-Carvalho RC, Boiteux LS, Fonseca MEN, Díaz-Pendón JA, Moriones E, Fernández-Muñoz R, Charchar JM, Resende RO (2010) Multiple resistance to *Meloidogyne* spp. and to bipartite and monopartite *Begomovirus* spp. in wild *Solanum* (*Lycopersicon*) accessions. Plant Disease 94: 179–185.

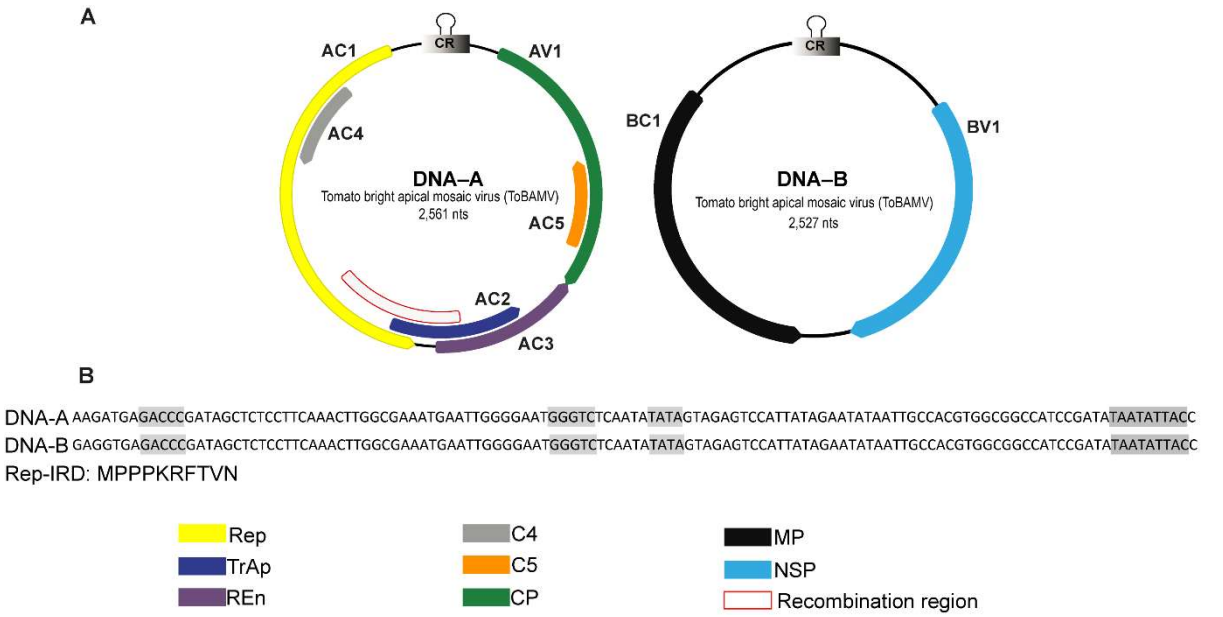

Fig. 1. Schematic of the genomic organization of the DNA–A and DNA – B segments of tomato bright apical mosaic virus (ToBAMV) and recombination region (**A**). GGGTC/GACCC iteron, TATABOX, TAATATT/AC nonanucleotide identified in the common region (CR) of the DNA–A and DNA–B segments and amino acid sequence identified in the Rep iteron-related domain (Rep–IRD): MPPPKRFTVN (**B**). Proteins synthesized by each ORF of both DNA segments are described in the figure legend: Viral replicase (Rep), coat protein (CP), transcription activator protein (TrAp), replication enhancer (REn), movement protein (MP) and nuclear transport protein (NSP).

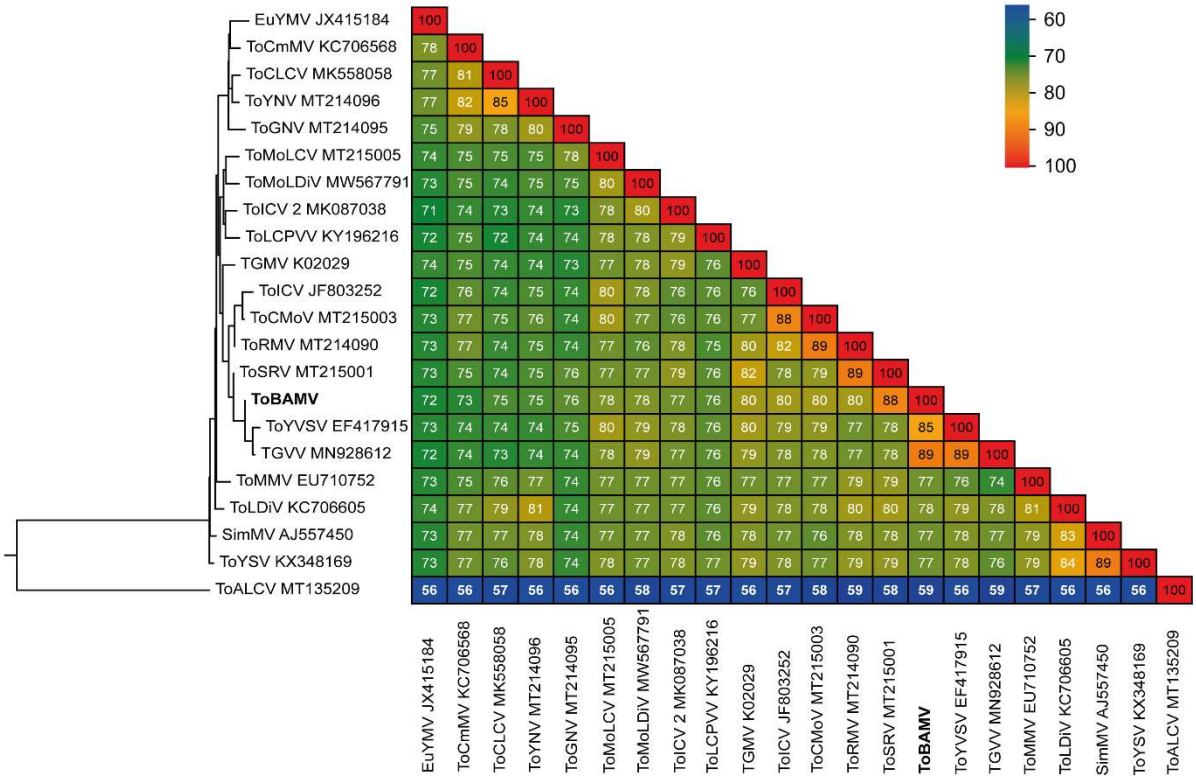

Fig. 2 Sequence Demarcation Tool (SDT) and phylogenetic reconstruction from sequences of the DNA–A segment of begomovirus isolates that infect tomato. Tomato bright apical mosaic virus (ToBAMV) is most phylogenetically related to isolates tomato yellow veins streak virus – ToYVSV (EF417915) and tomato golden vein virus – TGVV (MN928612). The highest percentage of identity obtained was 89% with the TGVV isolate (MN928612). Euphorbia yellow mosaic virus – EuYMV (JX415184), tomato common mosaic virus – ToCmMV (KC706568), tomato chlorotic leaf curl virus – ToCLCV (MK558058), tomato yellow net virus – ToYNV (MT214096),tomato golden net virus – ToGNV (MT214095), tomato mottle leaf curl virus – ToMoLCV (MT215005), tomato mottle leaf distortion virus – ToMoLDiV (MW567791), tomato interveinal chlorosis virus 2 – ToICV2 (MK087038), tomato leaf curl purple vein virus – ToLCPVV (KY196216), tomato golden mosaic virus – TGMV (K02029), tomato interveinal chlorosis virus – ToICV (JF803252), tomato chlorotic mottle virus – ToCMoV (MT215003), tomato rugose mosaic virus – ToRMV (MT214090), tomato severe rugose virus – ToSRV (MT215001), tomato mild mosaic virus – ToMMV (EU710752), tomato leaf distortion virus – ToLDiV (KC706605), Sida micrantha mosaic virus – SimMV (AJ557450), tomato yellow spot virus – ToYSV (KX348169). Outgroup, isolated from the genus *Topilevirus*, tomato apical leaf curl virus – ToALCV (MT135209)

CAPÍTULO 08

Begomoviroses do tomateiro no Brasil: Cenário atual, perspectivas & conclusões gerais.

Begomoviroses do tomateiro no Brasil: Cenário atual, perspectivas e conclusões gerais.

1. Cenário atual das begomoviroses do tomateiro:

Os begomovírus associados à cultura do tomateiro têm demonstrado uma constante expansão para novas áreas em todas as regiões brasileiras (comparação **Tabela 1 *versus* Tabela 2**). Os resultados da presente tese mostram claramente que tomato mottle leaf curl virus (ToMoLCV), Sida micrantha mosaic virus (SimMV) e tomato severe rugose virus (ToSRV) são os begomovírus mais disseminados dentro do território brasileiro, indicando adaptações às diferentes regiões. ToMoLCV e SimMV estão presentes em estados representativos das cinco regiões brasileiras (**Tabela 2**), enquanto ToSRV foi detectado em três regiões (Sul, Sudeste e Centro-Oeste).

Registramos a presença de begomovírus infectando tomateiros em unidades da federação até então ausentes antes dos nossos resultados (**Tabela 1 *versus* Tabela 2**). Por exemplo, SimMV havia sido identificado em três estados, agora este vírus, diante dos nossos resultados, ocorre em dez estados. ToMoLCV foi o begomovírus que mais ampliou o número de estados onde estava presente, passando de dez estados, onde havia sido anteriormente detectado, para 16 unidades de federação. ToSRV não havia sido formalmente relatado infectando tomateiros no estado do Paraná (PR); diante do cenário atual, o vírus foi detectado no referido estado, estando, atualmente, presente em nove estados da federação.

Além da expansão territorial dos begomovírus que infectam o tomateiro no Brasil, observa-se também a expansão em número de espécies (comparação **Tabela 1 *versus* Tabela 2**). Anteriormente a este estudo, haviam registradas 29 espécies de begomovírus associadas à cultura do tomateiro, agora com os dados gerados no presente estudo, este número aumentou para 35 espécies, sendo que algumas ainda permanecem por serem inteiramente caracterizadas. Com os resultados obtidos neste estudo, observa-se o surgimento de um subgrupo de cinco novas espécies de begomovírus (**destacadas na Tabela 2**) identificadas em tomateiro, entretanto, ainda endemicas, nas regiões Norte, Nordeste, Sul e Centro-Oeste.

Por fim, confirmou-se a presença de tomato chlorotic mottle Guyane virus (ToCMoGV) no Brasil (**Tabela 2**). Foi detectado infectando tomateiros na região Norte brasileira. Até então este patógeno havia sido relatado apenas na Guiana Francesa, onde estava geograficamente restrito. Os resultados da presente tese indicam a importância para os programas de melhoramento do tomateiro do constante monitoramento da população viral de begomovírus para compreensão de sua dinâmica espaço-temporal afim de manter atualizadas as informações realacionadas às espécies virais que ocorrem, onde ocorrem e dessa foma, antecipar em

estratégias de manejo frente ao surgimento de novas espécies potencialmente capazes de superar as principais fontes de resistência disponíveis.

Tabela 1. Distribuição das espécies de begomovírus descritas em associação com o tomateiro (*Solanum lycopersicum*) no Brasil (anterior a este estudo).

Espécie viral	Unidade da Federação	Referências
*Chino del tomate Amazonas virus**	AM	Fonseca et al. (2011)
Euphorbia yellow mosaic virus	DF, ES, GO, MG, RS & SP	Barreto et al. (2013); Macedo et al. (2018) e Duarte et al. (2020)
Sida common mosaic virus	RJ	Duarte et al. (2021a)
Sida micrantha mosaic virus	MG, DF & GO	Calegario et al. (2004) e Reis et al. (2020)
Sida mottle virus	SP	Cotrim et al. (2007)
Sida yellow net virus	AM & RJ	Fernandes (2015) e Duarte et al. (2021a)
*Tomato bright yellow mosaic virus**	BA	Fonseca et al. (2013)
*Tomato bright yellow mottle virus**	TO	Fonseca et al. (2013)
Tomato chlorotic leaf curl virus	PA	Quadros et al. (2019)
Tomato chlorotic mottle virus	BA, MG, DF, ES, PE & RJ	Ribeiro et al. (2003) e Ribeiro et al. (2007)
Tomato common mosaic virus	MG, RJ & ES	Castillo-Urquiza et al. (2008); Barbosa et al. (2016) e Mituti et al. (2019)
*Tomato golden leaf distortion virus**	TO	Fonseca et al. (2013)
*Tomato golden leaf spot virus**	TO	Fonseca & Boiteux (2013)
Tomato golden mosaic virus	BA, DF, MG, PR, RN, RJ & SP	Matyis et al. (1975) e Hamilton et al. (1984)
Tomato golden net virus	MG	Reis et al. (2023)
Tomato golden vein virus	GO, DF & MG	Albuquerque et al. (2012); Reis et al. (2020) e Reis et al. (2021)
Tomato interveinal chlorosis virus	PE	Albuquerque et al. (2012)
Tomato interveinal chlorosis virus-2	GO	Rêgo-Machado et al. (2019)
Tomato leaf curl purple vein virus	PI	Macedo et al. (2018)
Tomato leaf distortion virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mild mosaic virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mottle leaf curl virus	MG, GO, DF, ES, RJ, SP, BA, CE, PB & PE	Ribeiro et al. (2003); Chaves et al. (2017); Ferro et al. (2017) e Souza et al. (2022)
Tomato mottle leaf distortion virus	GO	Martins et al. (2021)
Tomato rugose mosaic virus	MG, GO, DF, SP, PR & BA	Ribeiro et al. (2003); Fernandes et al. (2006) e Souza et al. (2020)
*Tomato rugose yellow leaf curl virus**	RS	Fonseca et al. (2016)
Tomato severe rugose virus	DF, GO, MG, RJ, SP, PE, SC & RS	Rezende et al. (1997); Cotrim et al. (2007) e Duarte et al. (2021)
Tomato yellow net virus	GO	Reis et al. (2023)
Tomato yellow spot virus	MG	Calegario et al. (2007)
Tomato yellow vein streak virus	DF, GO, MG, RS, RJ & SP	Faria et al. (1997); Albuquerque et al. (2010) e Reis et al. (2021)

* Vírus ainda em fase de caracterização, mas com sequências depositadas no GenBank. Amazonas (AM), Distrito federal (DF), Goiás (GO), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Bahia (BA), Tocantins (TO), Espírito Santo (ES), Pernambuco (PE), Pará (PA), Paraná (PR), Rio Grande do Norte (RN), Piauí (PI), Ceará (CE), Paraíba (PB), Rio Grande do Sul (RS), Santa Catarina (SC).

Tabela 2. Distribuição das atuais espécies de begomovírus descritas em associação com o tomateiro (*Solanum lycopersicum*) no Brasil (informações geradas no presente estudo estão em negrito e em áreas em cinza).

Espécie viral	Unidade da Federação	Referências
*Chino del tomate Amazonas virus**	AM	Fonseca et al. (2011)
Euphorbia yellow mosaic virus	DF, ES, GO, MG, RS & SP	Barreto et al. (2013); Macedo et al. (2018) e Duarte et al. (2020)
Leonurus mosaic virus = Tomato yellow spot virus	MG**, TO, RR**	Calegario et al. (2007); **Oliveira (2024) – Presente Tese**
Sida common mosaic virus	RJ	Duarte et al. (2021a)
Sida micrantha mosaic virus	**AM, RR, TO, BA, PE, PR, SP,** MG, DF & GO	Calegario et al. (2004), Reis et al. (2020) e **Oliveira (2024) – Presente Tese**
Sida mottle virus	SP	Cotrim et al. (2007)
Sida yellow net virus	AM & RJ	Fernandes (2015) e Duarte et al. (2021a)
Tomato bright apical mosaic virus	**DF & GO**	**Oliveira (2024) – Presente Tese**
*Tomato bright yellow mosaic virus**	BA	Fonseca et al. (2013)
*Tomato bright yellow mottle virus**	TO	Fonseca et al. (2013)
Tomato chlorotic leaf curl virus	PA	Quadros et al. (2019)
Tomato chlorotic mottle Guyane virus	**AM**	**Oliveira et al. (2024)**
Tomato chlorotic mottle virus	**SP**, BA, MG, DF, ES, PE & RJ	Ribeiro et al. (2003), Ribeiro et al. (2007)
Tomato common mosaic virus	MG, RJ & ES	Castillo-Urquiza et al. (2008); Barbosa et al. (2016) e Mituti et al. (2019)
*Tomato golden leaf distortion virus**	TO	Fonseca et al. (2013)
Tomato golden leaf spot virus	**TO**	Fonseca & Boiteux (2013); **Oliveira (2024) – Presente tese**
Tomato golden mosaic virus	BA, DF, MG, PR, RN, RJ & SP	Matyis et al. (1975) e Hamilton et al. (1984)
Tomato golden net virus	MG	Reis et al. (2023)
Tomato golden vein virus	**SP**, GO, DF & MG	Albuquerque et al. (2012); Reis et al. (2020), Reis et al. (2021) e **Oliveira (2024) – Presente Tese**
Tomato interveinal chlorosis virus	PE	Albuquerque et al. (2012)
Tomato interveinal chlorosis virus-2	GO	Rêgo-Machado et al. (2019)
Tomato iridescent apical mosaic virus	**PR**	**Oliveira (2024) – Presente Tese**
Tomato iridescent golden mosaic virus	**TO**	**Oliveira (2024) – Presente Tese**
Tomato iridescent golden net virus	**PR**	**Oliveira (2024) – Presente Tese**
Tomato iridescent mottle virus	**CE & PE**	**Oliveira (2024) – Presente Tese**
Tomato leaf curl purple vein virus	PI	Macedo et al. (2018)
Tomato leaf distortion virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mild mosaic virus	MG & RJ	Castillo-Urquiza et al. (2008)
Tomato mottle leaf curl virus	**AM**, **RR**, **TO**, **PR**, **RS**, **SC**, MG, GO, DF, ES, RJ, SP, BA, CE, PB & PE	Ribeiro et al. (2003); Chaves et al. (2017); Ferro et al. (2017), Reis et al. (2020), Souza et al. (2022) e **Oliveira (2024) – Presente Tese**
Tomato mottle leaf distortion virus	GO	Martins et al. (2021)
Tomato rugose mosaic virus	MG, GO, DF, SP, PR & BA	Ribeiro et al. (2003); Fernandes et al. (2006) e Souza et al. (2020)
*Tomato rugose yellow leaf curl virus**	RS	Fonseca et al. (2016)
Tomato severe rugose virus	**PR**, DF, GO, MG, RJ, SP, PE, SC & RS	Rezende et al. (1997); Cotrim et al. (2007), Duarte et al. (2021) e **Oliveira (2024) – Presente Tese**
Tomato yellow net virus	GO	Reis et al. (2023)
Tomato yellow vein streak virus	DF, GO, MG, RS, RJ & SP	Faria et al. (1997); Albuquerque et al. (2010) e Reis et al. (2021)

***** Vírus ainda em fase de caracterização, mas com sequências depositadas no GenBank. Amazonas (AM), Distrito federal (DF), Goiás (GO), São Paulo (SP), Minas Gerais (MG), Rio de Janeiro (RJ), Bahia (BA), Tocantins (TO), Espírito Santo (ES), Pernambuco (PE), Pará (PA), Paraná (PR), Rio Grande do Norte (RN), Piauí (PI), Ceará (CE), Paraíba (PB), Rio Grande do Sul (RS), Santa Catarina (SC).

2. Perspectivas.

Capítulo 02.

Os resultados gerados a partir das pesquisas conduzidas deste capítulo fornecem subsídios aos programas de melhoramento do tomateiro quanto ao cenário atual do patossistema begomovírus–tomateiro no Brasil no sentido de estabelecer as melhores estratégias de controle varietal desses patógenos.

Capítulo 03.

O patógeno ToCMoGV identificado no Brasil (isolado AM–035) poderá representar um novo desafio para o controle genético das begomoviroses. Ensaios biológicos adicionais serão necessários para avaliar a eficiência dos principais fatores de resistência contra este isolado uma vez que essa informação não foi disponibilizada na descrição origina dessa espécie.

Capítulo 04.

Reforça a importância da caracterização molecular de begomovírus, através da identificação de iterons e motivos conservados, como ferramenta na correta identificação de espécies do gênero e mitigar as nomenclaturas errôneas de novas espécies em surgimento.

Capítulo 05.

O clone infeccioso da nova espécie tomato golden leaf spot virus (ToGLSV) é uma ferramenta de grande importância a ser utilizada em programas de melhoramento do tomateiro no *screening* de germoplasma visando a seleção de tomateiros como fontes de resistência a este novo vírus.

Além disto, poderá ser utilizado em ensaios biológicos para expressão de determinadas proteínas virais e compreender melhor os mecanismos moleculares de resposta à infecção viral em tomateiros que portem os fatores Ty-1/Ty-3, aplicando uma análise transcriptômica para identificar os genes expressos e os que estão sendo silenciados durante o processo infectivo.

Capítulos 06 & 07.

Clones infecciosos das novas espécies serão obtidos visando finalizar sua caracterização biológica, e posterior inoculação em tomateiros com resistência aos begomovírus a fim de avaliar seu espectro de ação frente às novas espécies relatadas.

3. Conclusões gerais.

1. A tecnologia HTS aliada aos ensaios de PCR com primers espécies-específicos se mostraram ferramentas eficazes no estudo da diversidade de vírus obtidos de tomateiros das cinco regiões brasileiras, proporcionando a obtenção de perfil mais preciso do cenário das begomoviroses associadas à cultura;

2. Os dados da presente tese sugerem que os fatores de tolerância do tomateiro *Ty*–1 e *Ty*–3 às begomoviroses, presentes em algumas variedades, podem impactar a diversidade de espécies de begomovírus capazes de infectar a cultura do tomateiro, pois inibem as infecções e reduzem os números de infecções mistas quando comparados às infecções que ocorrem em tomateiros que não apresentam tais fatores. Este reslutado portanto, confirma a hipótese de que as cultivares resistentes/tolerantes estão impactando a diversidade viral nas diferentes regiões do Brasil, contudo o impacto destes genes para formação de um padrão de distribuição e incidência das diferentes espécies de begomovírus ainda precisa ser plenamente estudando. Além disso, o impacto dos fatores *Ty*-1 e *Ty*-3 sobre as novas espécies recombinantes em surgimento não permite comprovar claramente a hipótese de que, a pressão desses dois fatores esteja conduzindo para recombinantes capazes de superar estes mecanismos/fatores de defesa da planta, para isto ensaios adicionais empregando clones infecciosos podem esclarecer essa hipótese;

3. O número de espécies de begomovírus continua em crescimento, com surgimentos de subgrupos de espécies endêmicas em diferentes regiões/biomas. Assim confirma-se a hipótese de que está ocorrendo um aumento da variabilidade genética/genômica espécies virais da família *Geminiviridae*, bem como a contínua emergência de novos begomovírus, contudo o surgimento de novas espécies estão retritas as regiões Norte, Nordeste, Sul e Centro-Oeste, onde o cultivo do tomateiro tem sido conduzido no Brasil;

4. A distribuição geográfica das diferentes espécies de begomovírus também apresenta uma expansão ocorrendo em novas áreas ou regiões produtoras de tomateiro do Brasil. Tal conclusão refuta a hipótese de que um processo de regionalização ecológica esteja ocorrendo ou que essas

espécies estejam se tornando mais frequentes em determinadas condições climáticas/ambientais do país. Assim, a distribuição de alguns desses patógenos está deixando de ser restrita ou endêmica a determinadas áreas geográficas do país;

5. A caracterização molecular e reconstruções filogenéticas de begomovírus associadas aos critérios de demarcação de espécies para o gênero são maneiras de se garantir a correta identificação de espécies, seja ela nova ou já descrita, e reduzir as possibilidades de nomenclaturas errôneas;

6. Agentes subvirais do tipo Alfasatélites continuam a serem detectados (ainda em baixa frequência) em associação com espécies de begomovírus que infectam o tomateiro, contudo ainda pouco se sabe sobre os efeitos dessa associação para a cultura do tomateiro;

7. O gênero *Topilevirus* (família *Geminiviridae*) está também se expandindo para novas regiões produtoras de tomate do Brasil, alcançando áreas até então livres da presença desse gênero viral;

8. Os resultados dessa tese (empregando um número relativamente pequeno de amostras por região), indica fortemente que a diversidade de espécies de begomovírus que infectam a cultura do tomate está sendo subestimada, principalmente nas regiões norte e sul do Brasil onde o estudo de viromas do tomateiro é pouco explorado.