Universidade de Brasília
Instituto de Ciências Biológicas
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Tese de Doutorado

Diversidade de vírus e agentes subvirais de DNA em plantas daninhas em cultivos do tomateiro e novos relatos em regiões Neotropicais

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Brasília – DF

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DIVERSIDADE DE VÍRUS E AGENTES SUBVIRAIS DE DNA EM PLANTAS DANINHAS EM CULTIVOS DO TOMATEIRO E NOVOS RELATOS EM REGIÕES NEOTROPICAIS

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SUMÁRIO

LISTA DE FIGURAS

Capítulo 1 – Revisão de Literatura

Chapter 2 - A metagenomics-based catalog of single-stranded DNA viruses in weeds and native flora associated with tomato fields in Neotropical areas

LISTA DE TABELAS

Capítulo 1 – Revisão de Literatura

Chapter 2 - A metagenomics-based catalog of single-stranded DNA viruses in weeds and native flora associated with tomato fields in Neotropical areas

RESUMO GERAL

Noronha, Priscila Alves. **Viroma de plantas daninhas associadas ao cultivo do tomateiro** 2026. Número de páginas (193). Tese (Doutorado em Biologia Microbiana) – Universidade de Brasília, Brasília, DF.

Os vírus constituem importantes agentes etiológicos de doenças emergentes em diversas culturas agrícolas, incluindo o tomateiro (*Solanum lycopersicum*). As plantas daninhas, por sua vez, exercem papel fundamental como reservatórios e hospedeiros alternativos, contribuindo para a persistência, diversidade e dispersão de vírus, especialmente aqueles que possuem genoma de DNA de fita simples (ssDNA), como os vírus dos gêneros *Begomovirus*, *Citlodavirus*, *Mulcrilevirus*, *Badnavirus*, *Caulimovirus*, *Gemycircularvirus* e *Gemykolovirus.* Informações sobre taxonomia características biológicas, etiologia de doenças, organização genômica, bem como transmissão e importância destes vírus e ferramentas de estudo são contempladas no **Capítulo 1** desta tese. Com a crescente diversidade de espécies, o uso de ferramentas como o Sequenciamento de Alto Rendimento (*High-Throughput Sequencing* - HTS) tem possibilitado o estudo detalhado da diversidade viral e tem sido amplamente utilizado de forma consistente. O desenvolvimento e a evolução de novas tecnologias de HTS estão revolucionando a descoberta de vírus, o diagnóstico, os estudos metagenômicos e evolutivos. Dessa forma, o objetivo geral deste trabalho é compreender o papel das plantas daninhas associadas ao cultivo do tomateiro como reservatório de vírus de ssDNA, bem como alterações genéticas das populações. Com essa finalidade, foi realizado um levantamento da diversidade genética das populações virais, incluindo begomovírus que ocorrem em plantas daninhas e outras espécies de plantas associadas ao cultivo do tomateiro, pertencentes a 15 famílias botânicas: Asteraceae, Bignoniaceae, Brassicaceae, Cactaceae, Capparaceae, Caricaceae, Cleomaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moraceae, Poaceae, Ruscaceae e Solanaceae. Para isso, foram coletadas 114 amostras foliares com sintomas típicos de begomovírus. O DNA total das amostras foi extraído e utilizado como molde para RCA (*Rolling Circle Amplification*). O conjunto de amostras a partir de RCA foi preparado na forma de um *pool* de amostras que foi enviado para sequenciamento usando a plataforma NovaSeq6000 na Agrega. Após o sequenciamento, os dados foram analisados utilizando ferramentas de bioinformática. Como resultado proveniente do HTS foram recuperados 19.575.404 *reads* e 62.444 *contigs*. Foi possível recuperar 103 sequências virais sendo um total de 99 genomas completos e quatro genomas parciais. As sequências se distribuíram em cinco famílias distintas: *Geminiviridae* (85 *contigs*), *Caulimoviridade* (13 *contigs*), *Genomoviridae* (3 *contigs*), *Alphasatellitidae* (1 *contig*) e *Circoviridade* (1 *contig*). A família *Geminiviridae* foi a mais representativa, com três gêneros *Begomovirus* (83 *contigs*), *Citlodavirus* (1 *contig*), e *Mulcrilevirus* (1 *contig*). No gênero *Begomovirus* (família *Geminiviridae*) foi possível recuperar sete espécies conhecidas e cinco potenciais novas espécies denominadas: espécie nova #1 (C493), espécie nova #2 (C218), espécie nova #3 (C64), espécie nova #4 (C08) e espécie nova #5 (C329). Além disso, outra espécie nova denominada espécie nova #6 (C1998) (gênero *Citlodavirus*, família *Geminiviridae*) foi recuperada nesse *pool*. Análises para confirmação da hospedeira foram realizadas usando os *primers* ITS2F e ITS2R (**Capítulo 2**). *Primers* espécie-específicos foram empregados para recuperação dos genomas por PCR em cada amostra individualmente. Os resultados obtidos são apresentados e discutidos. A descrição e análise de 06 espécies novas encontra-se organizada por capítulo. Desta forma, informações sobre uma nova espécie, de genoma monopartido, classificada no *Begomovirus* foi denominada aqui de New species #5 C329 e detectada na amostra PR-134 coletada em 2012 em Capitão Leonidas Marques, Paraná, sul do Brasil. A análise filogenética mostrou que a nova espécie compartilha 84–85% de identidade com outros begomovírus, sendo a identidade

filogeneticamente mais próxima de 85% com tomato severe rugose virus – ToSRV [MW596573] (**Capítulo 3**). No **Capítulo 4**, quatro novas espécies de begomovírus bipartidos recuperadas em HTS, tiveram a organização genômica analisada em detalhe quanto às ORFs e motivo, e foi encontrado o DNA-B correspondente a cada uma delas. A sequência parcial foi sequenciada via Sanger. O DNA-A foi recuperado via PCR com *primers* espécies-específicos. Isolados das espécies novas #1 (NS#1) e #2 (NS#2) foram detectados em amostras provenientes de Goiás - GO (RGO-835 e RGO-834). Dois isolados da espécie nova #3 (NS#3) foram detectados em amostras do Pará (PR-126 e PR-136). Sete isolados da espécie nova #4 (NS#4) foram identificados em amostras provenientes de três estados brasileiros: Goiás (RGO-836, RGO-838, RGO-834 e RGO-833), Distrito Federal (RDF-826 e RDF-827) e Rio Grande do Sul (RS-082). A análise filogenética demonstrou que NS#1 tem identidade filogenética mais próxima com BGMV (MN822294); NS#2 é filogeneticamente mais próxima de Cleome leaf crumple virus (CleLCrV) (FN4359999); NS#3 é mais próxima de tomato leaf distortion virus (ToLDV) (NC_038474), e NS#4 é mais próxima de Sidastrum golden leaf spot virus – SidGLSV (NC_038462). As análises de recombinação demonstraram existe evidência de recombinação entre as novas espécies NS#3 e NS#4. No **Capítulo 5** informações sobre o gênero *Mulcrilevirus* detectado em uma amostra coletada no Distrito Federal (RDF-831) ilustram a importância de estudos HTS para conhecer a diversidade viral no país. Trata-se do primeiro registro desse vírus fora da China. A sequência C93 apresentou 92–93% de identidade (95% de cobertura da consulta) com isolados de *Mulcrilevirus mori* (= mulberry crinkle leaf virus – MCLV), dentre eles o isolado MN240483.1. No **Capítulo 6**, informações da descrição de uma nova espécie do gênero *Citlodavirus* são apresentadas. Isolado deste gênero foi detectado em uma amostra do Mato Grosso (MT-012), coletada em 2010 em Cuiabá, na região Centro-Oeste brasileira. A análise filogenética mostrou que a espécie compartilha 72,50% de identidade com *Citlodavirus passiflorae* (Passion fruit chlorotic mottle virus) [NC_040706.1]. No **Capítulo 7** é relatada a identificação de CleLCrV em uma amostra coletada no Paraguai (PAR-005), em Mayor Otaño, no ano de 2010. Trata-se do primeiro registro desse vírus no Paraguai. Esse isolado apresentou de 94,33% de identidade (100% de cobertura) com o isolado de DNA-A de acesso FN435999.1. O **Capítulo 8** apresenta o primeiro relato de um vírus do gênero *Gemycircularvirus* em repolho e malva, coletadas em 2022, uma na região do Distrito Federal (DF-824) e outra no estado do Goiás (RGO-834). Esse é o primeiro registro nessas hospedeiras. O isolado apresentou 91.26% de identidade com *Gemycircularvirus mocha1* (*Momordica charantia associated Gemycircularvirus*; NC_075310.1). No **capítulo 9** relata-se a detecção de um vírus do gênero *Gemykolovirus* em uma amostra coletada em Urutaí, estado de Goiás, em 2022 (RGO-833). O isolado apresentou 88.44% de identidade (56% de cobertura) com um isolado do vírus *Gemykolovirus heris1* (Plant associated genomovirus 7 – PaGmV 7) [NC_076273.1]. Trata-se do primeiro relato de PaGmV 7 em *Digitaria catamarcensis* (Poaceae). No **Capítulo 10** realizou-se uma conclusão geral e levantamento das perspectivas deste trabalho. Esses resultados reforçam a importância do monitoramento contínuo de plantas daninhas e não cultivadas que crescem nas proximidades das culturas, uma vez que podem atuar como reservatórios de vírus que contribuem para sua disseminação.

Palavras-chaves: ssDNA, plantas daninhas, *High-Throughput Sequencing.*

ABSTRACT

Important viruses are etiological agents of emerging diseases in various agricultural crops, including tomato (*Solanum lycopersicum*). The plants to which they are transmitted, in turn, play a fundamental role as reservoirs and alternative hosts, contributing to the persistence, diversity, and dispersal of viruses, especially those with single-stranded DNA (ssDNA) genomes, such as viruses of the genera *Begomovirus*, *Citlodavirus*, *Mulcrilevirus*, *Badnavirus*, *Caulimovirus*, *Gemycircularvirus*, and *Gemykolovirus*. Information on taxonomy, biological characteristics, disease etiology, genomic organization, as well as transmission and importance of these viruses and study tools are covered in **Chapter 1** of this thesis. With an increasing diversity of species, the use of tools such as High-Throughput Sequencing (HTS) has enabled the detailed study of viral diversity and has been widely and consistently used. The development and evolution of new HTS technologies are revolutionizing virus discovery, diagnosis, metagenomic and evolutionary studies. Therefore, the overall objective of this work is to understand the role of plants, particularly tomato plants, as reservoirs of ssDNA viruses, as well as genetic alterations in these populations. To this end, a survey of the genetic diversity of viral populations was conducted, including begomoviruses occurring in specific plants and other plant species associated with tomato cultivation, belonging to 15 botanical families: Asteraceae, Bignoniaceae, Brassicaceae, Cactaceae, Capparaceae, Caricaceae, Cleomaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moraceae, Poaceae, Ruscaceae, and Solanaceae. For this purpose, 114 leaf samples with typical begomovirus symptoms were collected. Total DNA from the samples was extracted and used as a template for RCA (Rolling Circle Amplification). The sample set from RCA was prepared as a sample pool that was sent for sequencing using the NovaSeq6000 platform at Agrega. After sequencing, the data were analyzed using bioinformatics tools. As a result of the HTS, 19,575,404 reads and 62,444 contigs were recovered. It was possible to recover 103 viral sequences, totaling 99 complete genomes and four partial genomes. The sequences were distributed among five distinct families: *Geminiviridae* (85 contigs), *Caulimoviridae* (13 contigs), *Genomoviridae* (3 contigs), *Alphasatellitidae* (1 contig), and *Circoviridae* (1 contig). The *Geminiviridae* family was the most representative, with three genera: *Begomovirus* (83 contigs), *Citlodavirus* (1 contig), and *Mulcrilevirus* (1 contig). In the genus *Begomovirus* (family *Geminiviridae*), it was possible to recover seven known species and five potential new species: new species #1 (C493), new species #2 (C218), new species #3 (C64), new species #4 (C08), and new species #5 (C329). In addition, another species derived from new species #6 (C1998) (genus Citlodavirus, family Geminiviridae) was recovered in this pool. Host confirmation analyses were performed using ITS2F and ITS2R primers (**Chapter 2**). Species-specific primers were used for genome recovery by PCR in each sample individually. The results obtained are presented and explained. The description and analysis of the 6 new species are organized by chapter. Thus, information on a new species, with a single genome, defined in *Begomovirus*, was named here as New Species #5 C329 and detected in sample PR-134 collected in 2012 in Capitão Leônidas Marques, Paraná, southern Brazil. A phylogenetic analysis showed that the new species shares 84–85% identity with other begomoviruses, with the closest phylogenetic identity of 85% being with tomato Severe Rugose Virus – ToSRV [MW596573] (**Chapter 3**). In **Chapter 4**, four new species of bipartite begomoviruses recovered in HTS, the genomic organization provided details on ORFs and motifs, and the corresponding DNA-B for each of them was found. The partial sequence was sequenced via Sanger. The DNA-A was recovered via PCR with specific-specific primers. Isolates of the new species #1 (NS#1) and #2 (NS#2) were detected in samples from Goiás - GO (RGO-835 and RGO-834). Two isolates of the new species #3 (NS#3) were detected in samples from Pará (PR-126 and PR-136). Seven isolates of the new species #4 (NS#4) were identified in samples from three Brazilian states: Goiás (RGO-836, RGO-838,

RGO-834 and RGO-833), Federal District (RDF-826 and RDF-827) and Rio Grande do Sul (RS-082). Phylogenetic analysis showed that NS#1 has a closer phylogenetic identity with BGMV (MN822294); NS#2 is phylogenetically closer to Cleome leaf crumple virus (CleLCrV) (FN4359999); NS#3 is closer to Tomato leaf distortion virus (ToLDV) (NC_038474), and NS#4 is closer to Sidastrum golden leaf spot virus – SidGLSV (NC_038462). Recombination analyses demonstrated evidence of recombination between the new species NS#3 and NS#4. **Chapter 5** provides information on the genus *Mulcrilevirus* detected in a sample collected in the Federal District (RDF-831), illustrating the importance of HTS studies for understanding viral diversity in the country. This is the first record of this virus outside of China. The C93 sequence showed 92–93% identity (95% query coverage) with isolates of *Mulcrilevirus mori* (= mulberry crinkle leaf virus – MCLV), including isolate MN240483.1. **Chapter 6** presents information on the description of a new species of the genus *Citlodavirus*. An isolate of this genus was detected in a sample from Mato Grosso (MT-012), collected in 2010 in Cuiabá, in the Brazilian Midwest region. Phylogenetic analysis showed that the species shares 72.50% identity with *Citlodavirus passiflorae* (Passion fruit chlorotic mottle virus) [NC_040706.1]. Chapter 7 reports the identification of CleLCrV in a sample collected in Paraguay (PAR-005), in Mayor Otaño, in 2010. This is the first record of this virus in Paraguay. This isolate showed 94.33% identity (100% coverage) with the DNA-A isolate of accession FN435999.1. **Chapter 8** presents the first report of a *Gemycircularvirus* genus virus in cabbage and mallow, collected in 2022, one in the Federal District region (DF-824) and the other in the state of Goiás (RGO-834). This is the first record in these hosts. The isolate showed 91.26% identity with *Gemycircularvirus mocha1* (Momordica charantia associated Gemycircularvirus; NC_075310.1). **Chapter 9** reports the detection of a *Gemykolovirus* genus virus in a sample collected in Urutaí, Goiás state, in 2022 (RGO-833). The isolate showed 88.44% identity (56% coverage) with an isolate of *Gemykolovirus heris1* (Plant associated genomovirus 7 – PaGmV 7) [NC_076273.1]. This is the first report of PaGmV 7 in *Digitaria catamarcensis* (Poaceae). **Chapter 10** provides a general conclusion and outlines the perspectives of this work. These results reinforce the importance of continuous monitoring of weeds and non-cultivated plants growing near crops, as they can act as reservoirs of viruses that contribute to their spread.

Keywords: ssDNA, weeds, High-Throughput Sequencing.

INTRODUÇÃO GERAL

A cultura do tomateiro (*Solanum lycopersicum*) possui grande destaque no Brasil, principalmente devido sua importância econômica para o país. Em 2024, aproximadamente 4.407.502 toneladas (ton) de tomate foram produzidas, ocupando cerca de 55.767 hectares (ha), e gerando um rendimento médio de 72.760 kg por hectare (IBGE 2025). Embora os valores de produção e produtividade sejam expressivos, sabe-se que ambos poderiam ser maiores, uma vez que a cultura é acometida por diversos patógenos, destacando-se os vírus.

Os principais vírus que infectam a cultura do tomateiro no Brasil estão classificados em espécies de distintos gêneros, incluindo *Crinivirus*, *Orthotospovirus*, *Potyvirus, Topilevirus* e *Begomovirus* (Inoue-Nagata et al. 2016). Merecem destaque membros do gênero *Begomovirus*, uma vez que este é o maior grupo de vírus que infecta o tomateiro. Este gênero abriga mais de 221 vírus relatados em tomate, dentre as 300 espécies de vírus que são conhecidos por infectar essa cultura (GenBank 2025; Host DataBase 2025; Kitajima 2022; Fiallo-Olivé & Navas-Castillo 2023).

O gênero *Begomovirus* encontra-se classificado na família *Geminiviridae* e é composto por 463 espécies (ICTV 2025). Os vírus classificados nesse gênero são caracterizados por possuírem DNA circular de fita simples (ssDNA) e podem ser monopartidos (DNA–A apenas) ou bipartidos (DNA–A e DNA–B encapsidados em partículas diferentes) (Brown et al. 2015). Begomovírus são transmitidos por um complexo polífago e eficiente de espécies crípticas conhecida como mosca-branca (*Bemisia tabaci*), sendo duas espécies predominantes no Brasil: *B. tabaci Middle East Asia Minor* 1 – MEAM1 e *B. tabaci Mediterranean* –MED (Fernandes et al. 2023).

Desde o início da década de 1990, os begomovírus têm sido responsáveis por muitas doenças emergentes, tanto em hortaliças como em culturas alimentares, em todo o mundo, (Seal el al. 2006; Navas et al. 2011; Souza et al. 2022; Cai et al. 2023; Silva et al. 2024), constituindo um dos principais problemas fitossanitários de diversas culturas (Assunção et al. 2006; Fiallo-Olivé et al. 2020; Ma et al. 2024; Macedo et al. 2024). No Brasil, houve um aumento da incidência e severidade de begomoviroses, bem como descrição de novas espécies, principalmente em tomateiro, após a introdução de *B. tabaci* MEAM1 (Ribeiro et al. 2003; Gautam et al. 2022; Bello et al. 2023; Nogueira et al. 2024; Silva et al. 2024).

Além disto, espécies de plantas que crescem em habitats antrópicos, próximos às plantações, ou plantas daninhas dentro da plantação, têm sido estudadas como prováveis

reservatórios de vírus da família *Geminiviridae* (García-Arenal e Zerbini 2019; Batista et al. 2022; Chipiringo et al. 2022; Pereira-Silva et al. 2022), como nos trabalhos de Rodríguez-Negrete et al. (2019) que detectaram tomato yellow leaf curl virus (TYLCV), pepper huasteco yellow vein virus (PHYVV), Rhyncosia golden mosaic Sinaloa virus (RhGMSV) e Sida mosaic Sinaloa virus (SiMSiV) em uma ampla gama de plantas daninhas. Além disto, Queiroz-Ferreira et al (2024) que analisaram o complexo Sida micranta mosaic virus (SiMMV) em plantas daninhas e Lima (2023) que identificaram uma planta daninha como nova hospedeira de tomato bright yellow mottle virus (ToBYMV).

Em diversos países, incluindo o Brasil, espécies de plantas daninhas, pertencentes a famílias botânicas distintas, têm sido relatadas como hospedeiras de begomovírus. Dentre estas famílias podemos citar Sterculiaceae (Assunção et al. 2006); Capparaceae (Assunção et al. 2006); Fabaceae (Assunção et al. 2006; Rodríguez-Negrete et al. 2019; Batista et al. 2022; Kumar et al. 2025); Asteraceae (tomato common mosaic virus - ToCmMV) (Castillo-Urquiza et al. 2008); Amaranthaceae (tomato severe rugose virus - ToSRV) (Barbosa et al. 2011a); Euphorbiaceae (ToSRV) (Barreto et al. 2013; Santos et al. 2003; Dhasan et al. 2025); Malvaceae e Solanaceae (TYLCV) (Rodríguez-Negrete et al. 2019), Poaceae (TYLCV) (Kil et al. 2021), Icacinaceae (Pyrenacantha yellow mosaic virus - PyYMV) (Chipiringo et al. 2022) e Oxalidaceae (ToSRV) (Pereira-Silva et al. 2022). Essa ampla gama de hospedeiros dificulta o controle desses vírus.

Para o controle e manejo de begomovírus também podem ser adotadas estratégias como erradicação de plantas daninhas, eliminação de tomateiros espontâneos, monitorar e manter a população do inseto vetor em baixo nível durante todo o ciclo da cultivar, a utilização de cultivares híbridas ou tolerantes (Inoue-Nagata et al. 2009), além de períodos livres de tomate (Macedo et al. 2024). Segundo Boiteux et al. (2007), Zaidi et al. (2017), Reis et al. (2020) e Ma et al. (2024) a maneira mais eficaz de controlar vírus é o desenvolvimento de materiais que sejam resistentes tanto ao begomovírus quanto à mosca-branca, juntamente com outras medidas de controle que necessitam de informações a respeito do vírus.

Desta forma, o presente trabalho tem como objetivo principal entender o papel das plantas daninhas e outras plantas (associadas ao cultivo do tomateiro) como reservatórios virais.

Para isto realizou-se um levantamento da diversidade genética das populações de vírus de DNA que ocorrem em plantas daninhas e outras espécies de plantas associadas ao cultivo do tomateiro. Espera-se que os resultados do presente trabalho possam contribuir e atualizar

informações de monitoramento e levantamento de vírus de plantas daninhas e outras plantas associadas ao cultivo do tomateiro, bem como orientar os programas de melhoramento para resistência às espécies prevalentes em cada região.

HIPÓTESE

Considerando a grande diversidade de vírus presentes no país e a importância de plantas daninhas como reservatório de vírus e vetores, bem como as novas abordagens e técnicas para levantamento da diversidade viral, deve existir uma diversidade de vírus de DNA e agentes subvirais (satélites) em plantas daninhas associadas ao cultivo do tomateiro no Brasil maior do que a descrita até o momento.

OBJETIVO GERAL

Realizar o levantamento da diversidade genética de vírus de DNA e agentes subvirais em diferentes plantas daninhas e outras plantas associadas ao cultivo do tomateiro em regiões produtoras brasileiras e paraguaias.

OBJETIVOS ESPECÍFICOS

- Realizar levantamento da distribuição geográfica e da diversidade de vírus e agentes subvirais de DNA, em um conjunto de plantas daninhas e outras espécies (provenientes de 15 famílias botânicas) associadas ao cultivo do tomateiro;
- Realizar a identificação molecular das espécies de plantas daninhas e outras, coletadas próximas a cultivos de tomateiros, infectadas com vírus e/ou agentes subvirais;
- Caracterizar molecularmente seis novas espécies da família *Geminiviridae* (gêneros *Begomovirus* e *Citlodavirus*) e quatro espécies conhecidas (gêneros *Mulcrilevirus*, *Begomovirus*, *Gemycircularvirus*, *Gemykolovirus*) em plantas daninhas no Brasil e Paraguai.

CAPÍTULO 1

Revisão de Literatura

1. A cultura do tomateiro

O tomateiro (*Solanum lycopersicum*) possui grande relevância na agricultura, destacando-se não só por seu valor econômico, mas também por sua importância nutricional. Cultivado em diversas regiões tropicais e subtropicais ao redor do globo, o tomateiro desempenha um papel fundamental tanto na economia quanto na alimentação das populações. Essa hortaliça possui origem na zona andina da América do Sul, porém, foi domesticado no México e introduzido na Europa em 1544, disseminando-se pela Ásia meridional e oriental, África e Oriente Médio, e mais recentemente para outras partes da América do Sul e México (Naika et al. 2006; Peralta et al. 2008; Caspermeyer 2020; Klee e Rezende 2020).

A produção da cultura tem experimentado um crescimento constante ao longo das décadas. Comparando os dados entre 1994 e 2021, podemos notar um aumento significativo na produção global. De fato, os números revelam um aumento de cerca de 231 mil toneladas nesse período. Nesse cenário, a América é o terceiro maior produtor, perdendo apenas para a Ásia e Europa. No *ranking* mundial dos 10 maiores produtores de tomate, a China ocupa a 1ª posição, com produção de 70.119.694 milhões de toneladas, seguida pela Índia (20.425.000), Turquia (13.300.000), Estados Unidos (12.370.057) Egito (6.211.016), Itália (6.016.050), México (4.394.807), Brasil (4.166.017), que ocupa a 8ª posição, seguido da Espanha (3.968.460) e Nigéria (3.803.598) (**Figura 1**). Vale destacar que no levantamento anterior da Organização das Nações Unidas para a alimentação e a Agricultura - FAO o Brasil ocupava a 10ª posição (FAOSTAT 2024), representando um aumento significativo em relação a períodos anteriores.

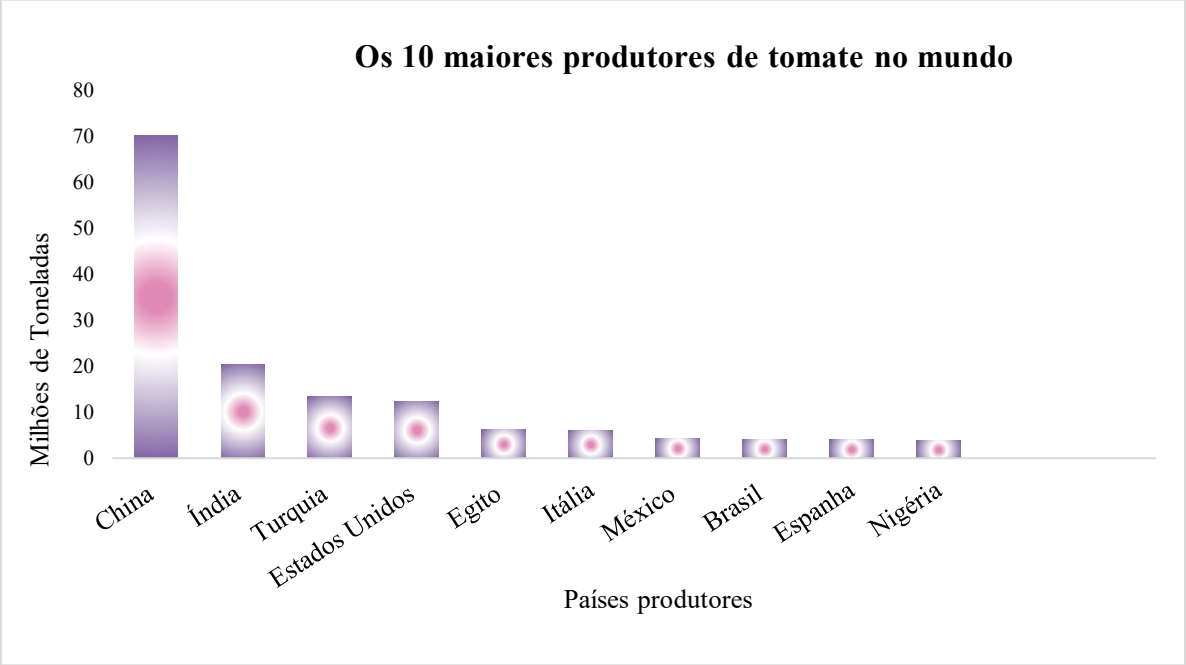


Figura 1. Dez maiores países produtores de tomate no mundo. Informações baseadas em dados obtidos FAOSTAT (2025).

Na horticultura, o tomate é um dos produtos mais cultivados no Brasil, possuindo uma grande importância econômica na demanda industrial e *in natura* que é predominantemente cultivado pela agricultura familiar (Cardoso et al. 2024).

No cenário brasileiro, de acordo com o último levantamento do Instituto Brasileiro de Geografia e Estatística (IBGE), ocorrido em 2024 – o Brasil produziu cerca de 4.407.502 toneladas de tomate, com área colhida de 60.576 hectares, destacando-se o estado de Goiás (GO) como o maior produtor, seguido por São Paulo (SP) e Minas Gerais (MG) (IBGE 2025), que juntos somam mais da metade da área e da produção nacional dessa hortaliça (Cardoso et al. 2024). Além disso, GO recebe destaque como maior produtor no segmento industrial, com cultivo em grandes áreas e com alta tecnologia. No segmento mesa (*in natura)*, MG e SP são os maiores produtores e consumidores do fruto (Cardoso et al. 2024; IBGE 2025).

Segundo o último Relatório de Informações Agropecuárias do Distrito Federal do ano de 2024, elaborado pela Empresa de Assistência Técnica e Extensão Rural do Distrito Federal (EMATER-DF), a produção de tomate aparece em destaque como uma das três principais horticulturas do Distrito Federal (EMATER 2024).

Vários problemas fitossanitários afetam a cultura, dentre eles destacam problemas causados por vírus e que serão abordados a seguir.

2. Doenças de etiologia viral do tomateiro

O tomateiro é considerado uma das plantas mais suscetíveis a doenças, principalmente quando se trata de viroses. De acordo com Rashid et al. (2016), a produção pode enfrentar um declínio de 70 a 95%. O tomateiro está associado ao maior número de vírus e viróides conhecidos para qualquer espécie de planta, totalizando atualmente 312 espécies conhecidas (Rivarez et al. 2021). Foram descobertas em tomate, na última década, 45 novas espécies de vírus e vários vírus conhecidos foram associados ao tomate pela primeira vez (Rivarez et al. 2021).

Os principais vírus para a cultura do tomateiro no Brasil estão classificados em diferentes gêneros, incluindo vírus de ssRNA como *Crinivirus* (Mituti et al. 2019; Yang et al. 2023; Favara et al. 2025; Ibañez et al. 2025)*, Orthotospovirus* (Rivarez et al. 2021; Gorayeb et al. 2023) e *Potyvirus* (Lucena et al. 2025), bem como vírus de ssDNA como *Begomovirus* (Inoue-Nagata et al. 2016; Reis et al. 2020; Duarte et al. 2021; Keller et al. 2023; Estrada et al. 2024; Macedo et al. 2024; Idrees et al. 2024; Queiroz-Ferreira et al. 2024; Oliveira et al. 2024).

Além disto, há relatos de isolados de duas espécies de topilevírus: 1. *Topilevirus solani* (= *tomato apical leaf curl virus*) (Batista et al. 2019; Souza et al. 2020) e 2. *Topilevirus lycopersici* (=*tomato associated geminivirus* 1) (Fontenele et al. 2017). É importante mencionar que em levantamentos realizados pela equipe, observa-se as frequências de topilevírus em condições de campo (Batista et al. 2019).

A maioria dos relatos de vírus em tomateiro refere-se a representantes do gênero *Begomovirus* (família *Geminiviridae*), que apresentam uma ameaça significativa a uma ampla gama de culturas (Reis et al. 2020; Oliveira et al. 2024; Torralba et al. 2024) especialmente o tomateiro. Alguns destes begomovírus relatados em tomate no Brasil foram elencados na **Tabela 1**. O ToSRV é o begomovírus bipartido mais disseminado no país, prevalecendo na região Centro-Oeste e Sudeste (Duarte et al. 2021; Reis et al. 2020; Souza et al. 2020), enquanto o begomovírus monopartido tomato mottle leaf curl virus (ToMoLCV), é prevalente na região Nordeste do país (Souza et al. 2022). As demais espécies têm uma distribuição geográfica geral mais restrita, e muitas endêmicas (Inoue-Nagata et al. 2016; Reis et al. 2020).

Tabela 1: Begomovírus relatados em tomate (*Solanum lycopersicum)* no Brasil, contendo as informações sobre o tipo de genoma, organismo, espécie viral, acrônimo e respectivas referências bibliográficas.

Genoma	Nome binomial da espécie Vírus (Acrônimo)	Referências
Bipartido	*Begomovirus costai* Bean golden mosaic virus (BGMV)	Souza et al. (2020)
	Begomovirus solanumamazonasense Chino del tomate Amazonas virus	Fonseca et al. (2011)
	Begomovirus cleomecrispi Cleome leaf crumple virus (CleLCrV)	Reis et al. (2019)
	Begomovirus euphorbiamusiviflavi Euphorbia yellow mosaic virus	Barreto et al. (2013)
	Begomovirus sidaflavaneti Sida yellow net virus (SiYNV)	Fonseca et al. (2016)
	Begomovirus solanummusivi Tomato bright yellow mosaic virus	Fonseca et al. (2013)
	Begomovirus solanumpallidi Tomato chlorotic leaf curl virus	Quadros et al. (2019)
	Begomovirus solanumpallidiguyanense Tomato chlorotic mottle Guyane virus	Oliveira et al. (2024)
	Begomovirus solanumpallidivariati Tomato chlorotic mottle virus	Ribeiro et al. (2007)
	Begomovirus solanumvulgarismusivi Tomato common mosaic virus	Castillo-Urquiza et al. (2008)
	Begomovirus solanumaureicontorsionis Tomato golden leaf distortion virus	Fonseca et al. (2013)
	Begomovirus solanumaureimusivi Tomato golden mosaic virus (TGMV)	Matyis et al. (1975)
	Begomovirus solanumintervenae Tomato interveinal chlorosis virus	Albuquerque et al. (2012)
	Tomato interveinal chlorosis virus-2 (ToICV2)*	Rêgo-Machado et al. (2019)
	Begomovirus solanumcontorsionis Tomato leaf distortion virus (ToLDV)	Castillo- Urquiza et al. (2008)
	Begomovirus solanumtenuimusivi Tomato mild mosaic virus (ToMMV)	Castillo- Urquiza et al. (2008)
	Begomovirus solanumseverparvi Tomato mosaic severe dwarf virus	Reis et al. (2020)
	Begomovirus solanumdistorsionis Tomato mottle leaf distortion virus	Martins et al. (2021)
	Begomovirus solanumrugosi Tomato rugose mosaic virus (ToRMV)	Ribeiro et al. (2003)
	Begomovirus solanumrugosiflavi Tomato rugose yellow leaf curl virus	Fonseca et al. (2016)

	Begomovirus solanumseverugosi Tomato severe rugose virus (ToSRV)	Cotrim et al. (2007)
	Begomovirus solanumflavusdepravationis	Pereira-Carvalho et al. (2019)
	Begomovirus solanumflavusmaculae Tomato yellow spot virus (ToYSV)	Calegario et al. (2007)
	Begomovirus solanumflavusvenae Tomato yellow vein streak virus	Albuquerque et al. (2010); Oliveira et al. (2024)
	Begomovirus solanumaureimaculae Tomato golden leaf spot virus	Fonseca e Boiteux (2013)
	Begomovirus solanumaureivenae Tomato golden vein virus (TGVV)	Reis et al. (2020)
Monopartido	*Begomovirus solanumaureusreti* Tomato golden net virus (ToGNV)	Reis et al. (2023)
	Begomovirus solanumflavusreti Tomato yellow net virus (ToYNV)*	Reis et al. (2023)
	Begomovirus solanumvariatuminvolutionis	Souza et al. (2022)
	Tomato iridescent apical mosaic virus (ToIAMV)*	Oliveira (2024)
	Tomato iridescent mottle virus (ToIMoV)*	Oliveira (2024)
	Begomovirus solanumviolavenae Tomato leaf curl purple vein virus	Macedo et al. (2018)

* Não possui nomenclatura binomial da espécie até o presente momento (ICTV 2025).

Existem hipóteses de que a grande diversidade de begomovírus que infectam tomateiros no Brasil têm origem em hospedeiros silvestres locais, de onde surgiram diferentes vírus, abrangendo áreas geográficas distintas e proporcionando variados níveis de adaptação ao hospedeiro e/ou ao ambiente obtidas por meio de mutações, recombinações e pseudo-recombinações (García-Arenal e Zerbini 2019; Duarte et al. 2021; Wang et al. 2021).

Devido a importância do grupo de begomovírus e relatos recentes de membros da família *Geminiviridae* para o tomateiro, maiores informações serão apresentadas a seguir. Assim como vírus de outras famílias: *Caulimoviridae* (gêneros *Badnavirus* e *Caulimovirus*), *e Genomoviridae* (gêneros *Gemycircularvirus* e *Gemykolovirus*).

3. Família *Geminiviridae*: taxonomia e organização genômica

A família *Geminiviridae* é composta atualmente por 548 espécies de vírus distribuídas em 15 gêneros, sendo considerada a maior dentre as famílias de vírus de plantas (ICTV 2025).

Os critérios de classificação dos gêneros da família *Geminiviridae* são estabelecidos de acordo com a variedade de hospedeiros, vetores de insetos, organização do genoma e identidades de nucleotídeos (nts) (Brown et al. 2015; Zerbini et al. 2017; Rougmanac et al. 2022 e ICTV 2025) (**Tabela 2**). Os vírus da família *Geminiviridae* possuem uma ampla distribuição geográfica, praticamente global, infectando plantas monocotiledôneas e eudicotiledôneas (Varsani et al. 2017; Debat 2023). De todos os gêneros classificados em *Geminiviridae*, *Begomovirus* é o maior da família, totalizando 463 espécies, seguido do *Mastrevirus*, com 50 espécies (ICTV 2025).

Os vírus da família *Geminiviridae* possuem partícula icosaédrica geminada, sendo que cada partícula geminada contém apenas um ssDNA circular (DNA–A ou DNA–B) (Zerbini et al. 2017 e Mietzsch et al. 2025).

Os vírus dos gêneros *Becurtovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus*, *Turnvurtovirus* e *Welvivirus* possuem genomas monopartidos (Zerbini et al. 2017; Bejerman e Debat 2023). Informações sobre os gêneros classificados nesta família encontram-se descritas na **Tabela 2 e Figura 2**. Membros de *Geminiviridae* podem codificar ORFs (*Open Reading Frames*) tanto no sentido viral (V1, V2 e V3) como no sentido complementar (C1, C2, C3, C4, C5, C6 e C7). No componente DNA–A de vírus monopartidos e bipartidos podemos encontrar algumas das seguintes ORFS: no sentido viral a ORF V1 codifica a *Coat Protein* - CP (capa proteica); a V2 codifica a *Movement Protein* – MP (proteína de movimento); a V3 codifica uma proteína responsável pela regulação e o movimento. No sentido complementar, a C1 é responsável pela proteína *Replication-associated protein* – Rep (iniciadora de replicação); a C2 codifica a proteína *Transcriptional activator protein* – TrAP (ativação da transcrição); a C3 codifica a *Replication enchancer protein* – Ren (intensificadora de replicação) e C4 codifica uma proteína que atua na determinação de sintomas (Zerbini et al. 2017; Kulshreshtha et al. 2019; Kumar e Dasgupta 2023 e Li et al. 2024), enquanto a C5 codifica uma proteína determinante de patogenicidade e supressão do silenciamento gênico pós-transcricional (Li et al. 2015 e Zhao et al. 2023); a C6 codifica uma proteína de função desconhecida (Wang et al. 2022) e a C7 codifica uma proteína que aumenta a patogenicidade e atua também como supressora de silenciamento de RNA (Liu et al. 2023). No componente DNA–B dos begomovírus bipartidos encontram-se as ORFs BV1, que codifica a proteína *Nuclear Shuttle Protein* – NSP (proteína de transporte nuclear), responsável pelo transporte nucleocitoplasmático de DNA viral, e a BC1, responsável pela proteína *Movement Protein* –

MP (proteína de movimento), envolvida na disseminação célula a célula e sistêmica de DNA viral (Rojas et al. 2005; Li et al. 2015 e Kumar 2019).

A maioria dos vírus dessa família possui uma ou mais regiões intergênicas (IRs). Nas IRs é possível encontrar a origem de replicação e a estrutura de *stem-loop*, que contém um motivo nonanucleotídeo (TAATATTAC) invariante na replicação do círculo rolante (Hanley-Bowdoin et al. 1999). Nas sequências de isolados de becurtovírus e eragrovírus existem diferenças nucleotídicas na quarta e oitava posição do nonanucleotídeo, tendo um nonanucleotídeo atípico "5′-TAA**G**ATT**C**C-3′" na origem de replicação (Varsani et al. 2014).

O processo de replicação ocorre dependendo apenas de fatores do hospedeiro, pois os vírus desta família não codificam uma DNA polimerase, contando com fatores do hospedeiro durante os estágios iniciais de replicação. O dsDNA (*double strand* DNA) é sintetizado a partir do DNA de sentido complementar, tendo início a partir da clivagem da fita de senso-viral feita pela Rep, em uma sequência 5'-TAATATTAC-3' (Hanley-Bowdoin et al. 2013). As regiões de codificação, das duas fitas, são diferentes da região intergênica longa (RIL), sendo uma transcrição bidirecional.

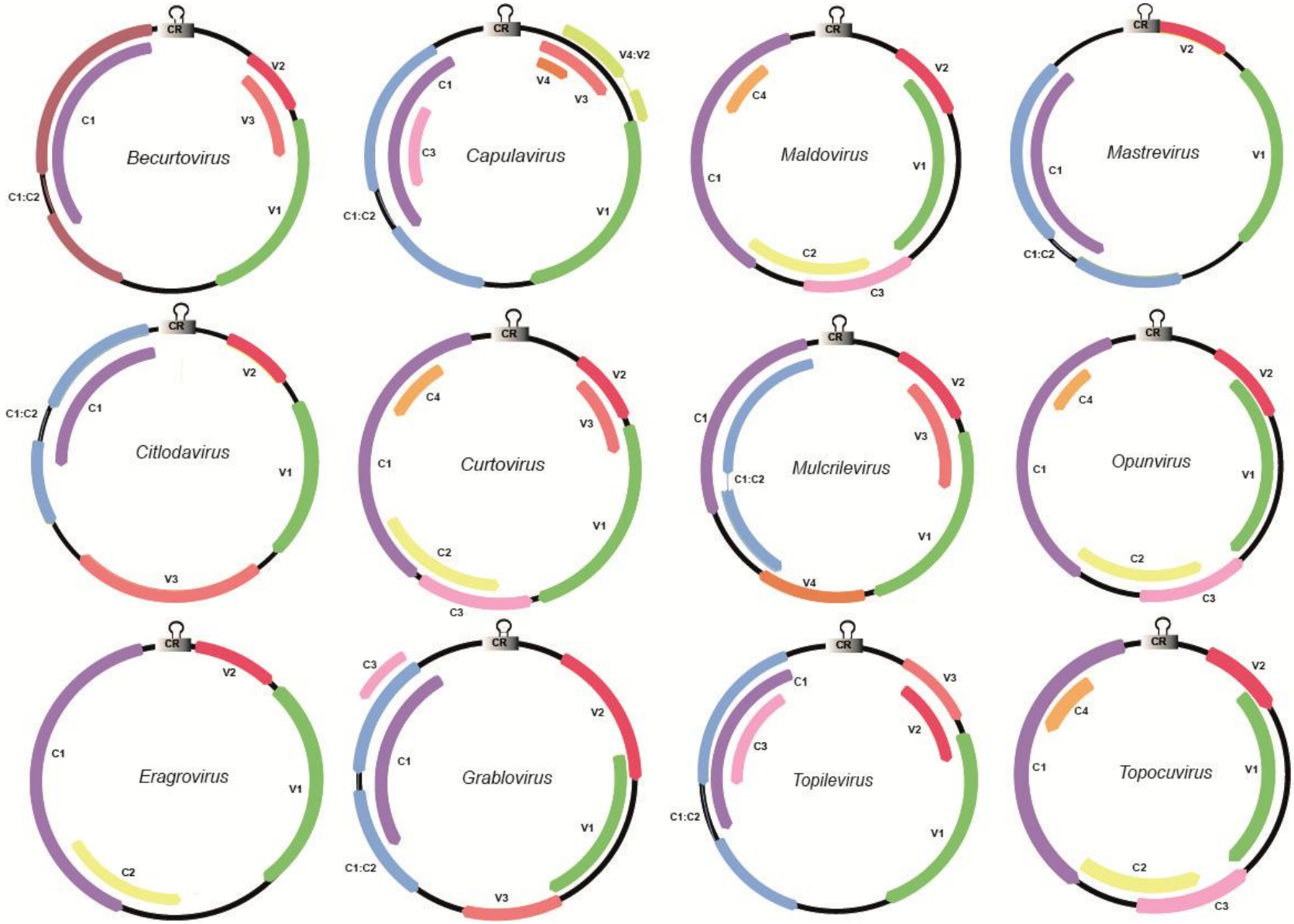
Conforme mencionado anteriormente, *Begomovirus* corresponde a um importante gênero da família *Geminiviridae*, com relato de várias espécies causando doenças e perdas significativas em tomateiro. Dada sua importância, este gênero será tratado de forma mais detalhada. Além disso, outros gêneros da família *Geminiviridae*, como *Citlodavirus* e *Mulcrilevirus*, também podem ocorrer em ambientes agrícolas, frequentemente em plantas daninhas que crescem próximas as áreas de cultivo do tomate e serão brevemente abordados a seguir.

Tabela 2. Características gerais de membros da família *Geminiviridae* quanto à espécie hospedeira, número de espécies, vetor e organização genômica, de acordo com *International Committee on Taxonomy of Viruses* (ICTV 2025).

Hospedeiras	Gênero (NE[1])	Vetor	Organização genômica			Referências
			N[3]	RI[4]	ORFs[5]	
Dicotiledôneas	*Becurtovirus* (3)	*Circulifer haematoceps*	TAA**G**ATT**C**C	2 = RIL e RIC.	V1, V2, V3, C1 e C2.	Heydarnejad et al. (2013); Hernandez-Zepeda et al. (2013); Razavinejad et al. (2013); Varsani et al. (2014a) e ICTV (2025).
	Begomovirus[2] (463)	*Bemisia tabaci*	TAATATTAC	1= RIL e 1= RIC.	AV1, AV2, AC1, AC2, AC3, AC4, AC5, BV1 e BC1.	Hanley-Bowdoin et al. (2000) ; Rojas et al. (2005); Fiallo-Olivé et al. (2020) e ICTV (2025).
	Capulavirus (5)	*Aphis craccivora* e *Dysaphis plantaginea*	TAATATTAC	2= RIL e RIC.	V1, V2, V3, V4, C1, C2 e C3.	Roumagnac et al. (2015); Susi et al. (2019); Bernardo et al. (2013); Bernardo et al. (2016); Ryckebusch et al. (2020) e ICTV (2025).
	Citlodavirus (6)	Desconhecido	TAATATTAC	2= RIL e RIC.	V1, V2, V3, C1 e C2.	Loconsole et al. (2012); Fontenele et al. (2018); Zhang et al. (2018); Qiu et al. (2020) e ICTV (2025).
	Curtovirus (3)	*Circulifer tenellus*	TAATATTAC	2= RIL e RIC.	V1, V2, V3, C1, C2, C3 e C4	Strausbaugh et al. (2008); Soto e Gilbertson (2003); Hanley-Bowdoin et al. (2013) e ICTV (2025).
	Grablovirus (3)	*Spissistilus festinus*	TAATATTAC	2= RIL e RIC.	V1, V2, V3, C1, C2 e C3.	Krenz et al. (2014); Sudarshana et al. (2015); Bahder et al. (2016) e ICTV (2025).
	Mulcrilevirus (2)	*Tautoneura mori*	TAATATTAC	1= RI.	V1, V2, V3, V4, C1 e C2.	Lu et al. (2015); Qiu et al. (2020); Lu et al. (2022) e ICTV (2025).
	Opunvirus (1)	Desconhecido	TAATATTAC	1= RI.	V1, V2, C1, C2, C3, C4.	Fontenele et al. (2020) e ICTV (2025).
	Topilevirus (2)	Desconhecido	TAATATTAC	1= RIL.	V1, V2, V3, C1, C2 e C3.	Fontenele et al. (2017); Vaghi Medina et al. (2018); Batista et al. (2019); Roumagnac et al. (2022) e ICTV (2025).
	Topocuvirus (1)	*Micrutalis maleifera*	TAATATTAC	1= RI.	V1, V2, C1, C2, C3 e C4.	Briddon et al. (1996) e ICTV (2025).

	Turncurtovirus (3)	*Circulifer haematoceps*	TAATATTAC	1= RI.	V1, V2, C1, C2, C3 e C4.	Razavinejad et al. (2013); Kamali et al. (2016); Hasanvand et al. (2018) e ICTV (2025).
Monocotiledôneas	*Eragrovirus* (1)	Desconhecido	TAA**G**ATT**C**C	2= RI-1 e RI-2.	V1, V2, C1 e C2.	Varsani et al. (2009); Varsani et al. (2014b) e ICTV (2025).
Dicotiledôneas e Monocotiledôneas	*Maldovirus* (3)	Desconhecido	TAATATTAC	1= RI.	V1, V2, C1, C2, C3 e C4.	Claverie et al. (2018) e Liang et al. (2015), Al Rwahnih et al. (2017); Roumagnac et al. (2022) e ICTV (2025).
	Mastrevirus (50)	*Cicadulina mbila* e *C. storeyi*	TAATATTAC	1= RIL e RIC.	V1, V2, C1 e C2.	Shepherd et al. (2010); Batista et al. (2021) e ICTV (2025).
Gimnosperma	*Welwivirus* (2)	Desconhecido.	TAATATTAC	2 = RIL e RIC.	V1, V2, V3 e C1.	Bejerman e Debat (2023) e ICTV (2025).

[1]NE: número de espécies correspondente a cada gênero.
[2]Único gênero da família *Geminiviridae* que possui genoma bipartido e monopartido, os demais gêneros são monopartidos.
[3]N: nonanucleotídeo (nucleotídeos que diferem entre os gêneros estão destacados em vermelho).
[4]RI: número de região intergênica (região intergênica longa – RIL; região intergênica curta – RIC).
[5]ORFs: *Open Reading Frame.* Sentido viral (V) e sentido complementar (C).
*V1/AV1 (proteína do capsídeo); AV2/V2 (Proteína de movimento); AC1/C1 (proteína iniciadora de replicação); AC2/C2 (proteína ativadora de transcrição); AC3/C3 (proteína intensificadora de replicação); AC4/C4 (proteína determinante de sintomas); BC1 (proteína de movimento envolvida no movimento viral célula a célula); BV1 (proteína de transporte nuclear); AC5 (proteína determinante de patogenicidade); V3 (proteína supressora de silenciamento gênico pós-transcricional e silenciamento gênico transcricional).


Becurtovirus
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Capulavirus
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Maldovirus
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Mastrevirus
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Citlodavirus
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Curtovirus
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Mulcrilevirus
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C3

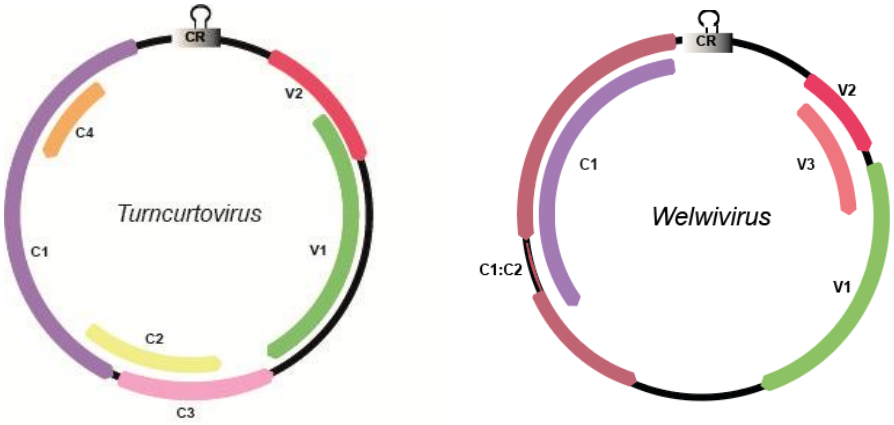

Figura 2. Organização genômica de membros de distintos gêneros pertencentes à família *Geminiviridae*, exceto o gênero *Begomovirus*. Os círculos representam os genomas virais e as setas indicam a posição das distintas *Open Reading Frames* – ORFs nas direções: viral (V) e complementar (C). A V1, codifica a capa proteica – CP (*Coat Protein*); a V2 codifica a proteína de movimento – MP (*Movement Protein*); a C1 codifica a proteína associada à replicação viral – Rep (*Replicase*); a C2, codifica a proteína ativadora da transcrição – TrAp (*Transactivator Protein*); a C3, codifica a proteína potencializadora da replicação – Ren (*Replication Enhancer Protein*); a C4 codifica proteína relacionada a indução de sintoma; a C5 codifica proteína determinante de patogenicidade e supressora do silenciamento gênico; a C6 codifica proteína com função desconhecida e a C7 codifica proteína associada a patogenicidade e supressora do silenciamento de RNA.

4. Gênero *Begomovirus*: organização genômica, características biológicas e evolução

O gênero *Begomovirus* encontra-se atualmente representado por 463 espécies descritas, sendo o gênero mais numeroso da família *Geminiviridae* (Brown et al. 2015, ICTV 2025).

Os begomovírus possuem uma (espécies monopartidas) ou duas (espécies bipartidas) móleculas de DNA circular. Begomovírus com genomas monopartidos possuem aproximadamente 2.500 a 3.000 nucleotídeos na molécula de DNA, enquanto os begomovírus bipartidos possuem em torno de 5.200 nucleotídeos (DNA–A e DNA–B) (Zerbini et al. 2017; Kumar 2019) e são considerados os menores genomas de vírus de plantas (Rojas et al. 2005).

O DNA–A de begomovírus monopartidos possuem de cinco a seis ORFs, sendo uma ou duas no sentido viral e quatro no sentido complementar (Fiallo-Olivé et al. 2021). No sentido viral, a ORF V1/CP (*Coat Protein*) é responsável por codificar a proteína da capa proteica, e V2 (encontrada em begomovírus monopartidos e em bipartidos do "Velho Mundo") codifica a proteína de movimento (MP- *Moviment Protein*) (Zerbini et al. 2017; Brown et al. 2012; Devendran et al. 2022). No sentido complementar: C1/Rep (*Replication-associated protein*) codifica a proteína iniciadora de replicação, C2/TrAP (*Transcriptional Activator Protein*) é responsável pela proteína ativadora de transcrição, C3/REn (*Replication Enchancer protein*) codifica a proteína intensificadora de replicação e C4 codifica uma proteína envolvida na determinação de sintomas, podendo atuar como supressora do silenciamento gênico (Brown et al. 2012; Kulshreshtha et al. 2019; Kumar e Dasgupta 2023 e Li et al. 2024). A função de movimento célula-a-célula dos begomovírus monopartidos é coordenada pela V1/CP, V2 e C4 (**Figura 3**) (Rojas et al. 2001). Já os begomovírus bipartidos codificam de cinco a seis ORFs no componente DNA–A, envolvidas na replicação viral, encapsidação, transmissão e patogênese: AV1/CP, AC1/Rep, AC2/TrAP, AC3/REn, AC4 e, em alguns vírus, AV2 (Brown et al. 2012; Zerbini et al. 2017 e Devendran et al. 2022).

Outras ORFs estão sendo identificadas. A ORF C5/AC5 pode ser encontrada no DNA–A de algumas espécies, tanto de begomovírus monopartidos quanto bipartidos. Localizada no sentido complementar, a jusante da C3 e sobreposta a uma porção da CP (Li et al. 2015 e Zhao et al. 2023). A C5 é composta por em média 100 nucleotídeos e é uma determinante de patogenicidade e potente supressora do silenciamento gênico (Li et al. 2015; Zhao et al. 2023). Outra ORF, relatada recentemente em um vírus da China, é a V3. Wang et al. (2021) identificaram essa ORF em Malvastrum yellow vein Honghe virus (MaYVHhV), localizada a jusante da ORF V2, porém, com função desconhecida. Gong et al. (2021) demonstraram que V3 é conservada em begomovírus e essencial para a infectividade total em *N. benthamiana* e

tomate, codificando uma proteína com 77 aminoácidos, localizada no complexo de Golgi, contribuindo significativamente para a virulência, pois atua como um supressor de Silenciamento gênico pós-transcricional – SGPT (*Post-Transcriptional Gene Silencing* – PTGS) e Silenciamento Gênico Transcricional – SGT (*Transcriptional Gene Silencing* – TGS). ORF C6 também foi identificada em *N. benthamiana*, em clones com tomato leaf curl China virus (ToLCCNV), e está presente em 36% dos begomovírus. Esta ORF está localizada no sentido complementar, sobreposta parcialmente às ORFs CP e V2, e codifica um polipeptídeo de 97 aminoácidos com localização na mitocôndria (Wang et al. 2022). A outra ORF, denominada C7, localiza-se no núcleo e no citoplasma, demonstrando interação com C2 e V2, formando grânulos conspícuos e desenvolvendo sintomas de mosaico mais graves (Liu et al. 2023). Ainda de acordo com Liu et al. (2023), além de codificar para um fator de patogenicidade, a C7 atua também como supressor de silenciamento de RNA e desempenha um papel crítico durante a infecção por TYLCV.

O componente DNA–B codifica duas proteínas envolvidas no movimento célula a célula e movimento a longa distância: BC1/NSP *Nuclear Shuttle Protein* – proteína de transporte nuclear e BV1/MP *Movement Protein* –proteína de movimento (**Figura 3**) (Rojas et al. 2005 eLi et al. 2015). A NSP é responsável pelo transporte nucleocitoplasmático (Rojas et al. 2005 e Kumar 2019), e a MP está envolvida no movimento célula a célula e sistêmica (Rojas et al. 2005; Kumar 2019 e Breves et al. 2023). Para a identificação de fatores do hospedeiro em processos celulares explorados por begomovírus, as funções de transporte intra e intercelular da NSP e MP são de grande relevância, tendo em vista que essas proteínas interagem com fatores do hospedeiro em várias organelas tornando-se potenciais centros na rede de interação proteína-proteína hospedeiro-vírus (Gouveia-Mageste et al. 2021; Breves et al. 2023). Em begomovírus monopartidos, como em *Begomovirus coheni* (*=Tomato yellow leaf curl vírus)*, a função da NSP é assumida pela CP, com o auxílio de outras proteínas, como a V2 e a C4 (Rojas et al. 2001 e Devendran et al. 2022).

Os dois componentes DNA–A e DNA–B não compartilham identidade de sequência nucleotídica, com exceção de uma sequência de ~200 nucleotídeos, que totalizam mais de 85% de identidade, conhecida como região comum (CR) (Rojas et al. 2005 e Hanley-Bowdoin et al. 2013). A CR engloba uma estrutura conservada, contendo a sequência nonanucleotídeo (TAATATTAC), que marca a origem de replicação da fita de DNA, e sequências repetidas (iterons) – reconhecimento da ligação da proteína associada à replicação codificada por DNA–A (Rep) (Arguello-Astorga & Ruiz-Medrano 2001 e Briddon et al. 2010).

Baseando-se em estudos filogenéticos, espécies classificadas no gênero *Begomovirus*, são separadas em dois grupos: "Velho Mundo" (*Old World*) – Europa, Ásia e África; e "Novo Mundo" (*New Word*) – Américas. Por conseguinte, os begomovírus monopartidos são mais comuns no "Velho Mundo", ou seja, nas Américas há uma predominância dos genomas bipartidos (Briddon et al. 2010; Navas-Castillo e Fiallo-Olivé 2020; Reis et al. 2020; Reis et al. 2021). O *tomato leaf curl purple vein virus* (*=Begomovirus solanumviolavenae)* (Macedo et al. 2018) foi o primeiro begomovírus monopartido relatado no Brasil. Todavia, Souza et al. (2022) demonstraram que o *tomato mottle leaf curl virus* (*=Begomovirus solanumvariatuminvolutionis*) é um vírus de genoma monopartido e tem origem evolutiva no Nordeste do Brasil. Ademais, Reis et al. (2023) detectaram dois vírus com genomas monopartidos no Brasil: *tomato golden net virus* (= *Begomovirus solanumaureusreti*) e *tomato yellow net virus*. Oliveira (2024) relataram tomato iridescent mottle virus (ToIMoV), na região Nordeste, e tomato iridescent apical mosaic virus (ToIAMV) na região Sul, indicando que esses vírus podem compreender um grupo único de begomovírus monopartidos no Novo Mundo.

Os componentes de DNA–B são mais diversos do que seus correlatos de DNA–A. Algumas explicações são apresentadas: **1.** O componente DNA–B codifica duas proteínas, portanto possui menos funções, sendo permissivo a variações; **2.** O componente DNA–B evolui exclusivamente em resposta ao hospedeiro – enquanto o DNA–A mantém interação com o vetor artrópode e **3.** O DNA–B tem origem distinta do DNA–A provavelmente originado como um satélite adquirido ao longo do processo evolutivo (Briddon et al. 2010). Estes autores propõem que a explicação mais provável para a diferença em questão estaria relacionada à combinação desses fatores.

Para a classificação, demarcação e identificação de espécies de begomovírus, são utilizados critérios baseados na identidade das sequências do DNA–A. Desta forma, identidades de sequências do DNA–A da espécie em estudo com as espécies já conhecidas devem ser comparadas e como parâmetros utiliza-se os valores de 91% e 94% como limites de demarcação, sendo considerada nova espécie aquelas com identidade menor que 91% e nova estirpe aquelas menores que 94% de identidade nucleotídica (Brown et al. 2015). Brown et al. (2015) ainda afirmam que a grande diversidade de espécies de begomovírus está associada às relações de ordem natural baseadas nas características desse gênero, que os diferencia de muitos outros gêneros.

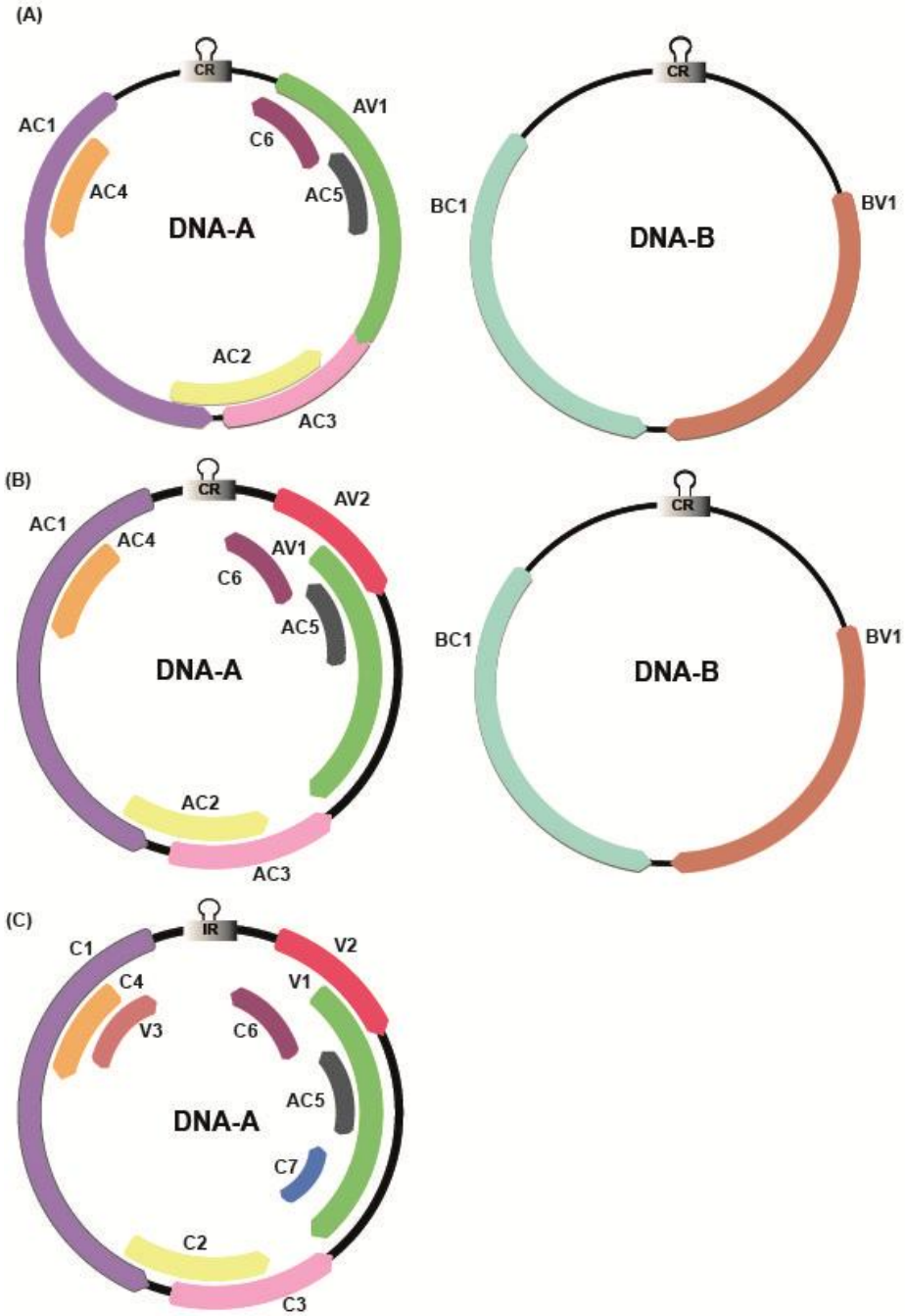

Figura 3. Organização genômica típica de begomovírus bipartidos e monopartidos. (A) Begomovírus bipartido do Novo Mundo. (B) Begomovírus bipartido do Velho Mundo. (C) Begomovírus monopartido. Os círculos representam os genomas virais e as setas indicam a posição das distintas Open Reading Frames (ORFs) na direção viral (V) e complementar (C). No componente DNA–A, A AV1 codifica a proteína do capsídeo; a AV2/V2 codifica a proteína de movimento; a AC1/C1 codifica a proteína iniciadora de replicação; a AC2/C2 codifica a proteína ativadora de transcrição; a AC3/C3 codifica a proteína intensificadora de replicação; a AC4/C4 codifica a proteína determinante de sintomas. No componente DNA–B, a BC1 codifica a proteína de movimento – MP (*Movement Protein*) envolvida no movimento viral célula a célula; a BV1 codifica a proteína de transporte nuclear; a AC5 codifica a proteína determinante de patogenicidade; a C6 codifica proteína com função desconhecida; a C7 codifica a proteína associada ao fator de patogenicidade e supressor de silenciamento de RNA e a V3 codifica a proteína supressora de Silenciamento gênico pós-transcricional – SGPT (*Post-Transcriptional Gene Silencing* – PTGS) e Silenciamento Gênico Transcricional – SGT (*Transcriptional Gene Silencing* – TGS).

4.1 Replicação de begomovírus

O processo de infecção é iniciado a partir da entrada do vírus, normalmente através do inseto vetor, na planta hospedeira. Desta forma, o material genético do vírus é transportado para o núcleo da célula. Para isso, o DNA de fita simples (single strand DNA - ssDNA) circular é convertido em DNA de fita dupla (dsDNA), que serve de modelo replicativo e transcricional, e em seguida são empacotados nos nucleossomos do hospedeiro, na forma de minicromossomos extracromossomais, com o auxílio das enzimas presentes no hospedeiro (Stanley et al. 1985; Hanley-Bowdoin et al. 1999; Gutierrez et al. 2002; Kumar 2019). A Rep é extremamente necessária para que se inicie o processo de replicação, pois apresenta funções de corte e ligação do DNA, auto repressão de sua transcrição e é responsável pela reprogramação do ciclo celular – induz a expressão de DNA polimerase dependente de DNA em células já diferenciadas (Hanley-Bowdoin et al. 1999), atuando como endonuclease sítio-específica (Pradhan et al. 2017). Além disso, após a identificação de uma pequena ORF, denominada C5 (Li et al. 2015), estudos recentes revelaram que uma das funções desta ORF é facilitar o movimento do DNA viral dentro e entre as células (Zhao et al. 2023).

Begomovírus assim como outros membros da família *Geminiviridae,* não possuem uma polimerase própria, necessitando desenvolver mecanismos que reativam a maquinaria de replicação em células hospedeiras. Desta forma, o processo de replicação ocorre no núcleo da célula hospedeira, por meio do mecanismo de "replicação por círculo rolante" (*Rolling Circle Replication* - RCR), que resulta em moléculas de ssDNA viral circular (Hanley-Bowdoin et al. Rojas et al. 2005; 2017). Contudo, além da RCR, há relatos de um modelo adicional de replicação, identificado como "replicação dependente de recombinação" (RDR), para alguns begomovírus e seus satélites (Jeske et al. 2001; Preiss e Jeske 2003; Alberter et al. 2005; Morilla et al. 2006; Jovel et al. 2007 e Erdmann et al. 2010). Diferentemente da RCR, a RDR não tem uma origem de replicação, podendo ser iniciada em qualquer extremidade 3', podendo levar à recombinação, caso dois vírus relacionados entrem no mesmo núcleo, sendo um fator importante para explicar a recombinação gênica comumente observada na família *Geminiviridae* (Jeske et al. 2001). O RDR ocorre no núcleo da célula hospedeira, onde um fragmento de ssDNA viral é inserido em qualquer lugar entre duas fitas de DNA circular covalente fechado - cccDNA (*covalently closed circular DNA*) gerado pela síntese de DNA circular de fita simples - csDNA (*circular single-stranded DNA*) (Tarasova e Khayat 2021).

Após o vírus ser introduzido no núcleo da célula, com o auxílio do inseto vetor, seu ssDNA é transportado até o interior do núcleo, auxiliado pela formação de um complexo

formado entre a CP e proteínas de cadeias de transporte da hospedeira (Priyadarshini et al. 2011). Dentro do núcleo, o processo de replicação pode ser divido em três etapas:

1. Conversão de ssDNA em dsDNA intermediário: com o auxílio da polimerase da hospedeira, o ssDNA é convertido em dsDNA intermediário, que requer a ativação da fita de origem do DNA, conhecido como forma replicativa (RF) (Kumar 2019). A forma intermediária servirá de molde para a transcrição viral e para a síntese de novos filamentos de ssDNA, através do mecanismo de círculo rolante (Stanley 1995; Gutierrez 2002; Monsalve-Fonnegra et al. 2002; Breves et al. 2023). Esse processo tem início com a ligação da Rep a uma sequência específica na região comum, composta por duas sequências repetidas, denominadas "iterons". Em seguida, a cadeia do ssDNA é clivada, iniciando-se assim o ciclo de replicação do vírus (Gutierrez 2002; Monsalve-Fonnegra et al. 2002).
2. Ocorre a RCR usando o dsDNA como molde para amplificação do ssDNA: a Rep é a proteína responsável pelo processo de clivagem dentro do nonanucleotídeo "TAATATTAC" (conservado na maioria dos membros da família *Geminiviridae*), localizado na haste da região intergênica, dando início ao processo (Gutierrez 1999; Monsalve-Fonnegra et al. 2002). Quando o ciclo é finalizado, a Rep atua separando a nova fita da antiga, assim, o novo ssDNA é produzido e circularizado usando a atividade de ligação da Rep (Pradhan et al. 2017).
3. A última etapa consiste na síntese de ssDNA a partir de dsDNA, com o acúmulo de genomas virais para encapsidação (Gutierrez 1999, 2002; Monsalve-Fonnegra et al. 2002). Quando os produtos dos genes CP e NSP estão sintetizados, eles se ligam ao ssDNA para a encapsidação ou transporte para fora do núcleo (Jeske et al. 2001). Após entrar no citoplasma da célula hospedeira, o vírus inicia o movimento a curta distância, que é facilitado pela ação da proteína MP. Esta proteína permite a passagem do vírus através dos plasmodesmas, estruturas que conectam células vegetais, facilitando assim a disseminação viral dentro da planta. Em begomovírus monopartidos, o transporte nuclear da NSP é substituído pela CP, que é auxiliada pela V2 e C5 (Devendran et al. 2022). A interação entre a V2 e C5 facilita a exportação nuclear e realocação da V2 para o plasmodesmo, evidenciando que a C5 interage com microfilamentos e promove a redistribuição da V2 para os plasmodesmos (Zhao et al. 2023). Posteriormente, o vírus é transportado a longas distâncias através do floema, o sistema vascular das plantas responsável pelo transporte de nutrientes e compostos orgânicos, permitindo assim a disseminação sistêmica da infecção (Hipper et al. 2013).

4.2 Transmissão de begomovírus

Os begomovírus são transmitidos por um complexo de espécies crípticas de *Bemisia tabaci* (De Barros e Ahmed 2011; Navas-Castillo et al. 2011; Rojas et al. 2018; Cantú-iris et al. 2019). A interação entre begomovírus e *B. tabaci* é caracterizada como circulativa não propagativa, no entanto, estudos de Pakkianathan et al. (2015) e Wang et al. (2016), com TYLCV, demonstraram a capacidade de replicação desse vírus no inseto vetor, quando submetido a condições específicas.

As espécies de *B. tabaci* predominantes no mundo são: *Middle East Asia Minor* 1 - MEAM1 (= biótipo B), Mediterranean -*MED* (= biótipo Q) (Brown et al. 1996; Carnero-Avilés et al. 2024; Li et al. 2023; Lestari et al. 2022; Peng et al. 2025) e a New Word – NW (=biótipo A) é dominante nas Américas tropicais e subtropicais, mas não é amplamente predominante o mundo todo (Perring et al. 2018).

MEAM1 foi relatada pela primeira vez no Brasil em 1991 (Moraes et al. 2018), enquanto MED foi relatada pela primeira vez em 2014 (Barbosa et al. 2015). Após a introdução da MEAM1 (=biótipo B) no Brasil, houve aumento na incidência e severidade de begomoviroses em tomateiro, bem como descrição de novas espécies. Presume-se que begomovírus que antes estavam presentes apenas em plantas daninhas tenham sido transmitidos para o tomateiro a partir da entrada de *B. tabaci* MEAM1 (Ambrozevicius et al. 2002). As espécies crípticas de *B. tabaci* MEAM1 e MED são vetores eficientes de begomovírus e ambas estão presentes no Brasil, porém, MEAM1 continua sendo a espécie dominante em culturas de campo aberto no Brasil (Fernandes et al. 2022; De Souza et al. 2024). Apesar disso, estudos recentes afirmam que a MED pode estar começando a se estabilizar em áreas abertas de tomate no Brasil (Álvarez et al. 2024).

Acredita-se que MEAM1 esteja mais adaptada e apresente uma maior eficiência na transmissão de begomovírus.

4.3 DNAs satélites associados à begomovírus

Os begomovírus monopartidos do Velho Mundo são frequentemente encontrados em associação com DNAs satélites, denominados como alfasatélite, betasatélite (Zhou 2013 e Li et al. 2015) e deltassatélite (Nawaz-ul-Rehman et al. 2021) (**Figura 4**). Os alfasatélites

pertencem à família *Alphasatellitidae*, composta por 3 subfamílias, 18 gêneros e 85 espécies. Já os betassatélites e deltassatélites encontram-se classificados na família *Tolecusatellitidae*, com 2 gêneros e 131 espécies (ICTV 2025).

Os alfasatélites, anteriormente conhecidos como DNA 1, são moléculas de DNA circulares de fita simples (≈1400 bases), com aproximadamente metade do tamanho dos DNAs de begomovírus. Além disso, codificam uma proteína iniciadora (alfa-Rep) que é semelhante à proteína Rep mestre codificada pelo componente genômico (DNA–R) dos nanovírus. São capazes de se auto-replicarem em plantas hospedeiras, porém, requerem um begomovírus auxiliar para o movimento em plantas e transmissão pelo inseto (Zhou 2013).

O primeiro betasatélite de begomovírus foi isolado de plantas de tomate infectadas com tomato leaf curl virus (ToLCV), na Austrália (Dry et al. 1997), porém, atualmente é classificado no gênero *Deltassatellite* (Nawaz-ul-Rehman et al. 2021). Anteriormente conhecidos como DNAβ, os betasatélites são componentes subvirais com aproximadamente ≈1350 nucleotídeos e possuem uma região altamente conservada em todos eles: região conservada por satélite (SCR) com ≈120 nucleotídeos (Zhou 2013), além de uma região rica em adenosina (A-rich region) e um pequeno quadro de leitura abertos que codifica uma proteína chamada βC1 (Nawaz-ul-Rehman et al. 2021). Estabelecem uma relação de dependência com seus vírus auxiliares para replicação, para a disseminação célula a célula e sistêmica em todo o hospedeiro, para encapsidação e transmissão a novas plantas hospedeiras por meio de insetos vetores. Sua função essencial é a indução de sintomas típicos de doenças e têm importantes papéis na supressão de silenciamento gênico transcricional e silenciamento gênico pós-transcricional, afetando outras plantas (Zhou 2013 e Nawaz-ul-Rehman et al. 2021). Inicialmente, os betasatélites foram encontrados associados a begomovírus monopartidos, porém, atualmente são identificados em associação com begomovírus bipartidos (Nawaz-ul-Rehman et al. 2021).

Vale ressaltar que alfasatélites e betasatélites não possuem semelhança na organização genômica com os begomovírus, exceto pela estrutura de grampo *stem-loop*, que é necessária para a proteína iniciadora RCR (*Rolling-Circle Replication*) (Zhou 2013).

Os deltasatélites estão associados tanto a begomovírus monopartidos do Velho Mundo (Dry et al. 1997) quanto aos bipartidos do Novo Mundo (Fiallo-Olivé 2012) e têm cerca de 700 pb. Deltasatélites foram relatados no Novo Mundo, pela primeira vez, em 2012 nas ilhas do Caribe, e até o momento existem 11 espécies distintas, conhecidos na Índia, Vietnã, Filipinas, Espanha, Austrália, Cuba e Porto Rico. Eles não codificam nenhum gene em seu genoma, no

entanto, compartilham de características comuns: a maioria contém uma região rica em adenosina, um nonanucleotídeo de origem de replicação (TAATATTAC) e uma estrutura secundária de alça. Isto posto, os deltassatélites não são moléculas autorreplicantes, necessitando de um vírus auxiliar para sua replicação e movimento sistêmico (Nawaz-ul-Rehman et al. 2021).

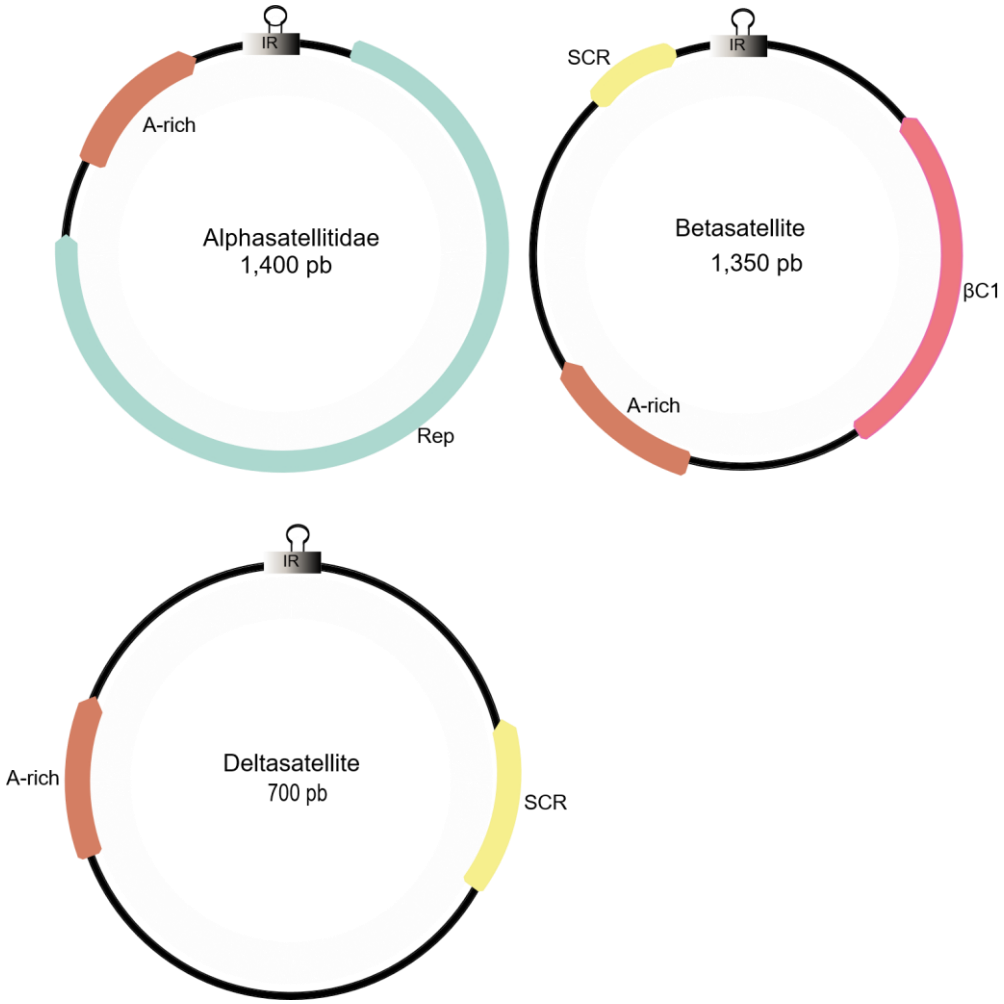

Figura 4. Representação genômica de DNA satélites (Alfasatélites, Betasatélites e Deltasatélites) associados a isolados de espécies de *Begomovirus*. Ilustração da organização genômica com as *Open Reading Frames* (ORFs) ilustradas. A Rep (proteína associada à replicação) em alfasatélites e βC1 e região rica em adenina (A-rich), está presente em todos os satélites de DNA e encontra-se marcada em cores distintas; SCR - *Satellite Conserved Region* (= Região conservada por satélite - RCS) e IR - *Intergenic region* (região intergênica).

4.4 Evolução e variabilidade de begomovírus

Três mecanismos principais impulsionam a evolução dos vírus: mutação (Roossinck 1997; Balol et al. 2010), recombinação (Roossinck 1997; Padidam et al. 1999 e Roossinck et al. 2007) e pseudo-recombinação (Roossinck 1997 e Butković et al. 2023). Esses mecanismos ocorrem nos begomovírus, causando uma alta variabilidade genética, resultando no surgimento de novas espécies, estirpes e isolados (Rocha et al. 2013).

O nível de diversidade genética aparenta ser uma propriedade intrínseca dos vírus, que ocorre independentemente da gama de hospedeiro ou da existência de infecção mista com outros vírus (García-Arenal e Zerbini 2019). Contudo, as recombinações são aumentadas na presença de plantas daninhas infectadas com várias espécies de vírus, aumentando a taxa de transmissão do vírus e ampliando ainda mais sua gama de hospedeiras (Wang et al. 2021).

Além disso, a probabilidade de eventos de recombinação e pseudo-recombinação é maior com a presença de dois ou mais begomovírus na mesma planta, podendo levar ao surgimento de novas espécies mais adaptadas ao novo hospedeiro (Padidam et al. 1999; Inoue-Nagata et al. 2006; Seal et al. 2006; Rojas et al. 2018 e Reis et al. 2020).

Portanto, há estudos que confirmam a ocorrência desses eventos, destacando a importância de compreender as interações entre diferentes vírus e seus hospedeiros na evolução e emergência de novas cepas virais. González-Aguilera et al. (2012) detectaram eventos de recombinação em ToSRV relacionados a isolados provenientes de plantas daninhas da espécie *Sida micrantha*, indicando a necessidade de monitoramento dessas populações virais no campo, para permitir uma estratégia de controle eficiente baseada na resistência a begomovírus. Assim, a ocorrência de vários isolados e espécies de vírus em uma mesma planta (infecção mista) é um dos fatores que colaboram com a diversidade genética de espécies (Reis et al. 2020).

As análises de Quadros et al. (2023) também revelaram eventos de recombinação em quatro vírus: entre o DNA–A de Sida micranta mosaic virus (SiMMV) e Sida common mosaic virus (SiCmMV) e outro entre o DNA–B de ToSRV e SiMMV. Os estudos de Nogueira et al. (2023), reafirmaram que ToSRV e tomato rugose mosaic virus (ToRMV) são pseudo-recombinantes, e verificaram que não só existe sinergismo entre estes dois vírus, mas uma interferência negativa de ToRMV na acumulação de ToSRV.

Recentemente Naveen et al. (2025) também identificaram eventos de recombinação tanto dentro de uma mesma espécie, em isolados que infectam espécies de plantas diferentes, quanto entre diferentes espécies de begomovírus: no DNA–A - entre diferentes isolados de tomato leaf

curl New Delhi virus (ToLCNDV); entre isolados de tomato leaf curl Patna virus (ToLCPaIV); ToLCPaIV com ToLCNDV; tomato leaf curl Bangalore virus (ToLCBaV) com tomato leaf curl Gujarat virus (ToLCGUV) e ToLCNDV, e no DNA–B – entre isolados de ToLCNDV e entre isolados de Coton leaf curl Multan alphasatellite (CLCuMuA).

4.5 Caracterização molecular: iterons e motivos conservados em begomovírus

Conforme mencionado anteriormente, um begomovírus bipartido típico apresenta dois componentes genômicos, DNA–A e DNA–B, enquanto um begomovírus monopartido possui apenas o DNA–A, responsável por funções essenciais como a replicação e a interação com o hospedeiro (Zerbini et al. 2017). Seu genoma apresenta uma sequência conservada TAATATTAC essencial para a origem de replicação (Silva et al. 2014), além do promotor transcricional TATA box (Pandey et al. 2010) e os iterons que regulam a replicação viral (Xu et al. 2019).

Iterons são sequências de DNA que servem como sítios de ligação para proteínas envolvidas na replicação do DNA viral. Nos begomovírus, os iterons são encontrados nas regiões de origem da replicação (Rep), que são locais específicos no genoma viral onde a replicação do DNA inicia. As proteínas virais se ligam aos iterons e iniciam o processo de replicação do DNA viral. Já os motivos conservados dos begomovírus são sequências de nucleotídeos que são altamente conservadas (mantêm-se similares) entre diferentes cepas e espécies de begomovírus. Esses motivos conservados geralmente desempenham papéis importantes na estrutura ou função das proteínas virais, como as envolvidas na replicação, transcrição e movimento viral. O estudo desses motivos conservados é crucial para compreender a biologia dos begomovírus e desenvolver estratégias de controle eficazes contra esses patógenos (Argüello-Astorga & Ruiz-Medrano 2001).

Os vírus da família *Geminiviridae* codificam uma proteína destinada a iniciação da replicação do vírus, a Rep. Essa proteína se liga de maneira específica a motivos iterados de DNA, chamados iterons, elementos essenciais para a replicação específica do vírus (Argüello-Astorga & Ruiz-Medrano 2001). Utilizando iterons de diversos membros da família *Geminiviridae* como dispositivo investigativo, Argüello-Astorga & Ruiz-Medrano (2001) identificaram subdomínios da Rep que são variáveis entre vírus de iterons diferentes, porém, são conservados em vírus com iterons idênticos, mesmo que sejam de diferentes hospedeiros e

vetores, ou até mesmo de origem geográfica e estrutura genômica distintos. Esses subdomínios são conhecidos como "*Iteron-Related Domains*" (IRD) e foram utilizados para a caracterização da diversidade genômica de begomovírus, analisando-se a estrutura primária da Rep-IRD e a sequência nucleotídica do iteron cognato. Com isso, Argüello-Astorga & Ruiz-Medrano (2001) concluíram que o estudo do IRD revelou uma correlação consistente entre sua sequência de aminoácidos e seus elementos de DNA cognatos previstos, sugerindo que seja um importante componente do domínio de reconhecimento de DNA específico da família *Geminiviridae*. Nogueira et al. (2023) investigaram se sítios divergentes na RC e no IRD estão envolvidos na interação entre ToSRV e ToRMV e descobriram que na inoculação mista contendo os mesmos nucleotídeos (IRD e RC) observou-se uma alta infectividade e alto acúmulo de DNA de ToSRV (RC) e diminuição do acúmulo viral de ToSRV (RC+IRD), indicando que diferenças na RC, mas não na IRD, são responsáveis pela interferência negativa de ToRMV no ToSRV.

Arguello-Astorga et al. (2004) também conduziram estudos de caracterização de uma região genômica conservada do fator de replicação da família *Geminiviridae* (AC1/Rep), atestando sua interação com retinoblastoma vegetal (pRBR). Mutações em uma sequência de 11 aminoácidos que compõem um motivo conservado, detectado na AC1/Rep de todos os membros da família, resultaram em comprometimento na interação com a pRBR em células, confirmando a importância desse motivo nas interações entre a proteína de replicação dos vírus da família *Geminiviridae* e a pRBR de plantas hospedeiras (Arguello-Astorga et al. 2004).

Motivos e sequências conservadas estão sendo utilizadas para a detecção, identificação e caracterização de novas espécies de begomovírus. Ao caracterizar uma nova espécie de *Blechum interveinal chlorosis virus*, Cantú-Iris et al. (2019) confirmaram a presença do nonanucleotídeo TAATATTAC e de três iterons GGGGGA, além de duas repetições de 15 nts incluindo um motivo (G) GGACCAC. Em sequências de begomovírus do Novo Mundo, os autores observaram regiões promotoras à CP viral que são curtas, com uma sequência central ACTT-N7-AAGT, uma sequência heptanucleotídica (N7) rica em GC e altamente variável e uma invariável associada ao TATA-box (TATA- *Associated Composite Element* – TACE) (Cantú-Iris et al. 2019). Reis et al. (2021) caracterizaram 45 isolados de tomato golden vein virus (TGVV) e tomato yellow vein streak virus (ToYVSV) utilizando análises de Rep-IRD, mostrando que esses dois vírus possuem distintos domínios relacionados à REP e confirmando a distinção entre as espécies.

Para caracterizar e identificar a função da ORF AC5 em begomovírus, Li et al. (2015) utilizaram domínios conservados, separando-os em dois grupos: (a) begomovírus mono e

bipartidos do Novo Mundo; (b) begomovírus do Velho Mundo. Com isso, concluíram que todas as proteínas AC5, dos grupos (a) e (b), têm um domínio conservado, ao qual os autores denominaram 'Gemini AC5-1' (pfam04807), porém, somente o grupo (b) possui um domínio adicional, 'Gemini AC5-2' (pfam08464), localizada na extensão do C-terminal (Li et al. 2015). Desta forma, os autores concluíram que a presença da ORF AC5 adicional é conservada em muitos begomovírus e a estrutura de domínio semelhante, sugeriram que a AC5 desempenha um papel importante, tanto no silenciamento gênico transcricional, quanto na intensificação de sintomas virais.

Além disso, outros gêneros da família *Geminiviridae*, como *Citlodavirus* e *Mulcrilevirus*, também podem ocorrer em ambientes agrícolas, frequentemente em plantas daninhas que crescem próximas as áreas de cultivo do tomate, assim como vírus de outras famílias: *Caulimoviridae* (gêneros *Badnavirus* e *Caulimovirus*), *e Genomoviridae* (gêneros *Gemycircularvirus* e *Gemykolovirus*) que serão brevemente abordados a seguir.

5. Gênero *Citlodavirus*

O gênero *Citlodavirus* (família *Geminiviridae*) foi proposto em 2021 (Rougmanac et al. 2021; Rougmanac et al. 2022). O nome do gênero deriva do vírus citrus chlorotic dwarf associated virus – CCDaV (espécie *Citlodavirus citri*), espécie tipo do gênero, identificada inicialmente na Turquia (Loconsole et al. 2012).

O gênero possui seis espécies descritas, sendo todas espécies monopartidas e contendo o nonanucleotídeo 5′-TAATATTAC-3′ que é altamente conservado na família *Geminiviridae* (Fontenele et al. 2018; Rougmanac et al. 2021; Rougmanac et al. 2022).

O genoma de citlodavírus varia de 3.639 a 3.763 nt (Roumagnac et al. 2022). Isso ocorre devido a sua MP, que é até três vezes maior do que geminivírus monopartidos, mas tem aproximadamente o mesmo tamanho de geminivírus bipartidos (Fontenele et al. 2018). Nos begomovírus bipartidos, a MP localizada no DNA-B, codifica uma proteína com homologia às MPs de *Citlodavirus passiflorae* (= Passion fruit chlorotic mottle virus- PCMoV), CCDaV e Camelia chlorotic dwarf associated virus (CaCDaV). Essa semelhança indica que PCMoV, CCDaV e CaCDaV podem representar um estágio intermediário na transição evolutiva entre os geminivírus monopartidos (~2,7–3,0 kb) e os begomovírus bipartidos (~5,3 kb) (Fontenele et al. 2018).

Citlodavírus possuem em sua organização genômica seis ORFS no sentido viral V1/CP (*Coat Protein*) – capa proteica, V2/MP (*Moviment protein)* – proteína de movimento e V3, e no sentido complementar C1/Rep (*Replication-associated protein*) – proteína de replicação (RepA e Rep) e a C2/TrAP (*Transcriptional activator protein)* - proteína ativadora de transcrição (**Figura 2**). Além do *stem-loop,* na região intergênica longa (RIL), contendo a região conservada do nonanucleotídeo e uma região intergênica curta (RIC) (Qiu et al. 2020).

Para a demarcação e identificação de espécies dentro desse gênero, adota-se o critério de 78% de identidade de sequência nucleotídica de todo o genoma (Roumagnac et al. 2022; ICTV 2025).

Os membros do gênero *Citlodavirus* ainda não têm um vetor natural formalmente identificado, apesar disso foi proposto que o vetor do CCDaV poderia ter como vetor mosca branca (*Parabemisia myricae*), em relação circulativa semi-persistente (Garnsey 1996; Loconsole et al. 2012).

A gama de hospedeiros naturais conhecidos para citlodavírus ainda é limitada, tendo sido identificado em: camélia comum (*Camellia japonica*) (Zhang et al. 2018); citrus (*Citrus* spp.) (Loconsole et al. 2012); amoreira-de-papel (*Broussonetia papyrifera*) (Qiu et al. 2020) e maracujá (*Passiflora edulis*) (Fontenele et al. 2018). Novas espécies de *Citlodavirus* vem sendo relatadas em outras hospedeiras: *Citlodavirus myricae* (Myrica rubra citlodavirus 1 - MRV1), em mirtilos-chineses (*Myrica rubra)*, na China (Gao et al. 2023) e *Citlodavirus apijamaicaense* (Apiscitlodal virus), em abelha da Jamaica, na Jamaica (Bandoo et al. 2024).

6. Gênero *Mulcrilevirus*

O gênero *Mulcrilevirus* possui duas espécies descritas até o momento (ICTV 2025). Esse gênero foi estabelecido e implementado pelo ICTV em 2021 (Rougmanac et al. 2022) após relato de *Mulcrilevirus mori* (= *Mulberry crinkle leaf virus*) em amoreiras na China (Lu et al. 2015; Ma et al. 2015; Qiu et al. 2020). Os integrantes desse gênero possuem genomas monopartidos, caracterizados por uma origem de replicação conservada, com a sequência 5'-TAATATTAC-3' (Qiu et al. 2020).

Semelhante a organização genômica de *Begomovirus*, a componente DNA–A de *Citoladavirus,* com tamanho aproximado de 2.950 nucleotídeos codifica seis ORFS: no sentido viral V1/CP, V2/MP, V3 e V4, e no sentido complementar C1/Rep (RepA e Rep) e a C2/TrAP

(**Figura 2**), e uma região intergênica (IR) (Qiu et al. 2020). Outras ORFs vêm sendo descritas em isolados de vírus do gênero *Mulcrilevirus*: a V5, codificada especificamente por *Mulcrilevirus mori*, é necessária para que infeção de *Nicotiana benthamiana* (Han et al. 2024). A V6 é uma ORF adicional, totalmente incorporada a V4, que regula positivamente a replicação do DNA, atuando em sinergia com a V4 (Han et al. 2025).

Como critério de demarcação de espécies dentro do gênero, utiliza-se 78% de identidade de sequência nucleotídica (Roumagnac et al. 2022 e ICTV 2025).

Os vírus do gênero *Mulcrilevirus* têm como vetor natural a cigarrinha (*Tautonera mori*) (Lu et al. 2021) e como hospedeira amoreiras, sendo descrito em: *Morus alba* - *Mulcrilevirus mori* (Lu et al. 2015; Ma et al. 2015) e amoreira-do-papel (*Broussonetia papyrifera*) - *Mulcrilevirus broussonetiae* (Qiu et al. 2020).

7. Família *Caulimoviridae*

A família *Caulimoviridae* possui 108 espécies distribuídas em 11 gêneros, sendo o gênero *Badnavirus* o maior gênero da família, com 74 espécies, seguido do gênero *Caulimovirus*, com 14 espécies (ICTV 2025). Os gêneros que compõem essa família são: *Badnavirus*, *Caulimovirus*, *Cavemovirus*, *Dioscovirus*, *Petuvirus*, *Rosadnavirus*, *Ruflodivirus*, *Solendovirus*, *Soymovirus*, *Tungrovirus* e *Vaccinivirus* (Krupovic et al. 2016 e ICTV 2025).

Alguns vírus dessa família são responsáveis por doenças de grande impacto econômico em culturas agrícolas das regiões tropicais e subtropicais. Elementos virais endógenos (EVEs; DNA viral integrado ao genoma nuclear do hospedeiro) são conhecidos para *Badnavirus*, *Caulimovirus*, *Cavemovirus*, Petuvirus e *Solendovirus* (Teycheney et al. 2020 e ICTV 2025).

Os membros da família *Caulimoviridae* não são envelopados e possuem uma única partícula de dsDNA circular não covalentemente fechados de aproximadamente 7,1–9,8 kbp (Bousalem et al. 2008 e Hohn & Rothnie 2013), além de possuir descontinuidades em regiões especificas da fita, tanto no sentido negativo (um), quanto no sentido positivo (um a três) (Teycheney et al. 2020).

A organização genômica varia de uma a oito ORFs, dependendo do gênero. As proteínas comuns a todos os gêneros são: proteína de movimento (MP), proteína do capsídeo (CP), proteína associada ao vírus, retropepsina (protease aspártica semelhante à pepsina) (AP) e transcriptase reversa (RT) com enzima RNAse H1 acoplada (Teycheney et al. 2020).

Os critérios de demarcação entre gêneros são baseados em: **a) morfologia do vírus** - os gêneros *Badnavirus* e *Tungrovirus* possuem formato de partícula baciliforme, enquanto os membros dos outros gêneros possuem partículas isométricas; **b) organização genômica** – os gêneros *Petuvirus* e *Vaccinivirus* possuem apenas uma ORF; os gêneros *Badnavirus*, *Dioscovirus* e *Tungrovirus* três ou quatro ORFs*;* os membros de outros gêneros de quatro a oito ORFs; **c) modo de transmissão e vetores**; e **d) hospedeiras** – monocotiledôneas (gênero *Tungrovirus*), dicotiledôneas para outros gêneros, exceto *Badnavirus*, que infectam mono e dicotiledônea (Teycheney et al. 2020).

A replicação dos vírus da família *Caulimoviridae* acontece de forma singular entre os vírus de plantas, envolvendo dsDNA e um intermediário de RNA retrotranscrito, caracterizando-os como pararetrovírus, porém, não requer obrigatoriamente a integração de seu genoma no DNA do hospedeiro como parte obrigatória de seu ciclo de replicação como acontece nos retrovírus (Hohon & Rothnie 2013). Apesar disso, existe evidência de que cepas ou vestígios deles podem estar integrados no genoma hospedeiro como elementos virais endógenos - EVEs (*Endogenous viral elements*) (Diop et al. 2018; Valli et al. 2023 & Liu et al. 2024). Após entrar na célula, um sinal de localização nuclear da extremidade N-terminal da proteína do capsídeo direciona o vírus ao núcleo. O DNA é associado a proteínas histonas, formando mini-cromossomos no núcleo e em seguida são transcritos pela RNA polimerase II dependente de DNA da célula hospedeira, gerando uma transcrição com redundância terminal de 35 a 270 nts, o RNA pré-genômico (pgRNA). O pgRNA atua como molde para a transcrição reversa da fita de DNA negativa e como mRNA policistrônico para a expressão de algumas ORFs (Pooggin & Ryabova 2018). A síntese da fita de DNA negativo é iniciada por um tRNA citosólico do hospedeiro, e ambas as fitas são sintetizadas pela transcriptase reversa viral e pela RNAse H1. As sequências polipurínicas resistentes à RNAse H1 servem como primer para sintetizar a fita de DNA positiva. As descontinuidades específicas ocorrem tanto na fita positiva quanto na negativa, sendo formadas devido ao deslocamento da fita existente, gerado pela nova fita (Hohn & Rothnie 2013).

8. Gênero *Badnavirus*

O gênero *Badnavirus* é o maior gênero da família *Caulimoviridae*, com 74 espécies descritas (ICTV 2025). O gênero foi estabelecido na década de 1990 para acomodar vírus de dsDNA transmitidos por cochonilhas (Lockhart 1990) e com formato diferente dos demais. O primeiro vírus classificado no gênero foi Commelina yellow mottle virus (CoYMV) em

Commelina diffusa, no Havaí (Medappa et al. 1985). Esse gênero difere dos demais gêneros da família, principalmente por seu formato baciliforme (Bhat et al. 2023).

As partículas de membros do gênero *Badnavirus* têm aproximadamente 30 nm x 120-150 nm e possui genoma de dsDNA circular de 7,2-9,2 kb, com descontinuidades em ambas as fitas, codificando no mínimo três ORFs (Geering & Hull 2012 e Bhat et al. 2016).

O genoma dos badnavírus possui uma sequência putativa do sítio de ligação do tRNA (TGGTATCAGAGCTTATAA), identificada dentro da região intergênica, assim como a caixa TATA (TATATAA). Membros de *Badnavirus* possuem três ORFs: ORF1/P1 – com função desconhecida; ORF2/P2 - codifica uma suposta proteína de ligação ao DNA e ORF3/P3 – poliproteína, que contém motivos-chave, incluindo aqueles envolvidos no capsídeo, movimento, protease aspártica, transcriptase reversa e RNAse H (Shahid et al. 2017). Estudos recentes mostraram que a ORF1/P1 do Citrus yellow mosaic Badnavirus (CBMV) apresentou uma atividade supressora de silenciamento de RNA que ainda não foi atribuída a nenhum ORF do CMBV (Vadlamudi et al. 2021). Alguns isolados possuem ORFs adicionais, como os que infectam cacau (*Theobroma cacao*), conhecidas como ORF4, ORFX e ORFY, cujas funções são desconhecidas até o momento (Ullah e Dunwell 2023).

O ICTV propõe como critérios para a demarcação das espécies dentro do gênero *Badnavirus*, os intervalos de hospedeiras, as especificações do vetor e uma identidade mínima de 80% na sequência nucleotídica da região que codifica a polimerase (RT+RNAse H) (ICTV 2025).

Os badnavírus infectam tanto monocotiledôneas quanto dicotiledôneas, e estão distribuídos em todo o mundo, causando doenças economicamente importantes em regiões tropicais e subtropicais (Bhat et al. 2016). Culturas como banana (*Musa* spp.) (Figueiredo et al. 2006), pimenta-do-reino (*Piper nigrum* L.) (Bhat et al. 2003), cítricos (*Citrus* sp.) (Venkataravanappa et al. 2024), cacau (*Theobroma cacao*) (Ameyaw et al. 2024), cana-de-açúcar (*Saccharum officinarum*) (Krishna et al. 2023) e até plantas daninhas (Teixeira et al. 2021) são afetadas por isolados de badnavírus. Por outro lado, muitas espécies do gênero têm uma gama de hospedeiros restrita e várias espécies infectam apenas uma única cultura (Bhat et al. 2023), como *Badnavirus etainflatheobromae* (= *Cacao swollen shoot virus*) que tem como hospedeiro natural o *Theobroma cacao* (Ameyaw et al. 2024), sugarcane bacilliform virus (SCBV) com o hospedeiro *Saccharum officinarum* (Krishna et al. 2023) e *Badnavirus mori* (= Mulberry badnavirus 1 – MBV-1) em amoreira (*Morus* spp) (Chiumenti et al. 2016). Há poucos

registros desse gênero infectando plantas daninhas, com uma predominância de relatos de infecção em *Commelina* spp. (família Commelinaceae) (King et al. 2011). Teixeira et al. (2021) foram os primeiros a relatar a ocorrência natural de infecção por uma espécie nova de *Badnavirus*, chamada de Centrosema bacilliform virus (CenBV), em uma planta daninha *Centosema brasilianum* (Fabaceae) no Brasil.

A disseminação primária, em larga escala, dos vírus do gênero *Badnavirus* ocorre através da propagação vegetativa, alguns vírus como *Badnavirus maculacommelinae* (= Commelina yellow mottle virus), *Badnavirus maculakalanchoes* (Kalanchoe top-spotting virus), *Badnavirus maculapiperis* (= Piper yellow mottle virus), *Badnavirus alphainflatheobromae* (= Cacao swollen shoot virus), e *Badnavirus alphacolocalasiae* (= Taro bacilliform virus) são transmitidos por sementes. A disseminação secundária, ou horizontal, ocorre por meio de várias espécies de cochonilhas e pulgões (Bhat et al. 2016).

9. Gênero *Caulimovirus*

O gênero *Caulimovirus* foi estabelecido pelo ICTV em 1978, com base nas características do primeiro vírus do gênero: cauliflower mosaic virus (CaMV), descoberto em 1933 na Alemanha, em couve-flor (*Brassica oleraceae* var. *botrytis*) (Shepherd 1933).

É o segundo maior gênero da família *Caulimoviridae*, com 14 espécies catalogadas (ICTV 2025). Os vírus desse gênero têm uma única molécula de dsDNA circular fechado não covalente, com tamanho de 7,8 a 8,2 kb. Suas fitas apresentam descontinuidades, a de sentido negativo uma única descontinuidade e a de sentido positivo, com duas ou três descontinuidades (Teycheney et al. 2020 e ICTV 2025).

Os genomas de membros do gênero *Caulimovirus* possuem de seis a sete ORFs que codificam: uma MP, um fator de transmissão de pulgões (Fator de transmissão por pulgões - ATF), uma proteína associada ao vírus, uma CP, uma protease + RT + RNAse H e uma proteína transativadora/viroplasmina (TAV), com duas ou quatro descontinuidades na fita (Teycheney et al. 2020).

Como critério para demarcação de espécies, o ICTV propõe que o intervalo de hospedeiras e a identidade nucleotídica mínima de 80% da região que codifica a polimerase (RT+RNAse H) determina as espécies do gênero (ICTV 2025).

Os principais vetores de espécies de *Caulimovirus* são os pulgões (família *Aphididae*), com o tipo de relação caracterizada como semipersistente (Hohn e Rothnie 2013). Uma proteína codificada pelo vírus (ATF/ ORF2), é necessária para que ocorra a transmissão (Uzest et al 2007). Segundo o ICTV (2025), todos os *Caulimovirus*, exceto *Caulimovirus venafragariae* (*Strawberry vein banding virus*) e *Caulimovirus deformatiolamii (Lamium leaf distortion virus*), são transmissíveis por inoculação mecânica, e não há relatos de transmissão via semente (ICTV 2025).

As plantas hospedeiras de membros do gênero *Caulimovirus* são restritas a dicotiledôneas, com relatos em cultivares como: figueira (*Caulimovirus tesselloscrophulariae = Figwort mosaic virus*) (Richins et al. 1987); couve-flor (*Caulimovirus tesselloscrophulariae = Cauliflower mosaic virus*) (Franck et al. 1980); morango (*Caulimovirus venafragariae = Strawberry vein banding virus*) (Petrzik et al. 1998); *Lamium maculatum* (*Caulimovirus deformatiolamii = Lamium leaf distortion virus*) (Zhang et al. 2008); *Metaplexis japônica* (*Caulimovirus metaplexis = Metaplexis yellow mottle-associated virus*) (Yang et al. 2021); *Pueraria montana* (*Caulimovirus puerariae = Pueraria virus* A) (Gudeta et al. 2022); algodão (*Cotton virus* A) (West-Ortiz et al. 2023; West-Ortiz et al. 2025); *Lilium lancifolium* (*Lancifolium caulimovirus* A e *Lancifolium caulimovirus* B) (Liang et al. 2025).

10. Família *Genomoviridae*

A família *Genomoviridae* possui 237 espécies distribuídas em 10 gêneros. Dentre eles, o gênero *Gemycircularvirus* é o maior gênero, com 126 espécies, seguido do *Gemykibivirus*, com 50 espécies e *Gemykolovirus* com 16 espécies (ICTV 2025). Os gêneros que compõem a família são: *Gemycircularvirus*, *Gemyduguivirus*, *Gemygorvirus*, *Gemykibivirus*, *Gemykolovirus*, *Gemykrogvirus*, *Gemykroznavirus*, *Gemytondvirus*, *Gemytripvirus* e *Gemyvongvirus* (Krupovic et al. 2012 e ICTV 2025).

Os vírus da família *Genomoviridae* possuem um genoma de ssDNA circular com tamanho aproximado de 1,8 – 2,4 kb e codificam duas ORFs: uma Rep e uma CP em orientação ambisense (Krupovic et al. 2016). Apesar da CP dos membros da família *Genomoviridae* não ser semelhante, em nível de sequência nucleotídica, a outros vírus conhecidos, a Rep é homóloga à de outros vírus ssDNA de seres eucarióticos e é mais próxima a dos vírus da família *Geminiviridae*, os quais compartilham vários motivos de sequências únicas e formam um grupo

irmão em análises filogenéticas (Kazlauskas et al. 2017; Kazlauskas et al. 2018 e Kazlauskas et al. 2019).

A identidade de 78% de nucleotídeos de todo o genoma é considerada para limite de demarcação de espécies dentro da família, e a filogenia de sequências da Rep foi utilizada para definir os gêneros (Varsani & Krupovic 2017).

Os vírus dessa família replicam-se através do mecanismo de replicação por círculo rolante (RCR), semelhante ao usado por plasmídeos bacterianos (Khan 1997; Chandler et al. 2013 & Ruiz-Maso et al. 2015). A RCR é iniciada pela Rep, clivando o dsDNA em uma sequência de nonanucleotídeo variável, presente em uma estrutura *stem–loop* de origem da replicação, o motivo nonanucleotídico de consenso é 'TRAKATTRC' (Varsani & Krupovic 2017). Logo, os gêneros classificados dentro da família *Genomoviridae* exibem assinaturas distintas no nonanucleotídeo, assim como motivos de nuclease e helicase conservados (Varsani & Krupovic 2017).

A grande maioria dos membros da família *Genomoviridae* foram descobertos por análises metagenômicas em amostras diversas, contudo, a extensão real de sua gama de hospedeiros permanece desconhecida (Varsani & Krupovic 2021).

11. Gênero *Gemycircularvirus*

O gênero *Gemycircularvirus* foi proposto por Rosario et al. (2012) ao detectar o vírus Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) em *Sclerotinia sclerotiorum*, um fungo patogênico que afeta plantas, nos Estados Unidos (Yu et al. 2010). O nome do gênero deriva de uma combinação de termos que refletem as características principais desses vírus: Gemini-like - semelhança com os geminivírus; Myco-associated - indica que infectam fungos; Circular virus - estrutura do genoma viral circular (Rosario et al. 2012 e Sikorski et al. 2013).

Maior gênero da família *Genomoviridae*, os *Gemycircularvirus* (126 espécies) (ICTV 2025) são caracterizados por genomas circulares de DNA de fita simples (CRESS-DNA), com aproximadamente 2,1 a 2,3 kb. Os vírus desse gênero possuem duas ORFs: uma codifica a CP, no sentido viral, e outra a Rep, no sentido complementar. A Rep possui domínios conservados cruciais para que ocorra a RCR e que também são encontrados nas Rep de outros vírus do gênero *Geminiviridae*. A origem da replicação tem uma estrutura de *stem–loop* onde se localiza

o nonanucleotídio conservado: TAATATTAT (Krupovic et al. 2016 e Varsani e Krupovic, 2017).

Os vírus pertencentes ao gênero *Gemycircularvirus* foram relatados em associação com uma grande variedade de organismos: *Sclerotinia sclerotiorum* (Yu et al. 2010); insetos (Dayaram et al. 2012; Rosario et al. 2012 e Li et al. 2015); plasma humano (Uch et al. 2015); ratos (Li et al. 2015b); águas de esgoto (da Silva Assis et al. 2016); líquido cefalorraquidiano de humanos (Anh et al. 2021); morcegos (Bollati et al. 2022 e Couto et al. 2024) e lêmures (Paietta et al. 2024).

Isolados de *Gemycircularvirus* também foram identificados em plantas, incluindo espécies como: mandioca (*Manihot esculenta*) (Dayaram et al. 2012); soja (*Glycine max*) (Dayaram et al. 2012; Male et al. 2015; Chiumenti et al. 2019 e Chabi-Jesus et al. 2020); *Hypericum japonicum* (Hypericaceae) (Du et al. 2014); em Poaceae (Male et al. 2015); gramínea selvagem (*Brachiaria deflexa*) e cana-de-açúcar (*Saccharum officinarum*) (Male et al. 2015); feijão (*Phaseolus vulgaris*) (Lamas et al. 2016); *Momordica charantia* (de Rezende et al. 2018); oliveiras (Olea europaea) (Chiumenti et al. 2019); cítricos (Chabi-Jesus et al. 2020); tomate (*Solanum lycopersicum*) (Reis et al. 2022) e arbusto ardente (*Euonymus alatus*) (Paudel 2023).

Para a demarcação de espécies dentro do gênero, foi adotado o critério de 78% de identidade pareada do genoma (Varsani & Krupovic 2017).

12. Gênero *Gemykolovirus*

O gênero *Gemykolovirus* foi proposto em 2016 por Krupovic et al. (2016), baseados nos vírus *Pteropus-associated gemykolovirus 1* e *Pteropus-associated gemykolovirus 2* que infectam morcegos frugívoros (*Pteropus*), coletados no Tonga (Male et al. 2016).

Possui apenas 16 espécies catalogadas (ICTV 2025) e constitui um grupo irmão do gênero *Gemycircularvirus,* em análises filogenéticas baseadas no genoma completo (Varsani & Krupovic 2017).

Isolados de *Gemykolovirus* são caracterizados por terem genomas circulares de ssDNA, com aproximadamente 2,2 kb, e por codificar duas proteínas, a Rep e a CP, em sentidos ambissenso, separadas por duas regiões intergênicas que contém sinais de poliadenilação bidirecionais (Varsani & Krupovic 2017).

Para a demarcação de espécies dentro do gênero, foi adotado o critério de identidade pareada de 78% em todo o genoma (Varsani & Krupovic 2017).

Isolados de *Gemykolovirus* foram relatados em organismos como carrapato (*Ixodes scapularis*) (Rosário et al. 2018), aranha (*Segestria pacifica*) (Rosário et al. 2018) e tartaruga do deserto de Sonora (*Gopherus agassizii*) (Orton et al. 2020). Isolados de três espécies foram descritos em associação com plantas: *Gemykolovirus citas1* (Citrus Tunisia genomovirus 1), em *Citrus sinensis* (Chabi-Jesus et al. 2020); *Gemykolovirus poaspe1* (Plant associated genomovirus 9) em *Avena byzantina* (Fontelene et al. 2020b); Tecoma stans-associated gemykolovirus em *Tecoma stans* (Nery et al. 2023).

13. Vírus em plantas daninhas e plantas associadas ao cultivo de tomateiro

Plantas daninhas constituem importantes e contínuos reservatórios de fontes de vírus (Ambrozevicius et al. 2002; García-Arenal & Zerbini 2019; Wang et al. 2021; Galbács et al. 2024). Neste trabalho utilizou-se a definição de plantas daninhas com base em dois artigos distintos. No primeiro deles, plantas daninhas são plantas que ocorrem em locais indesejados no sistema agrícola, que influenciam a composição vegetal e competem por recursos hídricos, luz, nutrientes e gás carbônico (Cruz et al. 2022). O segundo, inclui que as propriedades rústicas e inerentes das plantas daninhas inferem em sua boa adaptação, como dormência de sementes e germinação em tempos distintos (Lorenzi 2014).

Sabe-se que plantas daninhas têm sido relatadas como hospedeiras de vírus, incluindo begomovirus em diversos países, inclusive no Brasil (Ambrozevicius et al. 2002). Alguns exemplos de hospedeiras de begomovírus em distintas espécies/famílias botânicas são: *Triumfetta semitriloba* (família Sterculiaceae) (Assunção et al. 2006); *Cleome affinis* (família Capparaceae) (Assunção et al. 2006); *Macroptillium lathyroides* (L.), *Desmodium* sp. e *Macroptilium erytroloma* (família Fabaceae) (Assunção et al. 2006 e Batista et al. 2022); *Blainvillea rhomboidea* (família Asteraceae) (Castillo-Urquiza et al. 2008); *Amaranthus spinosus* (família Amaranthaceae) (Barbosa et al. 2011a); *Crotalaria* spp. *Euphorbia heterophylla* (família Euphorbiaceae) (Barreto et al. 2013; Santos et al. 2003*); Croton bonplandianum* (família Euphorbiaceae) (Pramesh et al. 2013); *Sida rombifolia* (Família Malvaceae) (Maurício-Castillo et al. 2014; Rodríguez-Negrete et al. 2019); *Datura stramonium*, *Nicotiana glauca*, *Nicotiana plumbanginifolia*, *Solanum elaegnifolium*, *Solanum rostrarum* e *Solanum verbascifolium* (família Solanaceae) (Rodríguez-Negrete et al. 2019);

Digitaria ciliares, Eleusine indica, Panicum dichotomiflorum, Setaria faberi e *Echinochloa crus-galli* (família Poaceae) (Kil et al. 2021); *Pyrenacantha* sp. (família Icacinaceae) (Chipiringo et al. 2022), *Oxalis latifolia* (família Oxalidaceae) (Pereira-Silva et al. 2022), *Macroptilium* spp. (família Fabaceae) (Melo et al. 2025), *Croton bonplandianus* Baill (família Euphorbiaceae) (Dhasan et al. 2025) e *Acalypha indica* (família Fabaceae) (Kumar et al. 2025). Estes são alguns exemplos que ilustram o papel de plantas daninhas como reservatório viral.

Interessante observar que existem vários relatos de vírus provenientes de plantas daninhas sendo transmitidos e infectando espécies cultivadas. Alguns exemplos são *Begomovirus bauri* (=*Abutilon mosaic virus*) e o *Begomovirus euphorbiamusivi* (= *Euphorbia mosaic virus*) (proveniente de *Euphorbia prunifolia*), os isolados destas espécies foram transmitidos para o tomateiro por *B. tabaci* (Costa e Carvalho 1960). Ademais, Cotrin et al. (2004) confirmaram infecção por *Begomovirus sidavariati* (= *Sida mottle virus*) em cerca de 10% das amostras de tomateiro com sintomas de mosaico, e Callegario et al. (2004) atestaram infecção natural do tomateiro por *Begomovirus sidamicranthae* (= *Sida micrantha mosaic virus*), além da detecção de *Begomovirus sidaflavaneti* (= *Sida yellow net virus*) e *Begomovirus sidavulgaris* (= *Sida common mosaic virus*), identificados por Duarte et al. (2021). García-Arenal e Zerbini (2019) também constataram a transmissão de ToSRV entre plantas de *Sida* spp. e tomate (*Solanum lycopersicon*). Recentemente, Reis et al. (2025) analisaram filogeneticamente o vírus CleLCrV proveniente de tomateiro, esse isolado se agrupou com isolados de CleLCrV que tinham como hospedeiro original planta daninha (*Cleome affinis*), corroborando para o fato de que eventos de salto de hospedeiro de begomovírus de plantas daninhas para tomateiros ocorrem naturalmente.

Ambrozevicius et al. (2002) já havia destacado a importância das plantas daninhas como reservatório de begomovírus. Além disso, estas hospedeiras podem promover a ocorrência de infecções mistas, promovendo a interação entre diferentes espécies e/ou isolados (Syller 2012; Reis et al. 2020), além disso a detecção de novos vírus da família *Geminiviridae* em diferentes hospedeiras tem sido cada vez mais frequente (Claverie et al. 2018).

Assim, embora haja pesquisas nesse campo, é crucial realizar estudos adicionais sobre o papel das plantas daninhas como reservatórios de vírus.

A seguir serão apresentados begomovírus já relatados em plantas daninhas associadas às famílias Fabaceae e Lamiaceae. Estas duas famílias foram escolhidas para exemplificar a variedade de begomovírus em plantas daninhas, bem como ilustrar um mapa representando a

localidade dos vírus relatados (**Figura 5)**. Em seguida, as demais famílias foram agrupadas em um levantamento complementar, visando ampliar a abrangência da análise.

13.1 *Begomovirus* em plantas daninhas da família Fabaceae

A família Fabaceae possui distribuição cosmopolita e representa uma das maiores famílias de Angiospermas, incluindo cerca de 650 gêneros e 19 mil espécies. No Brasil temos a ocorrência de 200 gêneros e 2.800 espécies, sendo a maior família em número de espécies no país (Souza & Lorenzi 2019). Várias espécies cultivadas e usadas para alimentação encontram-se classificadas em Fabaceae.

A seguir serão apresentados vírus relatados em plantas daninhas da família Fabaceae.

Kitajima (2020) realizou uma compilação de vírus ocorrendo em diferentes plantas no Brasil no período de 1926 a 2018. Ao todo, durante estes 92 anos, 213 vírus e seis viróides foram relatados em um total de 345 plantas. Deste total a família Fabaceae apresentou o maior número de relatos de ocorrências de vírus, mostrando a grande suscetibilidade das espécies de Fabaceae.

Segundo o banco de dados *Genbank* (2025), Kitajima (2020), *Host DataBase* (2025) e pesquisas em periódicos científicos, o número de begomovírus relatados infectando plantas daninhas da família Fabaceae é de 73, distribuídos em todo o mundo, sendo que, destes, **11** foram relatados no Brasil (**Figura 6**). Os gêneros com maior número de begomovírus relatados são: *Macroptillium*, *Rhynchosia*, *Cyamopsis* e *Desmodium*. Os gêneros *Albizia*, *Cassia*, *Cyamopsis*, *Medicago*, *Mimosa*, *Mucuna*, *Puerparia*, *Rhynchosia*, *Senna*, *Sesbania* e *Trogonella* não tiveram relatos de infecção por begomovírus, até o momento, no Brasil.

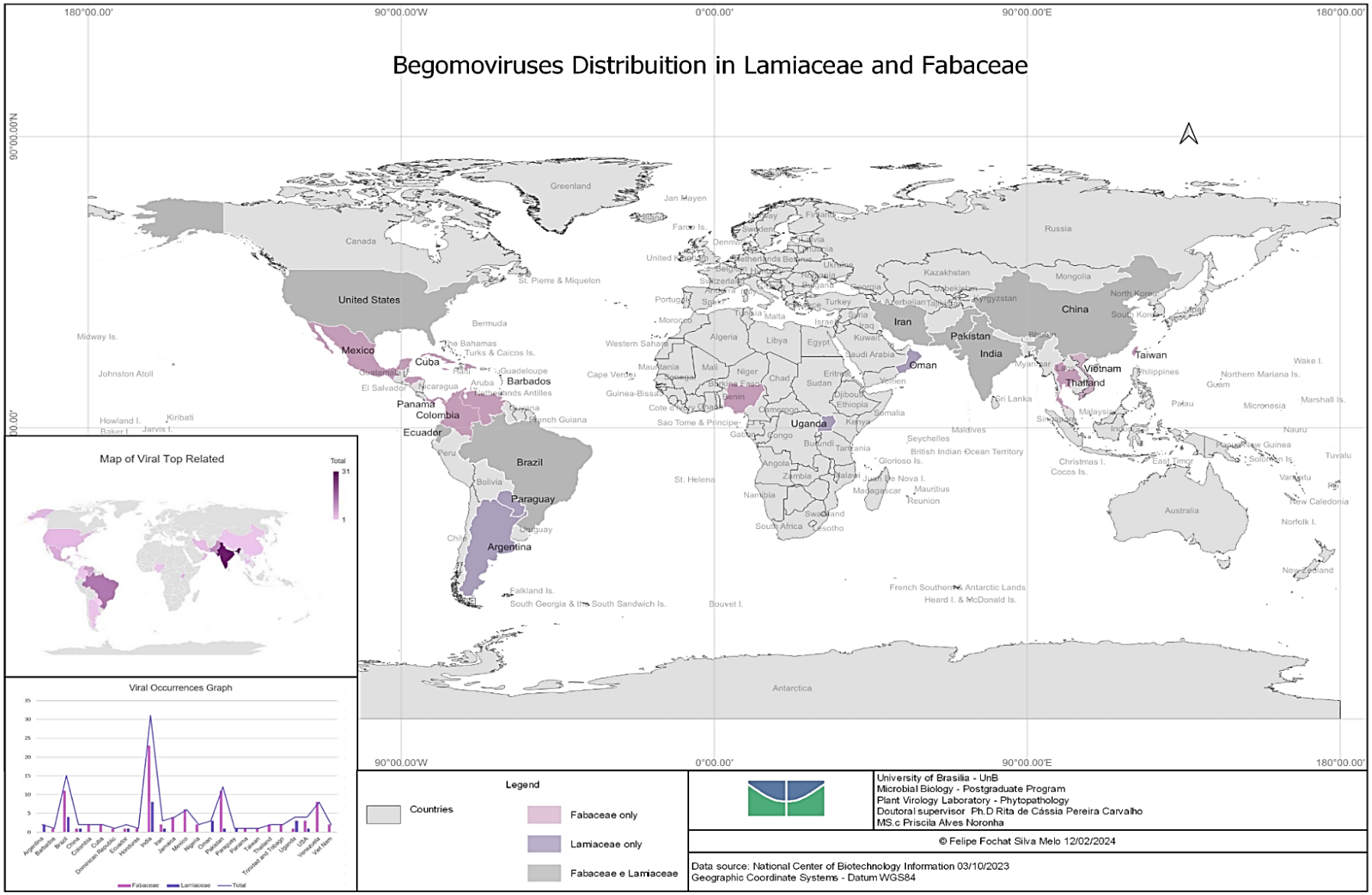

Figura 5. Mapa de distribuição de espécies virais classificadas no gênero *Begomovirus*, relatadas em hospedeiras de plantas daninhas das famílias botânicas Fabaceae e Lamiaceae, segundo o banco de dados do *GenBank* (2025), Kitajima (2020) e *Host DataBase* (2025).

Descrição do mapa:

Vírus relatados em Fabaceae

Barbados
Bean mosaic crinkle Barbados virus

Brasil
Bean golden mosaic virus
Euphorbia yellow mosaic virus
Macroptilium bright mosaic virus
Macroptilium bright yellow interveinal virus
Macroptilium common mosaic virus
Macroptilium yellow net virus
Macroptilium yellow spot virus
Macroptilium yellow vein virus
Soybean chlorotic spot virus
Tomato crinkle leaf yellows virus
Tomato severe rugose virus

China
Kudzu mosaic virus

Colômbia
Passionfruit leaf distortion virus
Rhynchosia golden mosaic Colombia virus

México
Rhynchosia golden mosaic Yucatan virus
Desmodium leaf distortion virus
Bean latent virus
Nigéria
African cassava mosaic virus
East African cassava mosaic Cameroon virus
Rhynchosia golden mosaic Sinaloa virus
Rhynchosia golden mosaic virus
Tobacco apical stunt virus

Cuba
Rhynchosia golden mosaic Havana virus
Rhynchosia rugose golden mosaic virus

República Dominicana
Macroptilium golden yellow mosaic virus

Equador
Cabbage leaf curl 60írus

Honduras
Rhynchosia golden mosaic virus

Índia
Ageratum enation virus
Cotton leaf curl Kokhran virus
Croton yellow vein mosaic virus
Cyamopsis tetragonoloba leaf curl Sikar virus
Cyamopsis tetragonoloba leaf curl virus
Dolichos yellow mosaic virus
Fenugreek yellow vein virus
Horsegram yellow mosaic virus
Mimosa yellow vein virus
Mungbean yellow mosaic India virus
Mungbean yellow mosaic India virus
Mungbean yellow mosaic virus
Panamá
Potato yellow mosaic Panama virus

Taiwan
Velvet bean golden mosaic virus
Papaya leaf curl virus
Radish leaf curl virus
Rhynchosia yellow mosaic India virus
Senna leaf curl virus
Squash leaf curl China virus
Tomato leaf curl Kerala virus
Tomato leaf curl New Delhi virus
Tomato leaf curl Palampur virus
Tomato leaf curl Patna virus
Tomato leaf curl virus
Velvet bean severe mosaic virus

Irã
Tomato yellow leaf curl virus
Watermelon chlorotic stunt virus

Jamaica
Macroptilium yellow mosaic virus
Macroptilium golden mosaic virus
Rhynchosia golden mosaic Yucatan virus
Tobacco yellow crinkle virus

USA
Macroptilium mosaic virus
Rhynchosia mild mosaic virus
Rhynchosia mosaic virus

Venezuela
Desmodium mosaic virus
Desmodium yellow spot virus
Macroptilium mottle virus
Bean leaf crumple virus
Cabbage leaf curl virus
Rhynchosia mottle virus
Cabbage leaf curl virus

Paquiestão
Mungbean yellow mosaic virus
Okra enation leaf curl virus
Papaya leaf curl virus
Pedilanthus leaf curl virus
Pedilanthus leaf curl virus
Pedilanthus leaf curl virus
Pedilanthus leaf curl virus
Rhynchosia yellow mosaic virus
Tomato leaf curl New Delhi virus
Tomato yellow leaf curl virus

Tailândia
Foetid cassia leaf curl virus-[Thailand]
Tomato yellow leaf curl Thailand virus

Trinidade e Tobago
Calopogonium mucunoides begomovirus – Trinidad
Rhynchosia Trinidad virus

Uganda
Desmodium mottle virus

Vietnã
Kudzu mosaic virus
Mimosa yellow leaf curl virus

Vírus relatados em Lamiaceae

Argentina
Sida mosaic Bolivia virus 2
Tomato yellow spot virus

Brasil
Euphorbia yellow mosaic virus
Leonurus mosaic virus
Tomato yellow mosaic virus
Tomato yellow spot virus

China
Tomato yellow leaf curl virus

Equador
Hyptis golden mosaic virus

Índia
Chilli leaf curl virus
Dolichos yellow mosaic virus
Mungbean yellow mosaic virus
Ocimum leaf curl virus
Papaya leaf curl virus
Tomato leaf curl New Delhi virus
Tomato leaf curl virus

Irã
Tomato yellow leaf curl virus

Oman
Chilli leaf curl virus
Tomato leaf curl Oman virus
Tomato yellow leaf curl virus

Paquistão
Chilli leaf curl India virus

Paraguai
Leonurus mosaic virus

Uganda
Ocimum golden mosaic virus
Ocimum mosaic virus
Ocimum yellow vein virus

USA
Clerodendrum golden mosaic China virus

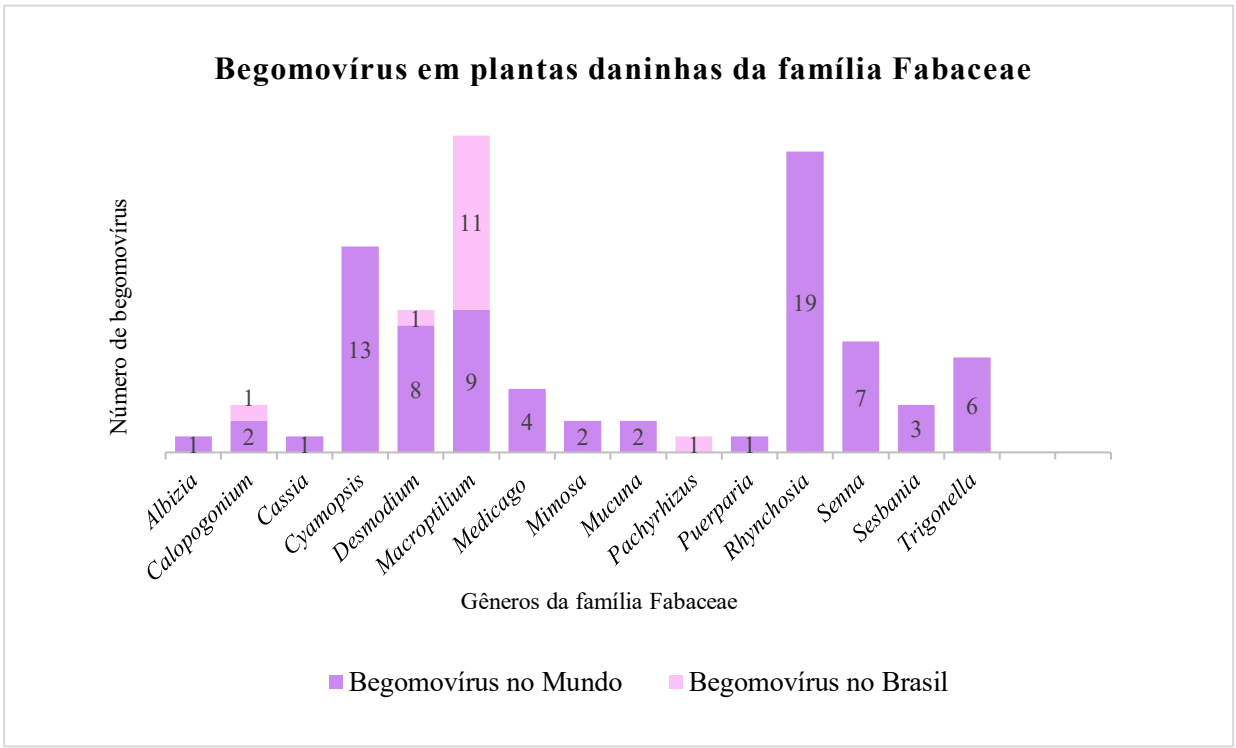

Figura 6. Número de begomovírus relatados em plantas daninhas classificadas em gêneros da família Fabaceae com base em pesquisas realizadas em periódicos nacionais e internacionais e banco de dados do *GenBank* (2025), Kitajima (2020) e *Host DataBase* (2025).

O gênero *Pachyrhizus* é o único relatado como infectado exclusivamente no Brasil pelo tomato severe rugose virus (ToSRV) (Silva et al. 2022)

O gênero *Macroptillium* foi o que apresentou o maior número de relatos, sendo encontradas 19 espécies de begomovírus, com dez ocorrendo no Brasil:

1. Bean golden mosaic virus – BGMV em *Macroptillium lathyroides* [acesso: MN822293] (Xavier et al. 2021);

2. Euphorbia yellow mosaic virus – EuYMV em *Macroptillium atropurpureum* [acesso: JN419000] (Silva et al. 2012);

3. Macroptilium mosaic virus – MacBMV em *Macroptillium lathyroides* [acesso: NC_031452] (Passos et al. 2016);

4. Macroptilium yellow interveinal virus – MaBYIV em *Macroptilium erythroloma* [acesso: MN146017] (Batista et al. 2021);

5. Macroptilium common mosaic virus – MacCMV em *Macroptillium lathyroides* [acesso: NC_031448] (Passos et al. 2016);

6. Macroptilium yellow net virus – MaYNV em *Macroptillium lathyroides* [acesso: NC_017001] (Silva et al. 2012);

7. Macroptilium yellow spot virus – MacYSV em *Macroptillium lathyroides* [acesso: MT627033] (Xavier et al. 2021);

8. Macroptilium yellow vein virus – MacYVV em *Macroptillium lathyroides* [acesso: KJ939898] (Sobrinho et al. 2014);

9. Soybean chlorotic spot virus – SoCSV em *Macroptillium lathyroides* [acesso: KJ939916] (Sobrinho et al. 2014) e

10. Tomato crinkle leaf yellows virus – TCrLYV em *Macroptilium atropurpureum* [acesso: JN419010] (Silva et al. 2012).

Tanto no gênero *Desmodium* quanto no gênero *Calopogonium*, no Brasil, os relatos correspondem somente ao vírus Macroptilium yellow spot virus – MacYSV, em *Desmodium glabrum* [acesso: KT779558] (Fontenele et al. 2016) e *Calopogonium mucunoides* [acesso: JN419015] (Silva et al. 2012) respectivamente.

13.2 *Begomovirus* em gêneros de plantas daninhas da família Lamiaceae

A família Lamiaceae possui distribuição cosmopolita e inclui cerca de 300 gêneros e 7.500 espécies. Destes, 38 gêneros ocorrem no Brasil, totalizando cerca de 500 espécies (Souza e Lorenzi 2019). Essa família abriga uma variedade de espécies, principalmente de cunho econômico na indústria alimentícia e farmacêutica (Karpinski 2020; Lorenzi e Matos 2008). Além disso abriga o gênero *Leonurus* que possui espécies daninhas como o rubim (*Leonurus japonicus*) e o cordão-de-frade (*Leonurus nepetifolia*) (Souza e Lorenzi 2019).

Ao todo, 20 begomovírus foram relatados em seis gêneros de plantas daninhas da família Lamiaceae (**Figura 7**). Destes, apenas quatro possuem relatos no Brasil, estando presentes nos gêneros *Leonurus* e *Leucas*, segundo o banco de dados *Genbank* (2025), Kitajima (2020) e *Host DataBase* (2025).

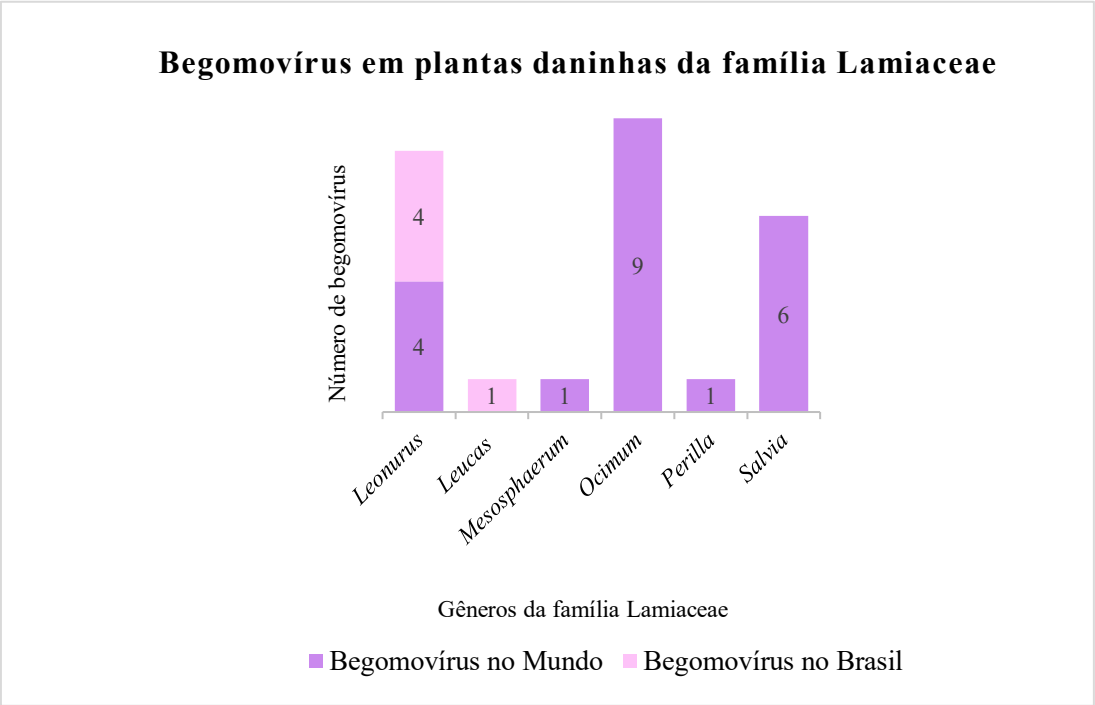

Figura 7. Número de begomovírus relatados em plantas daninhas classificadas em gêneros da família Lamiaceae com base em pesquisas realizadas em periódicos nacionais e internacionais e no banco de dados do *GenBank* (2025), Kitajima (2020) e *Host DataBase* (2025).

Associados ao gênero *Leonurus* seis espécies de begomovírus foram relatadas até o momento:

1. Dolichos yellow mosaic virus – DoYMV em *Leonurus cardiaca* [acesso: ON421038] (Akram et al. 2023);

2. Euphorbia yellow mosaic virus – EuYMV em *Leonurus sibiricus* [acesso: KX348182] (Ferro et al. 2017);

3. Leonurus mosaic virus – LeMV em *Leonurus sibiricus* [acesso: JX863081] (Boiteux et al. 2013);

4. Mungbean yellow mosaic virus – MYMV em *Leonurus cardiaca* [acesso: ON421019] (Akram et al. 2023);

5. Tomato yellow mosaic virus – ToYMV em *Leonurus sibiricus* [acesso: JX178670] (Carminatti et al. 2013) e

6. Tomato yellow spot virus – ToYSV em *Leonurus sibiricus* [acesso: JX513952] (Bornancini et al. 2019).

Associados ao gênero *Leucas*, há o relato de apenas um vírus: tomato yellow spot virus–ToYSV em *Leucas martinicensis* [acesso: JX178667] (Carminatti et al. 2013).

13.3 *Begomovirus* em outras plantas daninhas de distintas famílias botânicas

Nesta seção, apresentaremos no texto o número de begomovírus relatados em plantas daninhas pertencentes às famílias botânicas que serão foco do presente estudo (além de Fabaceae e Lamiaceae anteriormente descritas): Asteraceae, Bignoniaceae, Brassicaceae, Cactaceae, Capparaceae, Cleomaceae, Cucurbitaceae, Malvaceae, Moraceae, Poaceae e Solanaceae de acordo com o levantamento feito no banco de dados *GenBank* (2025), Kitajima (2020) e *Host DataBase* (2025) (**Figura 8**).

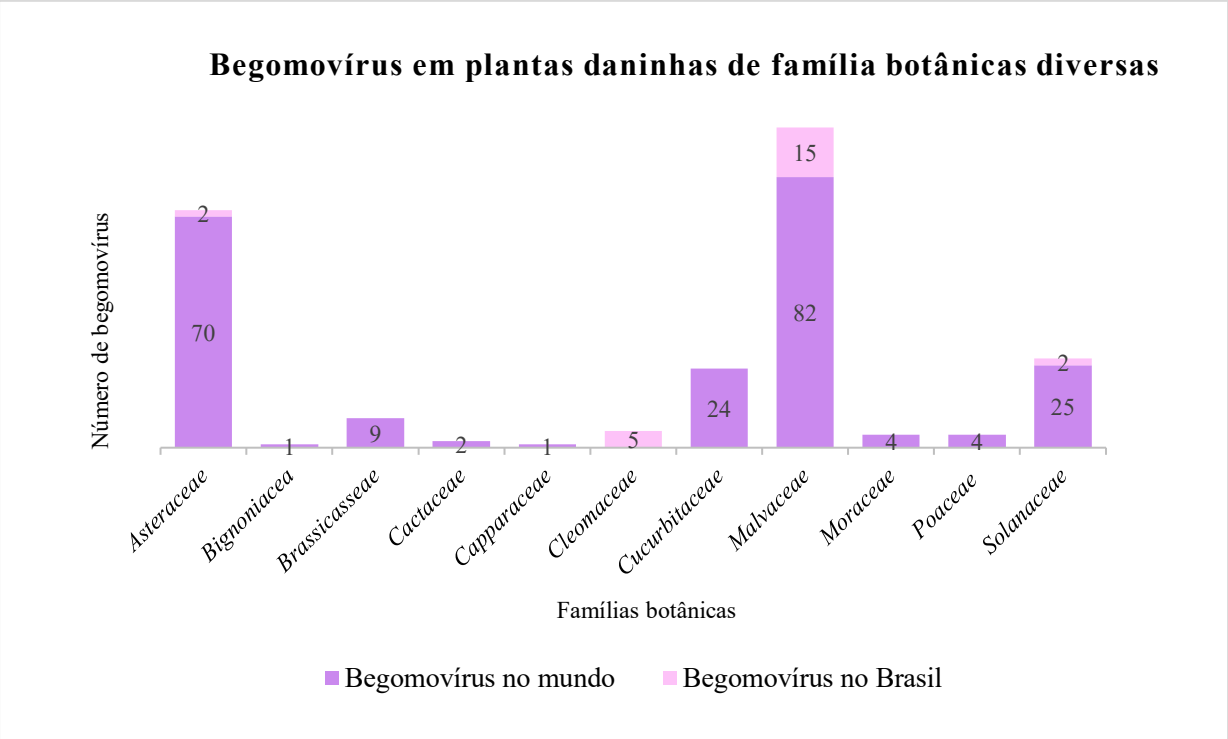

Figura 8. Número de begomovírus relatados em plantas daninhas classificadas nas famílias botânicas Asteraceae, Bignoniaceae, Brassicaceae, Cactaceae, Capparaceae, Cleomaceae, Cucurbitaceae, Malvaceae, Moraceae, Poaceae e Solanaceae, com base em pesquisas realizadas em periódicos nacionais e internacionais e no banco de dados do *GenBank* (2025), Kitajima (2020) e *Host DataBase* (2025).

Tanto Bignoniaceae, quanto Brassicaceae, Cactaceae, Capparaceae, Cucurbitaceae, Moraceae e Poaceae, não possuem begomovírus relatados, até o momento, no Brasil. Em contrapartida, a família Cleomaceae apresentou cinco relatos exclusivamente no Brasil:

1. Blainvillea yellow spot virus – BlYSV em *Cleome affinis* [acesso: JF694467] (Wyant et al. 2012);
2. Cleome bright yellow mosaic virus – CBYMV em *Cleome affinis* [acesso: DQ388447] (Boiteux et al. 2006);
3. Cleome golden mosaic virus – CIGMV em *Cleome* [acesso: NC_015397] (Fonseca et al. 2011);
4. Cleome leaf crumple virus – CleLCV em *Cleome* [acesso: MF072686] (Fontenele et al. 2017);
5. Cleome yellow mosaic virus – CIYMV em *Cleome* [acesso: HM357463] (Fonseca et al. 2010).

Na família Bignoniaceae, até o momento, o único vírus relatado é o *Tecoma stans leaf curl virus*, identificado em *Tecoma stans*, na Índia. Observa-se situação semelhando na família Capparaceae, onde o *Ageratum enation virus* foi identificado em *Gynandropsis gynandra*, também na Índia.

Em Malvaceae, 14 vírus foram relatados exclusivamente no Brasil:

1. Abutilon Brazil virus – AbBV em *Abutilon* [acesso: NC_014138] (Paprotka et al. 2010);
2. Abutilon mosaic Brazil virus – AbMV em *Sida rhombifolia* [acesso: NC_016574] (Wyant et al. 2012);
3. Corchorus mottle virus – CoMoV em *Corchorus hirtus* [acesso: JQ805780] (Fontenele et al. 2013);
4. Pavonia mosaic virus – PaMV em *Pavonia* [acesso: NC_040185] (Pinto et al. 2018);
5. Pavonia yellow mosaic virus – PaYMV em *Pavonia* [acesso: NC_037611] (Pinto et al. 2018);
6. Sida angular mosaic virus – SiAMV em *Sida acuta* [acesso: NC_031456] (Passos et al. 2016);
7. Sida chlorotic vein virus – SiCVV em *Sida urens* [acesso: NC_031454] (Passos et al. 2016);

8. Sida common mosaic viurs – SiCmMV em *Sida rhombifolia* [acesso: NC_038457] (Castillo-Urquiza et al. 2018);

9. Sida mottle Alagoas virus – SiMoAV em *Sida urens* [aceso: NC_020256] (Tavares et al. 2013);

10. Sida yellow blotch virus – SiYBV em *Sida urens* [acesso: NC_020254] (Tavares et al. 2013);

11. Sida yellow mosaic Alagoas virus – SiYMAV em *Sida urens* [acesso: C_020255] (Tavares et al. 2013);

12. Sidastrum golden leaf sport virus – SiGLSV em *Sidastrum* [acesso: NC_038462] (Fonseca et al. 2018);

13. Tomato mild mosaic virus – ToMiMV em *Sida urens* [acesso: KC706606] (Rocha et al. 2013);

14. Triumfetta yellow mosaic virus – em *Triumfetta* [acesso: 040188] (Nascimento et al. 2016);

Em Solanaceae existem apenas 2 relatos de begomovírus exclusivamente no Brasil:

1. Bean golden mosaic virus – BGMV em *Nicandra physalodes* [acesso: MT114400] (Reis et al. 2021);

2. Tomato severe rugose virus – ToSRV em *Nicandra physalodes* [acesso: JX415188] (Barreto et al. 2013);

Dado a importância dos relatos de infecções causadas por begomovírus nas famílias acima relacionadas, é fundamental o estudo desses vírus, identificando novidades referentes a potenciais espécies novas e hospedeiras.

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

Desde os primeiros usos de *Hight-Throughput Sequencing* (HTS) para descoberta de vírus em plantas (Kreuze et al. 2009; Al Rwahnih et al. 2009; Adams et al. 2009), sua aplicação na identificação de vírus e caracterização de viromas em diferentes espécies de plantas tem experimentado um avanço significativo (Maina et al. 2024; Jaksa-Czotter et al. 2024; González-Pérez et al. 2024; Pacheco-Dorantes et al. 2025;Mansour et al. 2025). O uso dessa tecnologia também revelou a presença de infecções virais mistas, destacando a complexidade dos viromas de plantas em ecossistemas agrícolas (Pacheco-Dorantes et al. 2025).

As tecnologias HTS foram introduzidas em meados de 2005 e desde então, diversas plataformas foram utilizadas. A plataforma 454 Life Sciences foi descontinuada pela Roche em 2016, sendo substituída por plataformas mais novas e competitivas, porém, foi a base para as atuais tecnologias HTS baseadas em PCR. Em 2006, a plataforma SOLiD da Applied Biosystems foi comercializada, contudo, foi descontinuada em 2016. Atualmente, somente as plataformas *Illumina* e *Ion Torrent* estão disponíveis na categoria de HTS. A *Illumina* oferece nove sequenciadores diferentes, produzindo de 4 milhões a 20 bilhões de leituras curtas, de 50 – 300 nts (Maliogka et al. 2018).

Segundo Varsani et al. (2017), o sequenciamento de alto desempenho, acompanhado da técnica de *Rolling Circle Amplification* (RCA), aceleraram a descoberta de vírus divergentes dentro da família *Geminiviridae*, gerando-se muitas sequências genômicas completas, permitindo a análise de identidades pareadas e filogenética.

Assim, o HTS tem sido utilizados em diversos estudos relacionados a viromas. A exemplo: em 2019, o mulberry crinivirus (MuCV) foi identificado através do HTS em uma amoreira com sintomas de mosaico, manchas e deformação das folhas (Zhang et al. 2023); em 2020, para identificar a diversidade viral em tomateiro e a diversidade de begomovírus e agentes subvirais que o infectam, Reis et al. (2020) utilizou essa técnica e identificou um gemycircularvírus, um novo alfasatélite e duas espécies novas de begomovírus em tomateiro. Recentemente, Fajardo et al. (2023) aplicaram a técnica para analisar os vírus que infectam videiras, reduzindo sua qualidade e rendimento, e identificaram a presença de quatro vírus com identidades genéticas de isolados previamente caracterizados. Ademais, Ferreira (2023) analisou a diversidade do complexo Sida micranta mosaic virus (SiMMV) e caracterizou três novas espécies de begomovírus em plantas daninhas da família Malvaceae utilizando a técnica de HTS como precursora. Lima (2023) além de recuperar um novo vírus com identidade de 85,87% com tomato bright yellow mottle virus – ToBYMV, o detectou em uma nova espécie hospedeira (*Bolusafra bituminosa,* família Fabaceae).

Em suma, os estudos mencionados destacam a relevância e eficácia da técnica de HTS na virologia vegetal contemporânea. Ao desvendar a complexidade do metagenoma viral em uma variedade de espécies vegetais, o HTS não apenas amplia nosso entendimento sobre a diversidade viral, mas também abre novas perspectivas para o desenvolvimento de estratégias de manejo e controle de doenças virais em cultivos agrícolas e ecossistemas naturais.

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CHAPTER 2

A metagenomics-based catalog of single-stranded DNA viruses in weeds and native flora associated with tomato fields in Neotropical areas

Work for submission to the Viruses

A metagenomics-based catalog of single-stranded DNA viruses in weeds and native flora associated with tomato fields in Neotropical areas.

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Abstract – Begomoviruses (*Geminiviridae* family) are single-stranded DNA (ssDNA) viruses transmitted by members of the *Bemisia tabaci* complex. Tomatoes (*Solanum lycopersicum*) and a wide array of associated weeds and native plants are major hosts of begomoviruses as well as other geminiviruses in Neotropical areas. Herein, High-Throughput Sequencing (HTS) was employed as a tool to provide an extensive catalog of ssDNA viruses in weeds and other native species naturally associated with tomato fields. The Illumina NovaSeq 6000 platform was used to characterize a pool of 114 leaf samples from 15 botanical families that were collected displaying begomovirus-like symptoms. The analyses of the sequencing data revealed 103 virus-like sequences with 99 of them representing complete genomes and four partial genomes. These sequences were distributed into five viral and subviral families: *Geminiviridae*, *Caulimoviridae*, *Genomoviridae*, *Alphasatellitidae*, and *Circoviridae*. The *Geminiviridae* was the most representative family with three genera (*Citlodavirus*, *Mulcrilevirus* and *Begomovirus*). Seven known begomoviruses and five putative new species (designated as new species #1 to #5) were recovered from this pool. A new species from the genus *Citlodavirus* was also recovered. This astonishing diversity adds even greater complexity in terms of managing ssDNA virus-induced diseases in the Neotropics.

Keywords: Asteraceae, Bignoniaceae, Brassicaceae, Cactaceae, Capparaceae, Cleomaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moraceae, Poaceae, Ruscaceae, Solanaceae.

1. Introduction

Plant-associated single-stranded DNA (ssDNA) viruses constitute a highly diverse group of pathogens that are of significant agricultural importance (Varsani et al. 2017; Fontenele et al. 2020; Lima 2023; Oliveira et al. 2024), belongin to families *Geminiviridae* (Varsani et al. 2017), *Caulimoviridae* (Varsani et al. 2017; Teycheney et al. 2020) and *Genomoviridae* (Varsani et al. 2017; Varsani e Krupovic 2017; Varsani e Krupovic 2021; Reis et al. 2022; Nery et al. 2023). The ssDNA viruses exhibit distinct genomic organization and ecological relationships with both primary and alternative hosts (e.g., weeds and native plants) as well as with their vectors. These biological features play a central role in their epidemiological dynamics in economically important host crops, such as tomato – *Solanum lycopersicum* (Ambrozevicius et al. 2002; Duarte et al. 2021a; Duarte et al. 2021b; Shahriari et al. 2023; Jo et al. 2023; Oliveira et al. 2024; González-Pérez et al. 2024).

The *Geminiviridae* is the largest among plant-associated families, comprising 15 genera (ICTV 2025). These genera are classified according to their host range, insect vectors, genome organization, and genomic pairwise identities (Brown et al. 2015; Zerbini et al. 2017; Rougmanac et al. 2022). Geminiviruses have icosahedral, twin particles, and circular genomic DNA (Zerbini et al. 2017; Mietzsch et al. 2025). This family is widely distributed; associated both monocot and dicot plants (Varsani et al. 2017; Bejerman and Debat 2023). The genus *Begomovirus* is the most numerous in the *Geminiviridae*, comprising more than 463 species (ICTV 2025). Begomoviruses can be either monopartite (only with DNA–A) or bipartite species (with DNA–A and DNA–B components) (Fiallo-Olivé et al. 2021). Begomoviruses are transmitted in nature by the *Bemisia tabaci* species complex (De Barro et al. 2011; Rosen et al. 2015; Fernandes et al. 2023). Monopartite begomoviruses encode six Open Reading Frames (ORFs). Two ORFs display a viral direction: V1/CP (coat protein) and V2 (only found in monopartite and bipartite begomoviruses of the "Old World"; movement protein). The ORFs C1/Rep (replication initiator protein), C2/TrAP (transcription activator protein), C3/Ren (replication enhancer protein), and C4 (symptom determinant) are in the complementary direction. The viral movement in plant tissues is coordinated by V1/CP, V2, and C4 in monopartite species (Rojas et al. 2001; Zerbini et al. 2017; Kulshreshtha et al. 2019; Kumar and Dasgupta 2023; Li et al. 2024). Bipartite genomes share little nucleotide identity between the two components (DNA–A and DNA–B), except for a sequence of ~200 nucleotides, known as the common region (CR). In the CR we find the nonanucleotide (TAATATTAC) and repeated sequences (iterons) (Briddon et al. 2010; Arguello-Astorga and Ruiz-Medrano, 2001).

Additional ORFs are currently being identified in a wide array of begomovirus genomes. The ORF AC5/C5 was found in the DNA–A of some species, being responsible for determining pathogenicity and acting as a potent suppressor of gene silencing (Li et al. 2015; Zhao et al. 2023). The ORF V3 was detected in Malvastrum yellow vein Honghe virus (MaYVHhV), but its function is yet elusive (Wang et al. 2021). The ORF C6 was identified in tomato leaf curl China virus (ToLCCNV) and is now detected in ~36% of begomoviruses (Wang et al. 2022). The ORF C7 acts as a pathogenicity factor and suppressor of RNA gene silencing (Liu et al. 2023).

The *Caulimoviridae* family encompasses 108 species distributed in 11 genera. *Badnavirus* is the largest genus (comprising 74 species), followed by *Caulimovirus* with 14 species (ICTV 2025). Viruses in the *Caulimoviridae* family display non-covalently closed dsDNA genomes, with sizes between 7.1 and 9.8 kbp, which is one of the peculiar features of this group of pathogens (Bousalem et al. 2008; Hohn and Rothnie 2013). In addition, these viruses replicate via reverse transcription, being classified as plant pararetroviruses (Teycheney et al. 2020). The genome of these viruses encodes one to eight ORFs, depending on the genus, including the movement protein (MP), capsid protein (CP), multipurpose virion-associated protein (VAP), retropepsin (pepsin-like aspartic protease) (AP), and reverse transcriptase (RT) with coupled RNAse H1 enzyme (Teycheney et al. 2020). Members of this family form isometric or bacilliform particles, depending on the genus, and are transmitted by insect vectors, such as aphids (usually in a semi-persistent manner) as occurs with *Caulimovirus tessellobrassicae* (= Cauliflower mosaic virus – CMV) (Teycheney et al. 2020).

The *Genomoviridae* family comprises 237 species distributed in ten genera. The *Gemycircularvirus* (with 126 species) is the largest genus in the family, followed by *Gemykibivirus* with 50 and *Gemykolovirus* with 16 species (ICTV 2025). This family displays circular ssDNA genomes of approximately 1.8–2.4 kb. These viruses are phylogenetically related to geminiviruses, but they do not encode movement proteins (Krupovic et al. 2016). Their compact genomes carry only two essential ORFs: a Rep and a CP (in ambisense orientation). These viruses have been described typically in association with fungi, although they have also been detected in various environmental plant samples. However, the true range of potential hosts for these viruses remains yet unknown (Varsani and Krupovic 2021).

Weeds and native flora may represent important viral reservoirs, allowing the survival, diversification, and spread of ssDNA viruses (García-Arenal and Zerbini 2019). Over the years, several studies are reporting weed-associated viruses associated tomatoes (*Solanum lycopersicum*) and other crop species (Costa and Carvalho 1960; Duarte et al. 2021; García-

Arenal and Zerbini 2019; Reis et al. 2020; Oliveira et al. 2024). In this sense, High-Throughput Sequencing (HTS) has emerged as a superior tool for large-scale identification and characterization of viral pathogens naturally associated in weeds and crop plants (Fajardo et al. 2023; Kawsaki et al. 2023; Maliogka et al. 2018; Villamor et al. 2019). Among the different HTS technologies, the Illumina platform stands out for its high data production generated per sequencing cycle, high precision, minimum accuracy of 75%, diversity of applications and library preparation configurations, highlighting the NovaSeq-6000 sequencer (Kumar et al. 2019; Hu et al. 2021).

In this context, the major objective of the present study was to perform metagenomic analyses using HTS-based approach to characterize the virome of species classified as either weeds or native flora often found in association with the tomato crop in Neotropical areas. To this end, a set of 114 foliar samples displaying typical begomovirus symptoms were collected from plants of 15 botanical families across five macro-regions of Brazil and Paraguay between 2004 and 2022.

2. Material and Methods

2.1 Leaf samples of weeds, native flora, and companion crops present within and around tomato fields – Leaf samples exhibiting characteristic begomovirus-like symptoms (apical and interveinal chlorosis, yellowing, mosaic, golden mosaic, severe mosaic, leaf deformation, epinasty, rugosity, and dwarfism) were collected between 2004 and 2022 in ten Brazilian States, the Federal District, and Paraguay. Therefore, the present survey comprised 114 individual samples distributed among 15 botanical families collected in areas across all five macro-geographic regions of Brazil and Paraguay (**Table 1** and **Table 2**). The leaves were swept away to eliminate any possible insects. The modified 2X CTAB + organic solvents protocol was adopted for the total DNA extraction (Boiteux et al. 1999). The purified DNA of each sample was stored in a freezer at -20°C, comprising the Embrapa and UnB viral collection. For botanic identification of each sample, the total DNA obtained from each host plant was used as a template in PCR assays with specific primers targeting the barcoding genes rubisco, maturase K (Fazekas et al. 2012) and, when necessary, the ITS2 region (Yao et al. 2010).

Table 1. Total number of foliar samples (organized by botanical family) that were collected in five macro-geographical Brazilian regions and in Paraguay displaying begomovirus-like symptoms.

Botanical families	Number of samples	Brazilian regions					
		North	Northeast	Midwest	South	Southeast	Paraguay
Asteraceae	04	---	---	04	---	---	---
Bignoniaceae	01	---	---	01	---	---	---
Brassicaceae	09	---	01	06	02	---	---
Cactaceae	04	---	01	03	---	---	---
Capparaceae	04	03	01	---	---	---	---
Caricaceae	01	---	---	01	---	---	---
Cleomaceae	01	---	---	---	---	---	01
Cucurbitaceae	01	---	---	01	---	---	---
Fabaceae	45	05	13	17	06	04	---
Lamiaceae	36	---	---	02	32	---	02
Malvaceae	03	---	---	03	---	---	---
Moraceae	01	---	---	01	---	---	---
Poaceae	02	---	---	02	---	---	---
Ruscaceae	01	---	---	01	---	---	---
Solanaceae	01	---	---	01	---	---	---
Total: 15	**114**	**08**	**16**	**43**	**40**	**04**	**03**

2.2. Enrichment via Rolling Circle Amplification (RCA) of circular DNA molecules and confirmation of viral infection – To enrich the samples, part of the DNA obtained from each sample was used for viral DNA amplification by rolling circle amplification (RCA) (Inoue-Nagata et al. 2004) with the Illustra templiphi DNA amplification kit (GE healthcare) and phi-polymerase according to Reis et al. (2020). Subsequently, the RCA of each sample was combined in the pool.

Figure 1. Plant samples with begomovirus symptoms. **A**. Mosaic in guanxuma (*Sida rhombifoʟia*, family Malvaceae). **B.** Symptoms of Curl and yellowing in Malva (*Malva sylvestris*, family Malvaceae). **C.** Leaf deformation and yellowing in papaya (*Carica papaya*, family Caricaceae) and **D.** Leaf deformation, curl and chlorosis in okra (*Solanum gilo*, family Solanaceae). Photos: Felipe Fochat Silva Melo.

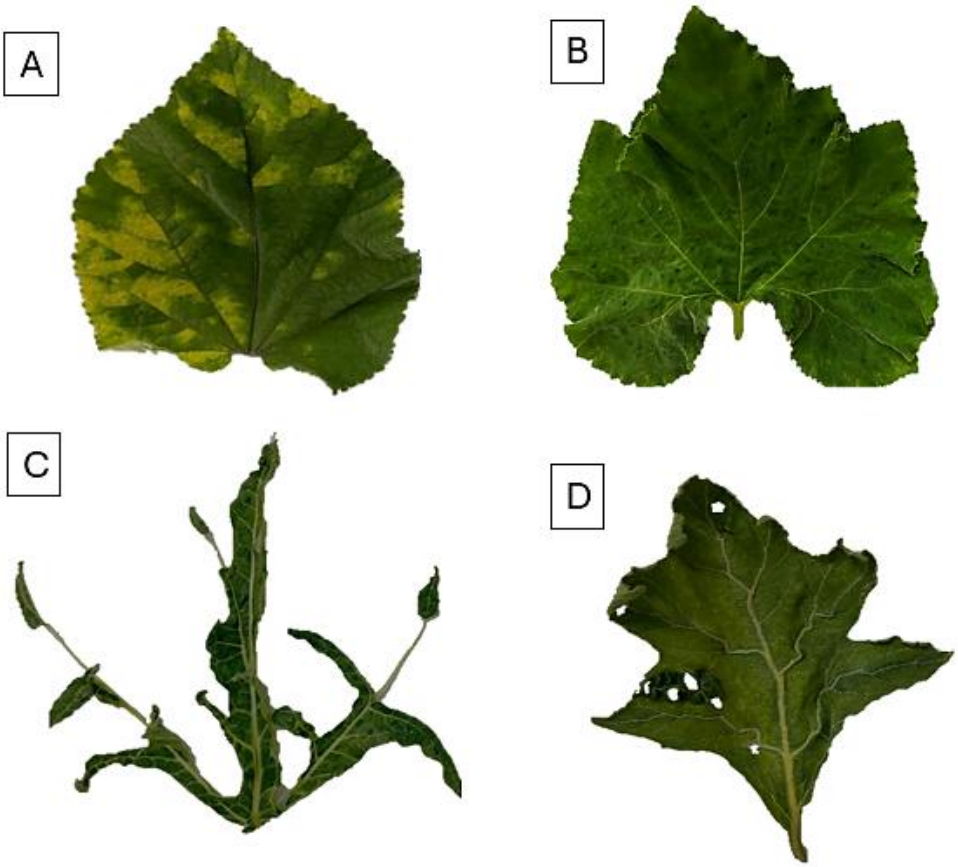

Table 2. Information on family, year of collection and code of the 114 leaf samples (isolates) collected in the five Brazil regions and in Paraguay, belonging to weed cultivars, used in the present study, which were collected exhibiting symptoms similar to those induced by begomoviruses.

Botanical family	Host Genus/species	Year and place of isolate collection	Code
Asteraceae	*Cichorium*	2022 – Brasília–DF	RDF-822, RDF-823, RDF-826, and RDF-827.
Bignoniaceae	*Handroanthus*	2022 – Brasília–DF (EEB)	RDF-817.
Brassicaceae	*Brassica*	2008 – Gama–DF (CNPH)	DF-245.
	Brassica	2008 – Reserva–PR	PR-083
	Brassica	2011 – Irecê–BA	BA-152.
	Eruca	2015 – Mauá da Serra–PR	PR-145.
	Brassica	2022 – Gama–DF (CNPH)	DF-804, RDF-820, RDF-821, RDF-824, and RDF-825.
Cactaceae	*Pilosocereus*	2007 – Irecê–BA	BA-022.
	Pereskia	2022 – Gama–DF (CNPH)	RDF-778, RDF-805, and RDF-808
Capparaceae	-	2005 – Palmas–TO	TO-027.
	-	2007 – Silves–AM	AM-008.
	-	2010 – Palmas–TO	TO-316.
	-	2013 – Praia do Forte–BA	BA-172.
Caricaceae	*Carica*	2022 – Urutaí–GO	RGO-839.
Cleomaceae	*Cleome*	2010 – Mayor Otaño (Paraguay)	PAR-005.
Cucurbitaceae	*Cucurbita*	2022 – Urutaí–GO	RGO-836.
Fabaceae	-	2004 – Gama–DF (CNPH)	DF-134.
	Senna	2005 – Guaraciaba do Norte–CE	CE-022.
	Arachis	2005 – Núcleo Rural São José–DF	DF-174.
	Phaseolus	2005 – Matinha–TO	TO-011.
	-	2007 – Rondonópolis–MT	MT-001.
	Crotalaria	2007 – Recife–PE	PE-007.
	Phaseolus	2007 – Comancin de São Félix–PE	PE-008.
	Phaseolus	2007 – Marilândia–PR	PR-024, and PR-030.
	Phaseolus	2007 – Barro Preto–PR	PR-043, PR-044, PR-046, and PR-047.
	Vigna	2007 – Guaraí–TO	TO-070.
	Phaseolus	2008 – Gama–DF (CNPH)	DF-258.
	Pterogyne	2008 – Gama–DF (CNPH)	DF-260.
	Glycine	2008 – Gama–DF (CNPH)	DF-263.
	Senna	2008 – UNITINS.	TO-118.
	Vigna	2008 – UNITINS.	TO-120.
	Phaseolus	2008 – Aragominas–TO	TO-210.

Fabaceae	*Phaseolus*	2009 – Venda Nova do Imigrante-ES	ES-006.
	Senna	2010 – Ibiapina-CE	CE-040.
	Phaseolus	2010 – Gama-DF (CNPH)	DF-361.
	Samanea tubulosa	2010 – Cuiabá-MT	MT-012.
	Phaseolus	2011 – Jaguaquara-BA	BA-076 and BA-077.
	Corchorus	2011 – Maracanã-BA	BA-080.
	Phaseolus	2011 – Comancin de S. Félix-PE	PE-008.
	Phaseolus	2011 – Capão Bonito-SP	SP-097.
	Mucuna	2012 – Gama-DF (CNPH)	DF-482.
	Phaseolus	2012 – Araguari.	MG-350 and MG-351.
	Crotalaria	2016 – Gama-DF (CNPH)	DF-629.
	Trifolium	2016 – Gama-DF (CNPH)	DF-660.
	Vigna	2017 – Petrolina-PE	PE-148.
	Cannavalia	2022 – São Miguel da Barra-AL	RAL-757, RAL-758, RAL-761 and RAL-762
	Bauhinia	2022 – Brasília-DF (Jardim IB)	RDF-767.
	Plathymenia	2022 – Brasília-DF (EAB)	RDF-768.
	Anadenanthera	2022 – Brasília-DF (Noroeste)	RDF-772 and RDF-773.
	Tamarindus	2022 – Brasília-DF	RDF-787.
	Desmodium	2022 – Brasília-DF	RDF-810.
Lamiaceae	*Leonurus*	2007 – São Gerônimo da Serra-PR	PR-011, PR-012, PR-014, and PR-015.
	Leonurus	2007 – Marilândia-PR	PR-028, PR-033, and PR-049.
	Leonurus	2007 – Barro Branco-PR	PR-034 and PR-035.
	Leonurus	2008 – São Gerônimo da Serra-PR	PR-087 and PR-088.
	Leonurus	2010 – Mauá da Serra-PR	PR-116 and PR-117.
	Leonurus	2011 – Gama-DF (CNPH)	DF-459 and DF-460.
	Leonurus	2011 – Mayor Otaño (Paraguay)	PAR-007 & PAR-008.
	Leonurus	2011 – Cruzmaltina-PR	PR-119, PR-120, PR-121, PR-122, PR-123, PR-124, PR-125, PR-126, PR-127, and PR-128.
	Leonurus	2012 – Capitão Leonidas Marques	PR-132, PR-133, PR-134, PR-135, and PR-136.
	Leonurus	2013 – São Sebastião do Caí-RS	RS-080, RS-081, and RS-082.
	Leonurus	2015 – Mauá da Serra-PR	PR-141.
Malvaceae	*Malva*	2022 – Urutaí-GO	RGO-832 and RGO-835.
	Abutilon theoprasti		RGO-834
Moraceae	*Morus alba*	2022 – Gama-DF (CNPH)	RDF-831.
Poaceae	*Digitaria*	2022 – Urutaí-GO	RGO-833.
	Saccharum	2022 – Urutaí-GO	RGO-838.
Ruscaceae	-	2015 – Brasília-DF (EAB)	RDF-814.
Solanaceae	*Solanum*	2022 – Urutaí-GO	RGO-837.

2.3. Preparation of the pool of samples and High-Throughput sequencing (HTS) – All 114 samples were grouped into a single pool were subjected to HTS sequencing on the Illumina platform at Agrega (Porto Alegre, Rio Grande do Sul, Brazil) using the Novaseq-6000 system (**Tables 1 and 2**).

2.4. Sequence analysis of the high-throughput sequencing (HTS) output – The reads generated during sequencing were analyzed according to the following methodological flow: **(1)** elimination of low-quality reads; **(2)** sequence reassembly using the CLC Genomics Workbench 23.0.3 program; and **(3)** contig validation using the BLASTn algorithm against the GenBank ssDNA virus database (https://www.ncbi.nlm.nih.gov/). Subsequently, contigs of potential viruses were mapped following previously described methodology (Nery et al. 2020; Reis 2020) with the aim of obtaining the entire contigs of the ssDNA virus-like genomes. These contigs were extended using the 'Map to reference' tool (available in Geneious® 11.0.5 software) with a parameter of 90 to 99% minimum overlap identity, which allowed mapping reads obtained via HTS that relate to each of the analyzed contigs (Kearse et al. 2012). After contig assembly, they were analyzed against the RefSeq viral database (NCBI) under very high stringency conditions (minimum match percentage = 98%) using the BLASTn algorithm. ORF annotation was performed based upon the GenBank reference genome, to which the contigs were subjected to the Muscle alignment option available in the 'Pairwise/Multiple align' tool, in Geneious® 11.0.5. Using the '*De Novo* Assemble' tool in Geneious® 11.0.5, the pooled contigs were subjected to taxonomic prediction analyses using the Kaiju web server (https://kaiju.binf.ku.dk/server), with default classification parameters (Tran and Phan 2020). These analyses are able to identify sequences of viral origin. The largest sequences were selected, assembled, and subsequently aligned to reference genomes displaying highest identities using the Muscle alignment option of the 'Pairwise/Multiple align' tool, present in Geneious® 11.0.5 software for ORF annotation. This same software was used to assemble the viral genome, annotate, and align the sequences. To characterize potential new species, the intergenic region (present in monopartite viruses) and the common region (present in bipartite viruses) were also analyzed. In the common region, nonanucleotide and interon motifs (repetitive units) were characterized as well as the REP-IRD (Rep Iteron–Related Domains), aiming to confirm if the detected DNA–A and DNA–B components are cognate (Arguello-Astorga & Ruiz-Medrano, 2001; Arguello-Astorga et al. 2004). To compare the isolates and viral species, the sequences were subjected to pairwise MUSCLE multiple alignment, using the SDT program (Muhire et al. 2014; Muhiere et al. 2025).

2.5. Design of species-specific primers for new species – Based upon the contig assembly results, PCR analyses were performed to detected the set of viruses identified after HTS in individual survey samples as previously described (Reis et al. 2020). A subset of viral species-specific primers designed in the present study were used in conjunction with the set of species-specific primers used by Reis et al. (2020) and by Batista (2020). The sequences of these primers as well as their corresponding PCR conditions are described in **Table 3.** Viral species identification—observed in sequence analyses obtained by HTS on individual samples—was confirmed using species-specific primers (for DNA–A and DNA–B genomic segments) designed in opposite and overlapping directions (forward and reverse). These primers were designed based on contigs assembled using the Geneious® 11.0.5 software, using the primer design function (Kearse et al. 2012). Virus specificity was verified *in silico* using the Primer-BLAST tool and in preliminary PCR assays using DNA samples from the reference collection of viral isolates identified by HTS and previously characterized (Reis 2020) as templates.

2.6. Use of species-specific primers for PCR detection of begomoviruses in individual samples – Aliquots of the pool or isolates previously characterized by Reis et al. (2020) and Batista (2020) were employed as positive controls. Detection was carried out in individual samples with the species-specific primers after initial detection using sample distribution a line versus column strategy (Oliveira et al. 2024). The total PCR mix (12.5 µL) was composed of 1.25 µL of 10X *Taq* Polymerase Buffer (100 mM Tris-HCl, pH 8.3 µL and 500 mM KCl, Invitrogen), 0.40 µL of $MgCl_2$ (50 mM, Invitrogen), 0.25 µL dNTPs (2.5 mM, Invitrogen), 0.25 µL of each primer (10 µM), 0.1 µL of *Taq* DNA Polymerase (5U/µL, Invitrogen), 8 µL of MilliQ water, and 2 µL of DNA (diluted in MilliQ water 1:10). PCR assays were performed from RCA products as templates and consisted of an initial denaturation step (94°C for 2 minutes, and then 35 cycles of 94°C for 30 seconds), followed by an annealing step for 55 seconds at a specific temperature for each primer (see Table 3,); and a final extension step (72°C for 1 minute). The PCR amplicons were visualized on a 1% agarose gel stained with ethidium bromide.

Table 3. Table of primers used in PCR assays, designed based on viral consensus sequences derived from High-Throughput Sequencing (HTS) for detection of begomoviruses and other genera, detailed by primer name, sequence and specific annealing temperature for each primer.

Viral species and genomic component	Primer name	(Foward) 5'-3'/ (Reverse) 3'- 5'	Annealing temperature °C
1. *Bean golden mosaic virus* DNA–A [1]	BGMV-For	GTGCGTGAATCCATGACCGT	55
	BGMV-Rev	ATTCACGCACAGGGGAACG	
2. *Cleome leaf crumple virus* DNA–A [1]	CILCrV-A-For	GACTCGACGTTCTGTGGT	51
	CILCrV-A-Rev	TCCTAGTCGGGGCTCACT	
3. *Cleome leaf crumple virus* DNA–B[1]	CILCrV-B-For	TAGGAAAGCAAAACGAGAATGGAA	58
	CILCrV-B-Rev	GCTTTCCTAAATCGCAATTGATC	
4. *Euphorbia yellow mosaic virus* DNA–A [1]	EuYMV-A-R-For	GGGGTTCCAAGTCCAATAAAGATGA	52
	EuYMV-A-R-Rev	CAGACACCTTATATTTGCCGGATTC	
5. *Euphorbia yellow mosaic virus* DNA–B[1]	EuYMV–B–RFor	GCCGAGGATAGAGGACACCAA	60
	EuYMV–B–RRev	CCAGGCCCAAACGCATTATATTTTATC	
6. *Tomato yellow spot virus* DNA–A [1]	F1A-ToY-For	ACGAAATCTTTTAGGAGCTAATGG	53
	F1A-ToY-Rev	CGTATTTCTGCAAAAAACTACTTCCT	
7. *Tomato yellow spot virus* DNA–B[1]	F3B-ToY-For	AATAAGGCGAAAGGTTAAAAGAATATGGCG	61
	R1A-ToY-Rev	GCCTTATTCACTTCACCTTCTTCGATTCAC	
8. *Mulberry crinkle leaf virus – Mulcrilevirus* [2]	F1 Mulcri- For	GGGAAGTGTGAGTCGATTGAGAGAAGG	53
	R1 Mulcri- Rev	ACACTTCCCACTCCGCTCCAGA	
9. *Cleome leaf crumple virus associated DNA 1- Clecrusatellite* [2]	F1 CLAp- For	GAACTGTAGCACAAATTCAACTAAAT	56
	R1 CLAp- Rev	CTACAGTTCCGGTTAATAGCACATATG	
10. *Passion fruit chlorotic mottle virus -Citlodavirus* [2]	F1C1998- For	TTTTGAGGAAGGAAAGGATGTATTC	58
	R1C1998- Rev	CCTCAAAAATAAACTCCAAGAATACGG	
11. *Banana streak CA virus (1) – Badnavirus*[2]	2BanaF – For	GCCATAATAACTCAGAAAGAA	
	BanaR - Rev	AACTCACGATTCTTCCTTTCCGA	
12. *Sugarcane bacilliform virus (1) - Badnavirus*[2]	2SugarF – For	AAGGTTACACTCAATGCAATCT	52
	SugarR - Rev	GCTCACGCTTCTGACTTTCCT	
13. *Plant associated genomovirus 7 – Gemykolovirus*[2]	GemykF – For	GCTCTTCGAATATCTCTTCCG	56
	Gemyk – Rev	GGTGACTGTTCGGACCATTC	
	MomoF – For	CCCACCCGAAAAAGCTCTTACG	

14. *Momordica charantia associated gemycircularvirus*	MomoR - Rev	GGGGTGAGGGATTTTCGGGT	56
15. *Cauliflower mosaic virus – Caulimovirus*[2]	FCauli – For	AGACGATCTACCCGAGCAATAAT	56
	RCauli - Rev	TGGTGATTAAAGGGAGATATACC	
16. *Espécie nova **#1** – Begomovirus*[2]	PC218F – For	TCTCAAACTTGCGATATGTATTGGAG	56
	PC218R – Rev	GTACGCAAAGGTCCTCAATG	
17. *Espécie nova **#2** – Begomovirus*[2]	PC08F – For	AATTGCGTCTTTTAAGCCTAGA	56
	PC08R – Rev	ATTAGCTCATGAAACCCCAG	
18. *Espécie nova **#3** – Begomovirus*[2]	PC64F – For	TTCTTCGAAATCCTGTGTTGCTGT	56
	PC64R – Rev	GGGACCACAAAAGCAGGAGAAA	
19. *Espécie nova **#4** – Begomovirus*[2]	PC329F – For	TTGAACATACACTTTACTTTTGC	56
	PC329R – Rev	TACTTATCCACAATGTACTCTTA	
20. *Espécie nova **#5** – Begomovirus*[2]	PC493F – For	GGTTAGCCGATCAGTAAACTTTTCC	56
	PC493R – Rev	AACTTCAACACCTACAAACACCG	
21. *Banana streak CA virus (2) – Badnavirus*[2]	BanaF – For	GAGTTAATCTTGCAGGTTATCGAAG	
	2BanaR - Rev	ATGGCATTAAGTAACTCATTCT	
22. *Sugarcane bacilliform virus (2) - Badnavirus*[2]	SugarF – For	AAACCCTCCAGAAGATTATTCATAC	
	2SugarR - Rev	GTGTAACCTTCCTCTCTTTG	

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

3. Results

3.1. Viral diversity present in the pool of samples – The HTS sequencing, conducted on the Illumina Novaseq-6000 platform, provided the following raw pool reads: 19,575,404 reads, 62,444 contigs with 103 of them corresponding to viruses as indicated by BLASTn analysis. The genomic information derived from HTS allowed the recovery of 27 viral genomes out of these 103 contigs distributed in four families: *Geminiviridae* (85 contigs), *Caulimoviridae* (13 contigs), *Genomoviridae* (3 contigs), *Circoviridae* (1 contig) and one *Alphasatellite* (Alphasatellitidae), four of which were partial genomes (**Tables 4, 5, and 6**).

The genus *Begomovirus* displayed the largest number of contigs (83), with 38 of them corresponding to DNA–A and to 45 DNA–B genomes. Three previously described bipartite *Begomovirus* species were recovered with both DNA–A and DNA–B components, namely: *Begomovirus costai* (*Bean golden mosaic virus*), *Begomovirus cleomecrispi* (*Cleome leaf crumple virus*) and *Begomovirus euphorbiamusiviflavi* (*Euphorbia yellow mosaic virus*) (**Tables 4 and 5**). Only the DNA–A component was recovered from the following previously characterized bipartite species: *Begomovirus leonuri* (*Leonurus mosaic virus*) (**Table 4**). On the other hand, only the DNA–B component was recovered from four known bipartite species: *Begomovirus macroptilimaculae* (Macroptilium yellow spot virus – MacYSV), *Begomovirus sidamicranthae* (*Sida micrantha mosaic virus*), *Begomovirus solanumpallidi* (*tomato chlorotic leaf curl virus* – ToCLCV) and *Begomovirus solanumflavusmaculae* (*tomato yellow spot virus* – ToYSV) (**Table 5**). The Euphorbia yellow mosaic virus (EuYMV) presented the highest read coverage in both DNA–A (contig 2827 - 2,466,136) and DNA–B (contig 1235 - 4,341,426). ToYSV was the virus with the highest number of isolates (*n*=30), followed by LeMV (*n*=19).

According to the taxonomic demarcation parameters defined for the genus *Begomovirus*, viruses that present identity of less than 91% are recognized as new species (Brown et al. 2015). In this context, five putative new *Begomovirus* species were recovered: New species #1 C493 (NS#1) (87% identity to BGMV, accession KJ939776.1); New species #2 C218 (NS#2) (88% identity to CleLCrV, accession FN435999.1); New species #3 C64 (NS#3) (83% identity to LeMV, accession JX863082.1); New species #4 C08 (NS#4) (81% identity to tomato leaf distortion virus – ToLDV, accession KC706605.1) and New species #5 C329 (NS#5) (85% identity with *Begomovirus solanumseverugosi* (tomato severe rugose virus – ToSRV, accession MW602388.1).

Other viral genera were also recovered in the *Geminiviridae* family (**Table 6**), contig #93 resembled to *Mulcrilevirus mori* (*Mulberry crinkle leaf virus*; genus *Mulcrilevirus*), with 92.22% identity and 94% coverage (accession MN240483.1). Contig #1998 represents a putative new species in the genus *Citlodavirus*, here designated New species #6 C1998 (NS#6), with 72.5% identity to *Citlodavirus passiflorae* (*Passion fruit chlorotic mottle virus*; accession NC_040706.1) and 88% coverage. Therefore, the contig has a nucleotide identity of less than 78%, constituting a new species according to the demarcation criteria established for the genus (Roumagnac et al. 2022).

Thirteen (13) contigs were recovered in the *Caulimoviridae* family. Eleven (11) contigs were identified within the genus *Badnavirus* with four of them (C803, C676, C3461, and C8279) displayed identity levels between 91% and 97% (100% coverage) to *Banana streak* CA virus–BSCAV (accessions NC_015506.1 and MW086553.1). In addition, seven contigs (C4093, C5930, C8303, C8789, C599, C12292, and C600) resembled to isolates of *Sugarcane bacilliform virus* with identities ranging from 90% to 94% and coverage of 99% (accession KT186240.1). In the genus *Caulimovirus*, contig #189 showed 98.14% identity (100% coverage) to *Caulimovirus tessellobrassicae* (*Cauliflower mosaic virus*) (accession KX434771.1).

It was also possible to recover representatives of the *Genomoviridae* family (three contigs) with two of them corresponding to members of the genus *Gemycircularvirus* (C1043 and C1045), including *Gemycircularvirus mocha 1* (*Momordica charantia associated gemycircularvirus*) with identities of 91% and 78% (accession NC_075310.1) and coverage of 99% and 100%, respectively. The remaining contig (C5696) resembled to *Gemykolovirus heris1* (Plant associated genomovirus 7), with identity of 88.44% and coverage of 56% (accession NC_076273.1), which is a member of the genus *Gemykolovirus*.

Four partial genomes were also recovered. In the *Caulimoviridae* family, the contig #16213 shared 69.79% identity with the species *Soymovirus maculavaccinii* (*Blueberry red ringspot virus*) (genus *Soymovirus*), with 94% coverage in BLASTn. In the *Circoviridae* family, the contig #4839 displayed 75.68% similarity to *Cyclovirus moosa* (Calfel virus LSF31_cyc880 – CalfelV880; genus *Cyclovirus*) with 77% coverage. In the *Geminiviridae* family, two partial *Begomovirus* contigs corresponding to the DNA–A and DNA–B component were also recovered. The contig #48842 presented nucleotide identity of 76.64% with *Begomovirus phaseolichlorosis* (*Bean chlorosis virus*) with 67% coverage, and in the DNA–B component, contig 15842 with 90.68% identity with *Begomovirus passifloraseveri* (*Passionfruit severe leaf distortion virus*) and 29% coverage.

In addition to the viral families described, a member of the *Alphasatellitidae* (genus *Clecrusatellite*) was also identified, with 96.15% identity and 100% coverage in relation to isolate of *Cleome leaf crumple virus associated DNA 1* (accession NC_014646.1).

Table 4. Contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of assembled viral genomes, E-value, description, and GenBank accession for the viruses obtained by High-Throughput Sequencing (HTS) for the DNA–A segment of the pool containing 38 contigs classified as *Begomovirus*. The contigs highlighted indicate potential new virus species, totaling 05 contigs.

Contig[1]	**Read coverage**	**Assembled genome size (nt)**	**Coverage (BLAST**) (%)**	**Identity (%)**	**E-value**	**Virus name (corresponding in latin)**	**Access GenBank**
48842*	9	660	67	76,64	0	Bean chlorosis virus *(Begomovirus phaseolichlorosis*)	NC_019569.1
493	1,292,633	2643	91	87	0	Bean golden mosaic virus (*Begomovirus costai*)	KJ939776.1
3338[2]	113,765	2617	100	96	0	Bean golden mosaic virus (*Begomovirus costai*)	KJ939766.1
218	605,816	2657	91	88	0	Cleome leaf crumple virus (*Begomovirus cleomecrispi*)	FN435999.1
7119	13,47	2730	100	94,33	0	Cleome leaf crumple virus (*Begomovirus cleomecrispi*)	FN435999.1
2827[3]	2,466,136	2610	100	98	0	Euphorbia yellow mosaic virus (*Begomovirus euphorbiamusiviflavi*)	JF756675.1
188[4]	69,521	2654	100	98	0	Leonurus mosaic virus (*Begomovirus leonuri*)	JX863082.1
64[5]	2,053,153	2661	100	83	0	Leonurus mosaic virus (*Begomovirus leonuri*)	KM887907.2
8[6]	2,972,340	2654	100	81	0	Tomato leaf distortion virus (*Begomovirus solanumcontorsionis*)	KU131588
329[7]	1,428,329	2593	100	85	0	Tomato severe rugose virus (*Begomovirus solanumseverugosi*)	MW596573

[1]Contigs indicated with a superscript number indicate that, after Muscle alignment (Brown et al. 2015) and analysis in Evolview software, these contigs showed similarities to other contigs. These details are described according to the corresponding superscript number. [2]Identities between 96% and 100% with contigs 338, 58, and 106. [3]Identities between 94% and 100% with contigs 6 and 442. [4]Identities between 93% and 100% with contigs 623, 68, 59, 197, 62, 165, 198, 55, 196, 171, 53, 202, 46455, 217, 52, and 208. [5]Identity of 95% with contig 159. [6]Identities between 95% and 100% with contigs 1139, 1379, 65, 78, and 12847. [7]Identity of 98% with contig 5250. *Virus not fully recovered. **Basic Local Alignment Search Tool.

Table 5. Contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of assembled viral genomes, E-value, description and accession in GenBank for viruses obtained by High-Throughput Sequencing (HTS) for the DNA–B segment of the pool containing 45 contigs of the genus *Begomovirus*.

Contigs[1]	**Read coverage**	**Assembled genome size (nt)**	**Coverage (BLAST**) (%)**	**Identity (%)**	**E-value**	**Virus name (latin binomial)**	**Access GenBank**
12357	396,888	2578	73	88,99	0	Bean golden mosaic virus (*Begomovirus costai*)	MT626872.1
222[2]	15,391	2581	100	92,37	0	Bean golden mosaic virus (*Begomovirus costai*)	MT626914.1
156	12,613	2662	100	91,56	0	Cleome leaf crumple virus (*Begomovirus cleomecrispi*)	FN436000.1
1235[3]	4,341,426	2578	100	99,34	0	Euphorbia yellow mosaic virus (*Begomovirus euphorbiamusiviflavi*)	KY559582.1
1064	43,631	2534	99	82,02	0	Macroptilium yellow spot virus (*Begomovirus macroptilimaculae*)	KT779558.1
5109	917,932	2533	100	83,39	0	Macroptilium yellow spot virus (*Begomovirus macroptilimaculae*)	MT627034.1
15842*	29	833	99	90,68	0	Passionfruit severe leaf distortion virus (*Begomovirus passifloraseveri*)	MT104018.1
1037	310	2605	99	93,23	0	Sida micrantha mosaic virus (*Begomovirus sidamicranthae*)	KC706534.1
2912[4]	889,739	2557	82	77,56	0	Tomato chlorotic leaf curl virus (*Begomovirus solanumpallidi*)	NC_055471.1
2972[5]	436,061	2617	100	93,97	0	Tomato yellow spot virus (*Begomovirus solanumflavusmaculae*)	KX348219.1

[1]Contigs indicated with a superscript number indicate that, after Muscle alignment, these contigs showed similarities to other contigs. These details will be described according to the corresponding superscript number. [2]Identities between 91.4% and 96.92% with contigs 89, 87 and 88. [3]Identities of 100% with contigs 83 and 1922. [4]Identities of 95.6% with contig 7. [5]Identities between 91.6% and 100% with contigs 48, 24, 28, 46, 49, 614, 2461, 9235, 1412, 45, 360, 26, 177, 133, 27, 44, 82, 176, 40, 25, 112, 33, 47, 94, 79, 324, 2914, 327 and 95. *Virus not fully recovered. **Basic Local Alignment Search Tool.

Table 6. Contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of assembled viral genomes, E-value, description, and GenBank accession for viruses obtained by High-Throughput Sequencing (HTS) for the complete genome segment of the pool containing 20 contigs belonging to 5 distinct families.

Contigs[1]	Read coverage	Assembled genome size (nt)	Coverage (BLAST**) (%)	Identity (%)	E-value	Family	Gênero	Virus name	Access GenBank
803[2]	1,791	7400	100%	95,71%	0	*Caulimoviridae*	*Badnavirus*	Banana streak CA virus	NC_015506.1
4093[3]	1,527	7413	99%	94,09%	0		*Badnavirus*	Sugarcane bacilliform virus	KT186240.1
189	11,649	8027	100%	98,14%	0		*Caulimovirus*	Cauliflower mosaic virus	KX434771.1
16213*	6	394	84%	72,65%	0		*Soymovirus*	Blueberry red ringspot virus	JF421559.1
48391*	6	475	77%	75,68%	4E-65	*Circoviridae*	*Cyclovirus*	Calfel virus LSF31_cyc880	NC_077208.1
530	364	1349	100%	96,15%	0	*Alphasatellitidae*	*Clecrusatellite*	Cleome leaf crumple virus associated DNA 1	NC_014646.1
1998	338	3707	88%	72,50%	0	*Geminiviridae*	*Citlodavirus*	Passion fruit chlorotic mottle virus	NC_040706.1
93	8,869	2968	94%	92,22%	0		*Mulcrilevirus*	Mulberry crinkle leaf virus	MN240483.1
1043	566	2195	99%	91,26%	0	*Genomoviridae*	*Gemycircularvirus*	Momordica charantia associated gemycircularvirus	NC_075310.1
1045	917	2186	100%	78%	0		*Gemycircularvirus*	Momordica charantia associated gemycircularvirus	NC_075310.1
5696	98	2208	56%	88,44%	0		*Gemykolovirus*	Plant associated genomovirus 7	NC_076273.1

[1]Contigs indicated with a superscript number indicate that, after Muscle alignment, these contigs showed similarities to other contigs. These details will be described according to the corresponding superscript number. [2]Identities between 93% and 99.07% with contigs 676, 3461, and 8279. [3]Identities between 94.07% and 100% with contigs 5930, 8303, 8789, 599, 12292, and 600. *Virus not fully recovered. **Basic Local Alignment Search Tool.

3.2. PCR detection of viruses from the pool with species-specific primers

The detections were systematized in **Table 7, Figure 2** and will then be presented and discussed according to the respective viral genera identified.

3.3. Genus *Begomovirus*

The following viruses (and their corresponding genomic components) were detected: BGMV (DNA–A), EuYMV (DNA–A + DNA–B), ToYSV (DNA–A + DNA–B), CleLCrV (DNA–A + DNA–B), in addition to five putative new species. Among these viruses, ToYSV (DNA–A) was the most prevalent, being present in ten (10) samples, followed by EuYMV (DNA–B), which was present in eight (8) samples. The BGMV was detected only in Fabaceae samples, in the Federal District (DF-361) and Minas Gerais (MG-350). EuYMV infections were identified predominantly in samples of the Lamiaceae family, but it was also collected in members of the families Brassicaceae, Fabaceae and Malvaceae, in the Federal District (DF-174 and RDF-824), Paraná (PR-145, PR-012, PR-117, PR-133, PR-126, and PR-127), and Goiás (RGO-835) States. In the case of ToYSV, its occurrence was limited to the Lamiaceae family and restricted to the Paraná region (PR-136, PR-135, PR-134, PR-133, PR-132, PR-128, PR-126, PR-125, PR-122, PR-121, PR-033, and PR-049). CleLCrV was detected in the families Cleomaceae and Fabaceae in the state of Bahia (BA-76), and also in Paraguay (PAR-005).

New species #1 and New species #2 were identified (in mixed infection) exclusively in Malvaceae, in three samples, and only in Goiás State (RGO-835, RGO-832 and RGO-834). On the other hand, the New species #3 (isolates PR-126 and PR-136) and New species #5 (isolate PR-134) were detected occurring only in Lamiaceae and in Paraná State. The New species #4, was recorded in seven samples from four different botanical families (Asteraceae, Cucurbitaceae, Poaceae and Malvaceae,), displaying a wide geographical distribution in Goiás (RGO-838, RGO-836, RGO-834 and RGO-833), Distrito Federal (RDF-826 and RDF-827) and Rio Grande do Sul (RS-082).

3.4. Genus *Clecrusatellite*

The alphasatellite *Cleome leaf crumple virus-associated DNA 1* was identified exclusively in species of the Lamiaceae family, with occurrence restricted to two samples (PR-035 and PR-087) collected in Paraná State.

3.5. Genus *Citlodavirus*

The New species #6 was detected in only one sample (MT-012), belonging to the Fabaceae family, in the Mato Grosso State.

3.6. Genus *Mulcrilevirus*

In the case of MCLV, its occurrence was limited to the detection of one sample (RDF-831) in a Moraceae tree, in the Federal District region

3.7. Genus *Badnavirus*

The *Sugarcane bacilliform virus* was identified in mixed infection with the new begomovirus species NS#4 only in one sample (RGO-838), belonging to the Poaceae family in Goiás State.

3.8. Genus *Caulimovirus*

CaMoV detection occurred only in Brassicaceae, in two samples from the Federal District (RDF-804 and DF-245).

3.9. Genus *Gemycircularvirus*

Gemycircularvirus mocha1 was detected in both Brassicaceae and Malvaceae. The two detections occurred in two distinct samples: one from Goiás (RGO-834) and the other from the Federal District (DF-824). This virus occurred in mixed infection with three of the new begomovirus species: NS#1, NS#2 and NS#4, in a sample (RGO-834) collected the Goiás.

3.10. Genus *Gemykolovirus*

The detection of *Gemykolovirus heris1* occurred exclusively in Poaeae species, in only one sample collected in the state of Goiás (RGO-833). This virus was detected in mixed infection with a new begomovirus species NS#4.

Table 7. Positive samples for ssDNA viruses detected by PCR, using species-specific primers in the pool samples, with the genus, acronym/virus name, sample code, host, botanical family, and year of collection.

Positive samples of *pool*					
Genus	**Acronym/species**	**Sample code**	**Host**	**Botanical family**	**Year of collection**
Begomovirus	BGMV DNA–A *Begomovirus costai* **(2)**	DF-361	*Phaseolus vulgaris*	Fabaceae	2010
		MG-350			2012
	EuYMV DNA–A *Begomovirus euphorbiamusiviflavi* **(1)**	DF-174	*Euphorbia heterophylla*	Fabaceae	2005
	EuYMV DNA–B *Begomovirus euphorbiamusiviflavi* **(8)**	PR-145	*Eruca vesicaria subsp. Sativa*	Brassicaceae	2015
		PR-012	*Leonurus sibiricus*	Lamiaceae	2007
		PR-117			2010
		PR-133			2012
		PR-126			2011
		PR-127			
		RGO-835	*Abutilon theophrasti*	Malvaceae	2022
		RDF-824	*Brassica oleracea var. capitata*	Brassicaceae	
	LeMV DNA–A *Begomovirus leonuri* **(10)**	PR-136	*Leonurus sibiricus*	Lamiaceae	2012
		PR-135			
		PR-134			
		PR-133			
		PR-132			
		PR-128			
		PR-126			2011
		PR-125			
		PR-122			
		PR-121			

	LeMV DNA–B *Begomovirus leonuri* **(4)**	PR-135	*Leonurus sibiricus*	Lamiaceae	2012
		PR-134			
		PR-033			2007
		PR-049			
	CleLCrV DNA–A *Begomovirus cleomecrispi* **(1)**	PAR-005	*Cleome affinis*	Cleomaceae	2010
	CleLCrV DNA–B *Begomovirus cleomecrispi* **(2)**	PAR-005	*Cleome affinis*	Cleomaceae	2010
		BA-76	*Phaseolus vulgaris*	Fabaceae	2011
	New species #1 **C493** **(3)**	RGO-835	*Abutilon theophrasti*	Malvaceae	2022
		RGO-834			
		RGO-832	*Sidastrum paniculatum*		
	New species #2 **C218** **(3)**	RGO-835	*Abutilon theophrasti*	Malvaceae	2022
		RGO-834			
		RGO-832	*Sidastrum paniculatum*		
	New species #3 **C64** **(2)**	PR-126	*Leonurus sibiricus*	Lamiaceae	2011
		PR-136			2012
	New species #4 **C08** **(7)**	RDF-826	*Cichorium intybus*	Asteraceae	2022
		RDF-827			
		RGO-836	*Cucurbita moschata*	Cucurbitaceae	
		RGO-838	*Saccharum officinarum*	Poaceae	
		RGO-833	*Digitaria catamarcensis*		
		RGO-834	*Abutilon theophrasti*	Malvaceae	
		RS-082	*Sida acuta*		2013
	New species #5 **C329** **(1)**	PR-134	*Leonurus sibiricus*	Lamiaceae	2012
Clecrusatellite	*Cleome leaf crumple virus associated DNA 1* **(2)**	PR-035	*Leonurus sibiricus*	Lamiaceae	2007
		PR-087			2008
Citlodavirus	New species #6 **C1998** **(1)**	MT-012	*Samanea tubulosa*	Fabaceae	2010

Mulcrilevirus	MCLV *Mulcrilevirus mori* **(1)**	RDF-831	*Morus alba*	Moraceae	2022
Badnavirus	*Sugarcane bacilliform virus* **(1)**	RGO-838	*Saccharum officinarum*	Poaceae	2022
Caulimovirus	CMV *Caulimovirus tessellobrassicae* **(2)**	RDF-804 DF-245	*Brassica oleraceae* *Brassica napus var. napus*	Brassicaceae	2022 2008
Gemycircularvirus	*Momordica charantia associated gemycircularvirus* **(2)**	RGO-834 RDF-824	*Abutilon theophrasti* *Brassica oleracea var. capitata*	Malvaceae Brassicaceae	2022
Gemykolovirus	*Plant associated genomovirus 7* **(1)**	RGO-833	*Digitaria catamarcensis*	Poaceae	2022

Begomovirus costai - Bean golden mosaic virus; *Begomovirus euphorbiamusiviflavi - Euphorbia yellow mosaic virus; Begomovirus leonuri – Leonurus mosaic virus*); *Begomovirus cleomecrispi - Cleome leaf crumple virus; Mulcrilevirus mori - Mulberry crinkle leaf virus; Caulimovirus tessellobrassicae - Cauliflower mosaic virus*.

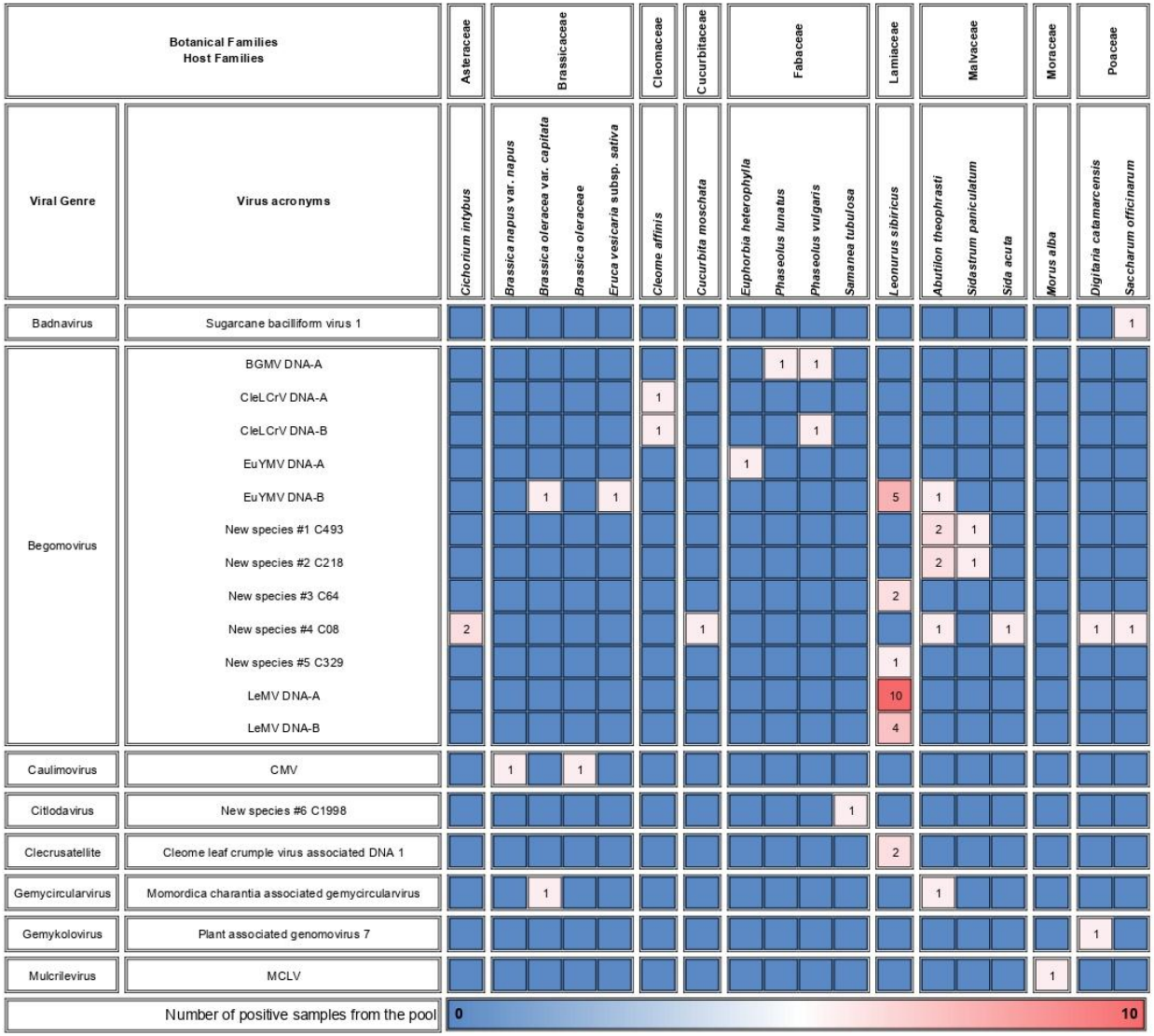

Figure 2. Quantitative of positive samples for viruses detected by PCR, using species-specific primers in the pool samples, with the genus, virus acronym, sample code, host, botanical family and year of collection.

4. Discussion

Studies conducted in different regions of the world have shown that tomatoes can be hosts of more than 300 different species of viruses, including those frequently associated with disease symptoms and production losses (Rivarez et al. 2021; Jo et al. 2023; GenBank 2025). Members of the genus *Begomovirus* were prevalent, accounting for the largest share of records ever made for this crop (Sastry et al. 2019; Kitajima 2020; Duarte et al. 2021a; Oliveira et al. 2024; GenBank 2025). This expressive diversity is directly related to the ability of these viruses to exploit different processes of genetic variation, such as natural mutation as well as and the exchange of genomic segments by recombination or pseudo-recombination. These genetic variation mechanisms can provide evolutionary adaptation (Seal et al. 2006; Fiallo-Olivé et al. 2023). The presence weed hosts associated with tomato crops constitutes important and continuous reservoirs of viruses that are transmitted to cultivated plants (García-Arenal and Zerbini 2019; Wang et al. 2021; Duarte et al. 2021; Rivarez et al. 2023). In addition, the common occurrence of mixed infections in weeds can favor the emergence of new species (Reis et al. 2020).

Recent advances using HTS technologies have boosted the identification and characterization of new plant viruses (Kawsaki et al. 2023; Maina et al. 2024; Jaksa-Czotter et al. 2024; González-Pérez et al. 2024; Queiroz-Ferreira et al. 2024; Pacheco-Dorantes et al. 2025; Mansour et al. 2025; Reis et al. 2025). Using HTS, we were able to recover viral genomes from seven distinct genera: *Begomovirus*, *Citlodavirus*, *Mulcrilevirus*, *Badnavirus*, *Caulimovirus*, *Gemycircularvirus*, *Gemykolovirus*, in addition to an alphasatellite (*Clecrusatellite*) distributed in the five macro Brazilian regions and in Paraguay.

The results revealed a remarkable diversity of viruses associated with weeds, suggesting that such species may function as natural reservoirs, favoring the persistence and flow of viruses between cultivated and non-cultivated plants (Wang et al. 2021; Galbács et al. 2024). Of this variety, more than 80% of the viral contigs belong to the genus *Begomovirus*. In addition to the detection of already known begomovirus species, five putative new species were identified, distributed in the Central-West and Southern regions of Brazil. NS#1 (2643 nts) shared 87% identity with BGMV (KJ939776.1) and was detected in three Malvaceae samples from the state of Goiás (RGO-835, RGO-834, and RGO-832). Interestingly, NS#2 (2657 nts), with 88% identity with CleLCrV (FN435999.1), was also found in the same samples, indicating a mixed infection between these new species. NS#3 (2661 nts) has 83% identity to LeMV (JX863082.1)

and was detected only in Lamiaceae, in the state of Paraná (PR-126 and PR-136). NS#4 (2654 nts) shares 81% identity with ToLDV (KC706605.1) and was detected in samples from the families Asteraceae, Cucurbitaceae, Poaceae, Malvaceae and Lamiaceae, in three different states: in the Federal District (RDF-826 and RDF-827), in the state of Goiás (RGO-836, RGO-838, RGO-834 and RGO-833) and in Rio Grande do Sul (RS-082). These four new species possess a typical bipartite DNA–A segment of begomoviruses, with cognate DNA–B segments of: NS#1 - 2545 nts, NS#2 - 2585 and 2578 nts, NS#3 - 2636 nts, and NS#4 - 2585 nts. Finally, NS#5 (2593 nts) has 85% identity with ToSRV (MW602388.1) and was detected in only one Lamiaceae sample in the state of Paraná (PR-134). For this species, its cognate DNA–B segment was not found, indicating that it is a putatively monopartite species. However, more extensive studies should be conducted to verify its putative monopartite nature. According to current parameters for demarcation of species of the genus *Begomovirus*, defined by nucleotide identity of less than 91% in the complete DNA–A genome, the five species detected in this study qualify as new species in the genus (Brown et al. 2015).

A new species of the genus *Citlodavirus* was also identified: NS#6. It has a typical *Citlodavirus* genome, with 3,707 nts and 72.50% identity with PCMoV (NC_040706.1). NS#6 was detected in only one sample, *Samanea tubulosa* (Fabaceae), a legume native to the North region of Brazil (Olival et al. 2022), collected in Cuiabá, Mato Grosso (MT-012) in 2010. According to the demarcation criteria established for the genus, this species has nucleotide identity of less than 78%, which confirms the hypothesis of a new species (Roumagnac et al. 2022).

Additionally, in this study, CLeLCrV (genus *Begomovirus*) was first reported in Paraguay, in a sample collected in 2010 in Mayor Otaño (PAR-005). Until now, reports of CLeLCrV were restricted to Brazil and, it was first reported associated the weed *Cleome affinis* (Paprotka et al. 2010) and more recently associated tomato (*Solanum lycopersicum*) (Reis et al. 2020). Similarly, MCLV (genus *Mulcrilevirus*) was first detected in Brazil, in mulberry (*Morus alba*), collected as an invasive plant growing near tomato crops in the Federal District (2022). Previously, *Mulcrilevirus* had only been reported in China (Lu et al. 2015; Lu et al. 2022), highlighting the importance of monitoring the potential introduction of exotic viruses into new mulberry production areas. Even in relatively well-studied agroecosystems, a significant portion of viral diversity remains unknown because it is concentrated in non-cultivated plants. This highlights the need for expanded studies of these plants to understand fully the risk, dynamics, and potential viral reservoirs (Hasiów-Jaroszewska et al. 2021; Rivarez et al. 2023).

Furthermore, *Gemycircularvirus mocha1* (*Gemycircularvirus*) was detected in two new hosts: *Abutilon theophrasti* (Malvaceae), collected in Urutaí, Goiás state (RGO-834), and cabbage (*Brassica oleracea* var. *capitata*), in the Federal District (RDF-824), both collected in 2022. *Gemycircularvirus mocha1* was first reported associated with a weed (*Momordica charantia*), in the municipality of Viçosa, Minas Gerais state (de Rezende et al. 2018), being reported again only in 2020 in eggplant (*Solanum melongena*), in the Federal District (Batista 2020). A new host for the virus *Gemykolovirus heris1* (*Gemykolovirus*) was also identified: the weed *Digitaria catamarcensis* (Poaceae), collected in Urutaí, state of Goiás, in 2022 (RGO-83). No published reports on this virus were found in scientific articles, the available information is limited to sequences deposited in GenBank/NCBI. Only five sequences of this virus were deposited, associated with: *Passiflora edulis* (MH939410.1), *Herissantia* (MH939376.1), *Ipoema* (MH939375.1), and Poaceae (MH939374.1 and NC_076273.1). This study represents the first report of the virus occurring in a species of the Poaceae family, although its detection had previously been recorded in the family. This result highlights the lack of studies on this virus.

The weed species with the highest number of detections was *Leonurus sibiricus* (Lamiaceae). In this study, three weed species (*Abutilon theophrasti* - RGO-835 and RGO834, *Sidastrum paniculatum* - RGO-832 and *Digitaria catamarcensis* - RGO-833), in addition to sugarcane (*Saccharum officinarum* - RGO-838), all collected in Urutaí, Goiás, in 2022, were also found containing mixed infection. These infections occurred among new species of Begomovirus (NS#1, NS#2 and NS#4) and between the new species with viruses from other genera: *Badnavirus* (RGO-838), *Gemykolovirus* (RGO-833) and *Gemycircularvirus* (RGO-834) (**Figure 3**). In nature, the same plant can simultaneously harbor multiple viruses, especially when dealing with weeds growing near crop fields (Rivarez et al. 2023). Studies by Ban et al. (2021), Reis et al. (2020), Galbács et al. (2024) and Oliveira et al. (2024) corroborate this observation by reporting the occurrence of mixed infections among begomoviruses. Mixed infections can intensify symptoms in the plant (Singhal et al. 2020, Ban et al. 2021). Furthermore, a range of important viral diseases in plants result from interactions between their causal agents (Xu et al. 2022) and can favor the emergence of new species (Reis et al. 2020).

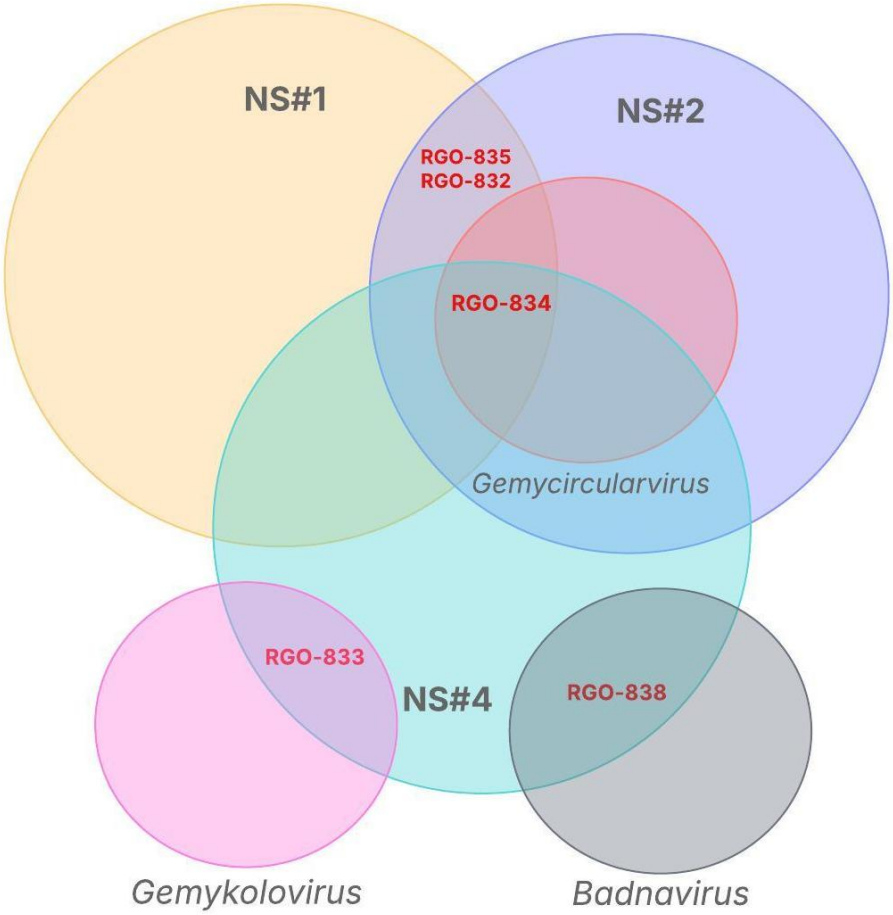

Figure 3. Euler-Venn diagram illustrating the detected mixed infections. The name sample of positive plants in mixed infections is shown, whether single, double, triple, or quadruple infections. Abbreviations: NS#1 - New species #1 (*Begomovirus*), NS#2 - New species #2 (*Begomovirus*), New species #4 (*Begomovirus*), RGO-835 e RGO834 – *Abutilon theophrasti*, RGO-832 – *Sidastrum paniculatum*, RGO-833 – *Digitaria catamarcensis* e RGO-838 – *Saccharum officinarum*.

5. Conclusions

The results obtained in this study provide a comprehensive overview of viral interactions involving weed species present in crop environments. This is the first exploratory study to portray viral diversity in weeds associated with tomato crops, bringing together such a wide range of samples from different botanical families. We discovered a wide variety of viruses, hosts, and novel species, as well as different multiviral complexes detected in different plant species, highlighting the high frequency of mixed infections. These findings reinforce the importance of considering such species as potential virus reservoirs, as they can contribute to the maintenance and spread of pathogens in agroecosystems (Hasiów-Jaroszewska et al. 2021; Rivarez et al. 2023).

These results also indicate an understudied diversity of novel viruses in an agroecosystem, especially in weeds that can contribute to the transmission of viruses to other crop species. Thus, the survey expands knowledge about viral diversity in non-cultivated plants and highlights the need for greater attention to weeds as key elements in the epidemiological dynamics of cultivated plant viruses. This virome dataset can also aid in future monitoring of viral diseases in tomato plants, potentially contributing to the monitoring and prevention of future viral epidemics in tomato plants.

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CHAPTER 3

A novel neotropical *Begomovirus* with monopartite genomes associated *Leonurus sibiricus*

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Work for submission to the Archives of Virology

Abstract

Weeds play a crucial role as reservoirs and sources of inoculum for various plant viruses, as well as harboring vectors, thus favoring the maintenance and dissemination of plant viruses in agroecosystems. High-Throughput Sequencing (HTS) technology has revolutionized plant virology by expanding our understanding of viral diversity and enabling the identification of emerging pathogens. Beside this, weed samples associated to tomato crops allowed the recovery of complete genome of a novel begomovirus. Using PCR with species-specific primers, this virus was detected in *Leonurus sibiricus* from Paraná State, Brazil. It shares 85% identity with *Begomovirus solanumseverugosi* (ToSRV, MW596573), being phylogenetically closer. The DNA-B was not found. PCR analyses with degenerate primers for the DNA-B segment detected the presence of mixed infection with *Begomovirus leonuri* (LeMV). This is a monopartite species, with a DNA-A genomic organization typical of New World monopartite begomoviruses, with ≈ 2.6 kb and the presence of 5 ORFs (V1, C1, C2, C3 and C4). Our results reinforce the importance of monitoring weeds near crop areas, as they act as viral reservoirs harboring a wide range of pathogens that are still poorly understood.

The *Geminiviridae* family (order *Geplafuvirales*) is composed of single-stranded DNA (ssDNA) viruses with wide genetic diversity and geographic distribution. Currently, it comprises 548 species distributed across 15 genera: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus*, *Turnvurtovirus* (Zerbini et al. 2017) e *Welwivirus* (Bejerman e Debat, 2023; ICTV, 2025). The delimitation of genera within the family are established in according to host range, vectors and genome organization (Brown et al. 2015; Zerbini et al. 2017; Rougmanac et al. 2022; Bejerman e Debat 2023). The *Begomovirus* genus stands out within the family for the largest number of species, totaling 463 currently described (ICTV, 2025). Begomoviruses may have a monopartite or bipartite ssDNA genomic component, with approximately 2.6 kb, encapsidad in twinned particles formed by two incomplete icosahedrons (Rojas et al. 2018; Kumar 2019). They are transmitted by a complex of cryptic species *Bemisia tabaci* (Hemiptera: Aleyrodidae), through a transmission characterized as circulative non-propagative (De Barro e Ahmed 2011; Navas-Castillo et al. 2011; Rojas et al. 2018; Krause-Sakate et al., 2020).

Monopartite begomoviruses associated tomato are more common in the "Old World," with a predominance of bipartite genomes in the Americas (New World) (Briddon et al. 2010; Navas-Castillo and Fiallo-Olivé, 2020). However, six monopartite begomoviruses have been identified in Brazil: 1. *Begomovirus solanumviolavenae* (= *tomato leaf curl purple vein virus*) (Macedo et al. 2018), in Northeast Brazil; 2. *Begomovirus solanumvariatuminvolutionis* (= *tomato mottle leaf curl virus*), with evolutionary origins in Northeast Brazil (Souza et al. 2022); 3. *Begomovirus solanumaureusreti* (= *tomato golden net virus*) and 4. *Begomovirus solanumflavusreti* (= *tomato yellow net virus*), in the Central-West region (Reis et al. 2023); 5. Tomato iridescent mottle virus (ToIMoV), in the Northeast region; and 6. Tomato iridescent apical mosaic virus (ToIAMV) in the South region (Oliveira 2024), indicating that these viruses comprise a unique group of monopartite *Begomovirus* species in the New World.

Begomoviruses are responsible for emerging diseases and currently constitute a major biotic problem in several crops, including tomato (*Solanum lycopersicum*). Associated with tomato cultivation, weeds have been observed to act as natural reservoirs for viruses, favoring the maintenance and spread of these pathogens (Ambrozevicius et al. 2002; García-Arenal e Zerbini 2019; Wang et al. 2021).

In addition, new species of begomoviruses that have been reported in weeds (Chipiringo et al. 2022). Furthermore, a *Begomovirus cleomecrispi* (Cleome leaf crumple virus - CleLCrV) isolate from tomato was phylogenetically analyzed, confirming that it was the closest to isolates from the original weed host (*Cleome affinis*), corroborating the fact that host-jumping events of begomoviruses from found weeds to tomato occur naturally (Reis et al. 2025). *C. affinis* has already been reported as a host for *Begomovirus cleomecrispi* (CleLCrV) (Paprotka et al. 2010), and alphasatellites such as *Clecrusatellite euphorbiae* (Euphorbia yellow mosaic virus associated DNA 1) and *Clecrusatellite cleomis* (Cleome leaf crumple alphasatellite) (Paprotka et al. 2010). In this study, a new putative monopartite species was described, exhibiting genomic organizations typical of New World *Begomovirus* species.

In the present study, 114 samples from 15 botanical families (**Suplemmentary Table 1**) of symptomatic weeds associated with tomato crops were collected from various regions of Brazil and Paraguay, from 2004 to 2022. These samples were submitted to total DNA extraction using the modified 2x CTAB protocol with organic solvents (Boiteux et al. 1999) and stored at -20°C. The samples were enriched for circular DNA through RCA (Rolling Circle Amplification) (Inoue-Nagata et al. 2004). The enriched DNA was subjected to High-Throughput Sequencing (HTS) on the Illumina NovaSeq-6000 platform at Agrega, located in

Rio Grande do Sul, Brazil. The ITS2 (ITS2F/ITS2R) primer was used to identify the host (Yao et al. 2010).

The HTS data were analyzed according to the following workflow: a) elimination of low-quality reads; b) sequence reassembly using the CLC Genomics Workbench 23.0.3 program; and c) validation of the contigs with the BLASTn algorithm, comparing them with the ssDNA Genbank virus database (https://www.ncbi.nlm.nih.gov/). Subsequently, the contigs of potential viruses were mapped following the methodology proposed by Nery et al. (2020) and Reis et al. (2020) to obtain the final contigs corresponding to the genomes of these viruses. The contigs were extended using the 'Map to reference' tool, available in the Geneious 11.0.5 software, with a minimum overlap identity parameter of 90% to 99%, which allowed mapping the reads obtained in the HTS that relate to each contig analyzed (Kearse et al. 2012).

As a result HTS sequencing yielded the following raw reads: 19,575,404 reads, allowing the assembly of 62,444 contigs, of which 103 sequences corresponded to viruses, as indicated by BLASTn analysis. Of these contigs, 85 of them consist of previously characterized *Begomovirus* species, as well as viruses from other genera: *Citlodavirus*, *Mulcrilevirus*, *Badnavirus*, *Caulimovirus*, *Gemycircularvirus*, *Gemykolovirus*, and an alphasatellite - *Clecrusatellite*). However, two contigs DNA-A showed an identity below 91%. According to the current taxonomic criteria of the genus *Begomovirus* (Brown et al. 2015), this isolate is characterized as a new species of the genus.

The DNA-A genome of the new species initially named new species #5 (NS#5) (1,428,329 reads, 100% coverage) showed 85% identity with *Begomovirus solanumseverugosi* (*tomato severe rugose virus*). A pair of specific primers was designed for this species: **PC329F** (5'-TTGAACATACACTTTACTTTTGC-3') and **PC329R** (5'-TACTTATCCACAATGTACTCTTA-3'). PCR assays with the specific primers were performed, using 56ºC temperature for alignment resulting in the detection of the NS#5 in a sample from Paraná (PR-134, *Leonurus sibiricus*), collected in 2012 in Capitão Leonidas Marques. The complete viral DNA-A sequence was confirmed by dideoxy Sanger sequencing using specific primer pairs. Further PCR assays with primers specific for eight *Begomovirus* and seven viruses from different genera were also performed, revealing that the NS#5 presents a mixed infection with *Begomovirus leonuri* (*Leonurus mosaic virus*).

For DNA-B detection, the universal primer pair 'PBL1v2040'/'PCRc1' (Rojas et al. 1993) was used. PCR assays for B-DNA were conducted and the amplicons were validated by Sanger dideoxy chain termination sequencing, confirming that only LeMV DNA-B was present due to mixed infection, indicating that it may be a monopartite virus.

This new species exhibits a DNA-A genomic organization typical of New World monopartite begomoviruses with ≈ 2.6 kb (**Fig. 1A**), displaying the ORFs V1 (756 nts), which encodes the viral coat protein - CP; C1 (1059 nts), responsible for the replicase - Rep; C2 (390 nts), which encodes the Transcription transactivator protein - TrAp; C3 (399 nts), which encodes the Replication enhancer protein - REn; and C4 (264 nts), involved in gene silencing. The ORFS V3, C5, C6, and C7 were not found. The nucleotide sequence 5'–TAATATT/AC–3' (conserved among begomoviruses) was also located in the intergenic region (IR). We also performed analyses of the iterons and iteron-related domains (Rep-IRD) in the sequence (Argüello-Astorga and Ruiz-Medrano, 2001) (**Fig. 1B**). The NS#5 has a genome of 2,593 nucleotides (nts) and exhibited the ATTGGTAG iteron (Rep-IRD = MPSKIRFKIN) (**Fig. 1B**).

We were unable to identify cognate sequences in silico analyses of the assembled HTS contigs. Despite this, LeMV DNA-B segments were detected in the positive sample for NS#5, indicating mixed infections. Comparing the Rep/CP intergenic regions of the A-DNA sequences with all B-DNA sequences (41) obtained by HTS, the identities ranged from 50.28% to 71.95%, indicating that none of them could be classified as cognate. Thus, through Sanger sequencing, we identified that the DNA-B segments refer to LeMV, confirming the aforementioned mixed infection.

To detect potential recombination events, the RDP5 program (Martin et al. 2020) was used. Recombination events were considered reliable only if detected by at least four of the seven methods implemented by the Program. Therefore, no significant recombination events were detected for the species in question.

The pairwise nucleotide sequence identities of the new species and other begomoviruses were calculated using SDT (Muhire et al. 2014). The analysis showed that the NS#5 shares 84–85% identities with other begomoviruses, with the closest phylogenetically related identity being 85% to ToSRV (MW596573) (**Fig. 2**).

Bipartite begomoviruses are predominant in Brazil (Reis et al. 2020; Reis et al. 2021). Despite this, six monopartite begomoviruses have been reported in the Northeast, Central-West, and Southern regions of Brazil (Macedo et al. 2018; Souza et al. 2022; Reis et al. 2023; Oliveira, 2024). In this study, we report a putative novel begomovirus detected in the subtropical state of Paraná, Southern Brazil. Analysis performed by PCR and HTS did not reveal the presence of a cognate DNA-B associated with this species, indicating that it is a monopartite species. Its genome is typical of New World monopartite begomoviruses, lacking the V2 movement protein present in all Old World monopartite begomoviruses (Rojas et al. 2018), indicating that the V1 (CP) and C4 proteins may be involved in systemic viral movement within cells, exhibiting a

function analogous to the BC1 protein (Rojas et al. 2001; Devendran et al. 2022). Despite having smaller populations compared to bipartite ones, the success of monopartite begomoviruses in the field is justified by their replicative efficiency and transmission by the vector, in addition to their independence from DNA-B, facilitating their dissemination in areas dominated by bipartite species (Souza et al. 2022).

In conclusion, the NS#5 is part of a unique group of Neotropical monopartite begomoviruses, presenting enormous biological and molecular interest. Additional biological assay studies with infectious clones are needed to further strengthen the evidence that this begomovirus is a genuinely monopartite species.

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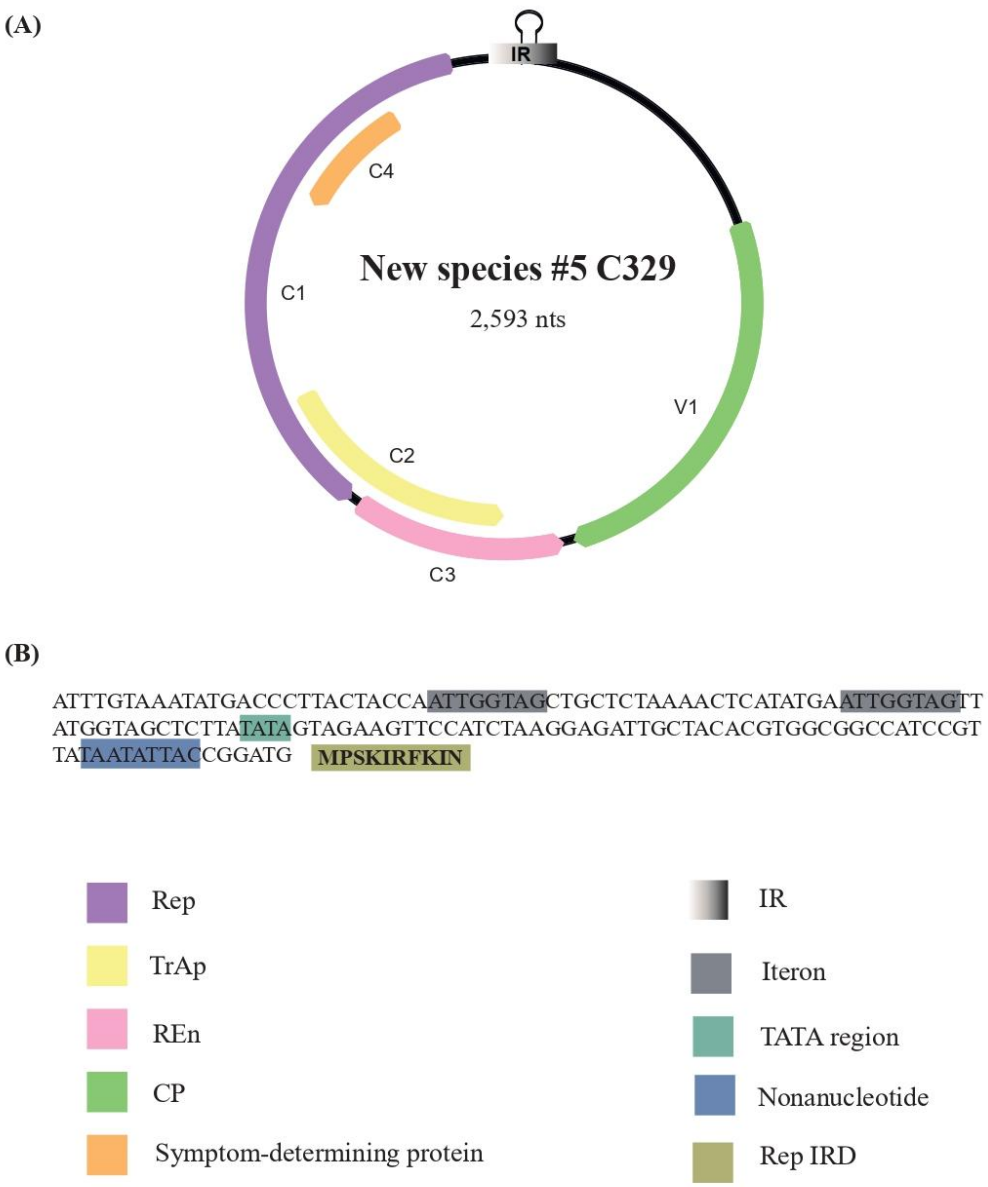

Figure 1. Genomic organization of the monopartite begomovirus species that infects weeds. **Panel A**: Diagrammatic representation of the circular genomes of NS#5 and their respective open reading frames (ORFs). The ORFs V1, C1, C2, C3, and C4 are color-coded according to the putative function of their protein products. CP = capsid protein; Rep = replication-associated protein; TrAp = transactivator protein; Ren = replication enhancer; C4 = putative symptom determinant and silencing suppressor; IR = intergenic region, encompassing the hairpin. **Panel B**: A segment of the intergenic region showing iterons, TATA region, nonanucleotide, and at the end Rep = IRD (Rep Iteron-Related Domain).

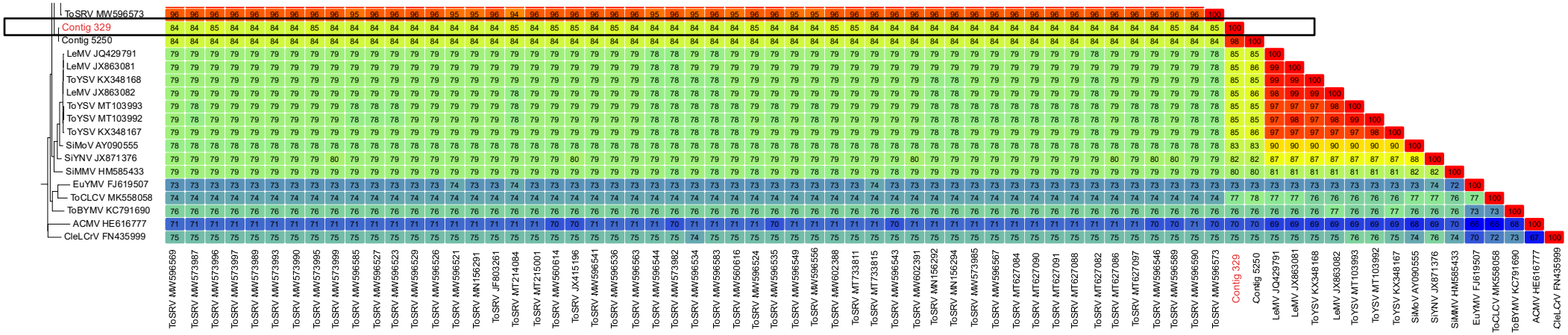
ToSRV MW596573
Contig 329
Contig 5250
LeMV JQ429791
LeMV JX863081
ToYSV KX348168
LeMV JX863082
ToYSV MT103993
ToYSV MT103992
ToYSV KX348167
SiMoV AY090555
SiYNV JX871376
SiMMV HM585433
EuYMV FJ619507
ToCLCV MK558058
ToBYMV KC791690
ACMV HE616777
CleLCrV FN435999

Figure 2. Pairwise identity in Sequence Demarcation Tool (SDT) analysis and phylogenetic reconstruction from DNA-A segment sequences of Begomovirus isolates associated tomato and weeds. Contig 329 (NS#5) is most phylogenetically related to the *Begomovirus solanumseverugosi* (virus tomato severe rugose virus - ToSRV) isolate (MW596573), sharing 85% pairwise identity, to which it is phylogenetically closest. Tomato severe rugose virus - ToSRV, Leonurus mosaic virus - LeMV, tomato yellow spot virus - ToYSV, Sida micrantha mosaic virus - SiMMV, Sida yellow net virus - SYNV, Euphorbia yellow mosaic virus - EuYMV, tomato chlorotic leaf curl virus - ToCLCV, Sida mottle virus - SiMoV, African cassava mosaic Burkina Faso virus - ACMV, tomato bright yellow mosaic virus - ToBMV, Cleome leaf crumple virus – CleLCrV.

Suplemmentary Table 1. Quantity of samples collected, organized by botanical families, and distributed by region.

Botanical families	Number of samples	Brazilian regions					
		North	Northeast	Midwest	South	Southeast	Paraguay
Asteraceae	04	---	---	04	---	---	---
Bignoniaceae	01	---	---	01	---	---	---
Brassicaceae	09	---	01	06	02	---	---
Cactaceae	04	---	01	03	---	---	---
Capparaceae	04	03	01	---	---	---	---
Caricaceae	01	---	---	01	---	---	---
Cleomaceae	01	---	---	---	---	---	01
Cucurbitaceae	01	---	---	01	---	---	---
Fabaceae	45	05	13	17	06	04	---
Lamiaceae	36	---	---	02	32	---	02
Malvaceae	03	---	---	03	---	---	---
Moraceae	01	---	---	01	---	---	---
Poaceae	02	---	---	02	---	---	---
Ruscaceae	01	---	---	01	---	---	---
Solanaceae	01	---	---	01	---	---	---
Total: 15	**114**	**08**	**16**	**43**	**40**	**04**	**03**

CHAPTER 4

Four new neotropical begomoviruses with bipartite genomes associated weeds, vegetable, and monocot field crops

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Work for submission to the Archives of Virology

Abstract

A high-throughput sequencing (HTS)-based survey of begomoviruses (*Geminiviridae*) in weed and crop samples recovery four new species (NS) in the genus *Begomovirus* (DNA–A identities with previously detected begomoviruses ranging from 81–88%). Using PCR with species-specific primers, the NS#1 and NS#2 were detected in Malvaceae in Central Brazil, whereas the NS#3 was detected only in *Leonurus sibiricus* in South Brazil. The atypical NS#4 displayed a large host range and wide geographical dispersion being detected in *Cucurbita moschata*, *Saccharum officinarum*, *Abutilon theophrasti*, *Digitaria catamarcensis*, *Cichorium intybus* in Central Brazil and in *Sida acuta* in South Brazil. Cognate DNA–B components was found for all sequences, demonstrating that they are bipartite species. The sequences were confirmed by dideoxy Sanger sequencing using specific primer pairs. This upsurge of Neotropical bipartite begomoviruses in terms of diversity and host range represents a constant threat to a wide range of economically important vegetable and field crops.

The genus *Begomovirus* is the largest genus of the family *Geminiviridae* with more than 450 species (ICTV 2025). Viruses of this genus have a circular, single-stranded DNA (ssDNA) genome (Zerbini et al. 2017; Kumar 2019; Mietzsch et al. 2025). Monopartite (only with DNA–A) and bipartite (with DNA–A and DNA–B) species are considered one of the smallest plant virus genomes (Rojas et al., 2005; Zerbini et al., 2017; Kumar, 2019). Components of bipartite species share a common region (CR) of approximately 200 nucleotides (nts), which allows the identification of cognate genomic components of the same bipartite species (Li et al., 2015). Furthermore, the CR presents a conserved structure, with the presence of iterons and the nonanucleotide sequence (TAATATTAC), which corresponds to the initial site of viral replication (Briddon et al., 2010; Arguello-Astorga and Ruiz-Medrano, 2001).

In Brazil, there is a predominance of begomoviruses with bipartite genomes (Briddon et al., 2010; Navas-Castillo and Fiallo-Olivé, 2020; Reis et al., 2020; Reis et al., 2021). They are transmitted, in a non-propagative circulatory manner, by a complex of cryptic *Bemisia tabaci* species (De Barros and Ahmed 2011; Navas-Castillo et al. 2011; Cantú-iris et al., 2019; Rojas et al., 2018). For the classification of a novel *Begomovirus* species, the identity of the paired DNA–A sequences must be lower than 91% (Brown et al., 2015).

Begomoviruses can infect a wide array of high-value agricultural crops (Torralba et al. 2024). Weeds are often present under neotropical areas and play a relevant role as potential

viral reservoirs (Wang et al. 2021; García-Arenal and Zerbini 2019). Currently, several weeds have been described as hosts of begomoviruses: *Sida rombifolia* (Malvaceae family) (Maurício-Castillo et al. 2014; Rodríguez-Negrete et al. 2019); *Datura stramonium*, *Nicotiana glauca*, *Nicotiana plumbanginifolia*, *Solanum elaegnifolium*, *Solanum rostrarum* and *Solanum verbascifolium* (family Solanaceae) (Rodríguez-Negrete et al. 2019); *Digitaria ciliares*, *Eleusine indica*, *Panicum dichotomiflorum*, *Setaria faberi* and *Echinochloa crus-galli* (family Poaceae) (Kil et al. 2021); *Pyrenacantha* sp. (family Icacinaceae) (Chipiringo et al. 2022), *Oxalis latifolia* (family Oxalidaceae) (Pereira-Silva et al. 2022), *Macroptilium* spp. (Fabaceae family) (Melo et al. 2025), *Croton bonplandianus Baill* (Euphorbiaceae family) (Dhasan et al. 2025) and *Acalypha indica* (Fabaceae family) (Kumar et al. 2025).

In the present study, a total of 114 symptomatic weed samples associated with tomato crops, belonging to 15 botanical families, were collected in different regions of Brazil and Paraguay, from 2004 to 2022. Total DNA was extracted from these samples using the modified 2x CTAB protocol with organic solvents (Boiteux et al., 1999), and then stored at -20°C. The samples were enriched with circular DNA by RCA (Rolling Circle Amplification) (Inoue-Nagata et al., 2004) and subjected to High Throughput Sequencing (HTS) on the Illumina NovaSeq-6000 platform from Agrega, located in Rio Grande do Sul, Brazil.

The HTS data were analyzed as follows: (***i***) low-quality reads were eliminated; (***ii***) sequence reassembly was performed using the CLC Genomics Workbench 23.0.3 program; and (***iii***) the contigs were validated with the BLASTn algorithm, comparing them with the ssDNA Genbank virus database (https://www.ncbi.nlm.nih.gov/). Subsequently, the contigs of potential viruses were mapped as previously proposed (Nery et al. 2020, Reis et al. 2020) to obtain the final contigs corresponding to the genomes of these viruses. The contigs were extended using the 'Map to reference' tool, in the Geneious® 11.0.5 software, the minimum overlap identity parameter used was 90% to 99%, allowing mapping of the reads obtained in the HTS of each contig analyzed (Kearse et al., 2012).

The following raw reads from the HTS sequencing were obtained: 19,575,404 reads, 62,444 contigs, of which 103 sequences corresponded to viruses, as indicated by BLASTn analysis. More than 80% of these contigs (*n*=83) comprised previously characterized *Begomovirus* species as well as viruses from other genera (*Citlodavirus*, *Mulcrilevirus*, *Badnavirus*, *Caulimovirus*, *Gemycircularvirus*, and *Gemykolovirus*) and an alphasatellite (*Clecrusatellite*). However, eleven DNA-A contigs presented identities below 91%. According to the current taxonomic criteria of the genus *Begomovirus* (Brown et al., 2015), these contigs are characterized as new species of the genus.

The DNA-A genome of four the new species initially designated as new species #1 C493 (NS#1) (1,292,633 reads, 91% coverage) showed 87% identity with *Begomovirus costai* (Bean golden mosaic virus - BGMV, accession KJ939776.1) in BLASTn; the new species #2 C218 (NS#2) (605,816 reads, 91% coverage), 88% identity with *Begomovirus cleomecrispi* (Cleome leaf crumple virus - CleLCrV, accession FN435999.1); the new species #3 C64 (NS#3) (2,053,153 reads, 100% coverage), 83% identity with *Begomovirus leonuri* (Leonurus mosaic virus - LeMV, accession JX863082.1) and the new species #4 C08 (NS#4) (2,972,340 reads, 100% coverage), 81% identity with *Begomovirus solanumcontorsionis* (Tomato leaf distortion virus - ToLDV, accession KC706605.1). We also performed analyses of the iterons and iteron-related domains (Rep-IRD) in the sequences (Argüello-Astorga and Ruiz-Medrano, 2001). The cognate DNA-B was found for all species (**Fig. 1A**).

PCR assays were performed using species-specific primers. Four pairs of species-specific primers were developed to detect these species: **NS#1 – PC493F** (5'-GGT TAG CCG ATC AGT AAA CTT TTC C-3') and **PC493R** (5'-AAC TTC AAC ACC TAC AAA CAC CG-3'); **NS#2 – PC218F** (5'-TCT CAA ACT TGC GAT ATG TAT TGG AG-3') and **PC218R** (5'-GTA CGC AAA GGT CCT CAA TG-3'); **NS#3 – PC64F** (5'-TTC TTC GAA ATC CTG TGT TGC TGT-3') and **PC64R** (5'-GGG ACC ACA AAA GCA GGA GAA A-3'); and **NS#4 – PC08F** (5'-AAT TGC GTC TTT TAA GCC TAG A-3') and **PC08R** (5'-ATT AGC TCA TGA AAC CCC AG-3'). Species NS#1 and NS#2 were detected only in Malvaceae, and in the same samples, *Abutilon theophrasti* (RGO-835 and RGO-834) and *Sidastrum paniculatum* (RGO-832), all from the state of Goiás, collected in 2022. NS#3 was detected only in Lamiaceae - *Leonurus sibiricus*, collected in the state of Paraná, in 2011 (PR-126) and 2012 (PR-136). NS#4 was detected in a greater number of samples: four in the state of Goiás - RGO-836 (*Cucurbita moschata*, Cucurbitaceae), RGO-838 (*Saccharum officinarum*, Poaceae), RGO-834 (*Abutilon theophrasti*, Malvaceae) and RGO-833 (*Digitaria catamarcensis*, Poaceae), collected in 2022; two in the Federal District, only in Asteraceae (*Cichorium intybus*) - RDF-826 and RDF-827, collected in 2022; and only one sample of Malvaceae (*Sida acuta*), collected in Rio Grande do Sul (RS-082), in 2013. The sequences were confirmed by dideoxy Sanger sequencing using specific primer pairs in ACTGene (Rio Grande do Sul State).

Additional PCR assays with primers specific for eight begomoviruses and seven viruses of different genera were also performed, revealing mixed infections between begomoviruses and other genera. Interestingly, all samples found with mixed infections were collected in the state of Goiás, in Urutaí, in 2022. Samples RGO-835 and RGO-832 were identified as having mixed infections between the new species NS#1 and NS#2. In sample RGO-834, the species

NS#1 and NS#2 were detected, in addition to *Momordica charantia-associated gemycircularvirus* (*Gemycircularvirus*). Sample RGO-833 was infected with NS#4 and *Plant-associated genomovirus* 7 (*Gemykolovirus*). And in sample RGO-838, NS#4 and *Sugarcane bacilliform virus 1* (*Badnavirus*).

These new species present a DNA-A genomic organization typical of New World bipartite begomoviruses (Fig. 1A), displaying the ORFs: V1/CP (756 nts), encoding the viral coat protein; C1/Rep (1080-1089 nts), responsible for the replicase; C2/TrAp (390 nts), encoding the transcription transactivator protein; C3/REn (399 nts), encoding the replication enhancer protein; and C4 (258-294 nts), involved in gene silencing and symptom determination. The nucleotide sequence 5'–TAATATT/AC–3' (conserved among begomoviruses) was also located in the intergenic region (IR). NS#1 has a genome of 2.643 nucleotides (nts) and exhibited the iteron GGTG (Rep-IRD = MPPKRFKIN) (**Fig. 1B**); NS#2 has 2.657 nts and iteron TCTCC/GGAGAC (Rep-IRD = MPPPKRFRVN) (**Fig. 1B**); NS#3 has 2.661 nts and iteron ACTC/GGAG/GGAGTA (Rep-IRD = MPSKPRRFRVQ) (**Fig. 1B**) and NS#4 has 2.654 nts and iteron TCTC/GGAGA (Rep-IRD = MTPPKRFKIQ) (**Fig. 1B**).

To detect potential recombination events, the RDP5 program (Martin et al., 2020) was used, and recombination events were considered reliable only if detected by at least four of the seven methods implemented by the program. Therefore, only one significant recombination event was detected between NS#3 and NS#4. The recombination region involves 1.239 nts and encompassed ORFs AC1, AC2, AC3, and AC4. The identified methods were as follows: RDP 1.049×10^{-43}, GENECONV 6.166×10^{-40}, BootScan 1.670×10^{-41}, MaxChi 1.138×10^{-23}, and SiScan 2.122×10^{-33}.

The pairwise nucleotide sequence identities of the new species and other begomoviruses were calculated using SDT (Muhire et al., 2014). Phylogenetic analysis showed that NS#1 shares 79% to 80% identities with other begomoviruses, with the closest phylogenetic identity being 80% with NS#4 and 87% with BGMV (MN822294); NS#2 is phylogenetically closest to CleLCrV (FN4359999) with 88% identity; NS#3 is phylogenetically closest to 86% with ToLDV (NC_038474), and NS#4 has 86% identity and is phylogenetically closest to Sidastrum golden leaf spot virus – SidGLSV (NC_038462) (**Fig. 2**).

In Brazil, most recorded begomoviruses correspond to bipartite genomes (Reis et al., 2020; Reis et al., 2021). It is hypothesized that the great diversity of begomoviruses associated vegetable and field plants in Brazil originates from weeds and local wild hosts might give rise to novel virus strains and species. These viral variants might display distinct patterns of ecogeographic distribution and well as distinct host adaptation (García-Arenal and Zerbini

2019; Wang et al. 2021; Duarte et al. 2021). Recently, a tomato-associated strain of *Begomovirus cleomecrispi* (Cleome leaf crumple virus – CleLCrV) was identified via HTS (Reis et al., 2025). This isolate clustered with CleLCrV isolates from the original weed host (*Cleome affinis*), corroborating the hypothesis that host-jumping events of begomoviruses from weeds to tomato plants are occurring under natural and agroecosystem conditions (Reis et al., 2025).

In the present study, we report four putatively new begomovirus species detected in weeds from four Brazilian regions: DF, GO, PR, and RS. Weeds have the capacity to harbor multiple begomovirus species simultaneously, potentially leading to the emergence of new viral species that can infect commercial crops. Furthermore, we detected the presence of mixed infections, which reinforces the natural predisposition for recombination that occurs in the Begomovirus genus, resulting in the emergence of new variants and species (García-Rodríguez et al., 2023), highlighting the complexity of plant viromes in agricultural ecosystems (Pacheco-Dorantes et al., 2025). This upsurge of Neotropical bipartite begomoviruses in terms of diversity and host range represents a constant threat to a wide range of economically important vegetable and field crops.

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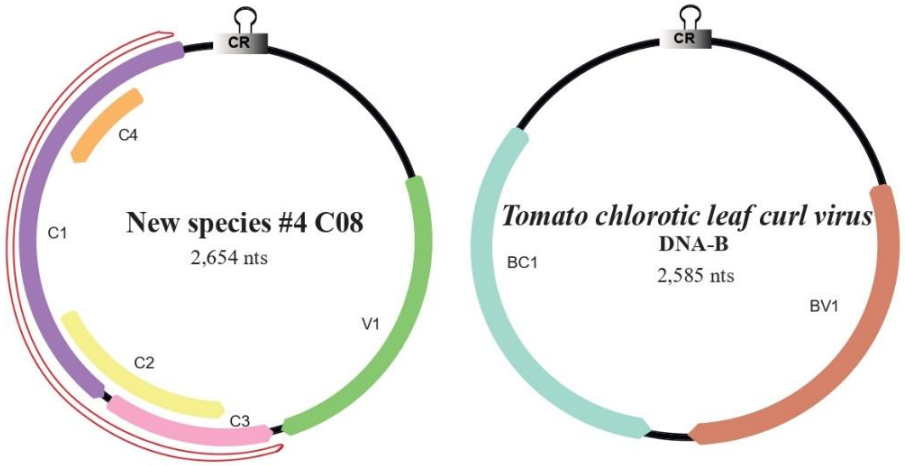

B)

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DNA-B GTGTCTCCAGATGAGTGTCTCCAATTGAGCTCCTCTCAAACTTGCGATATGTATTGGAGACTAGAGACAA
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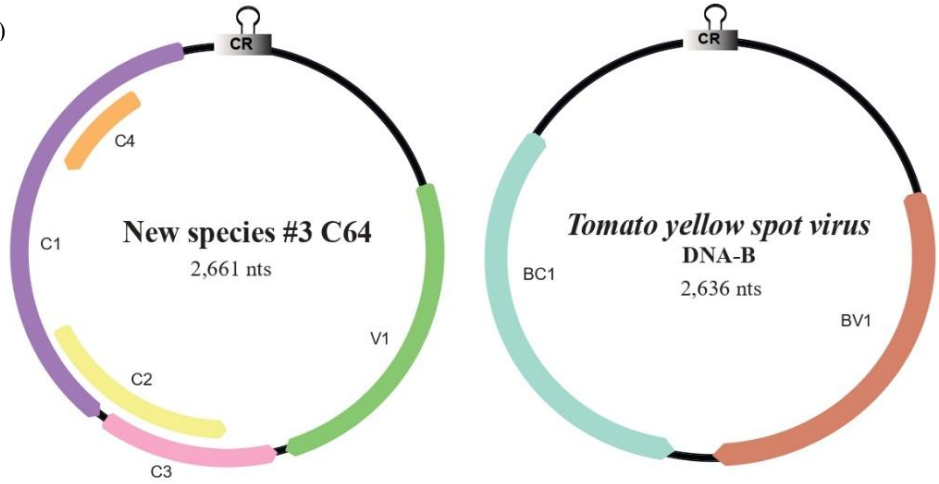

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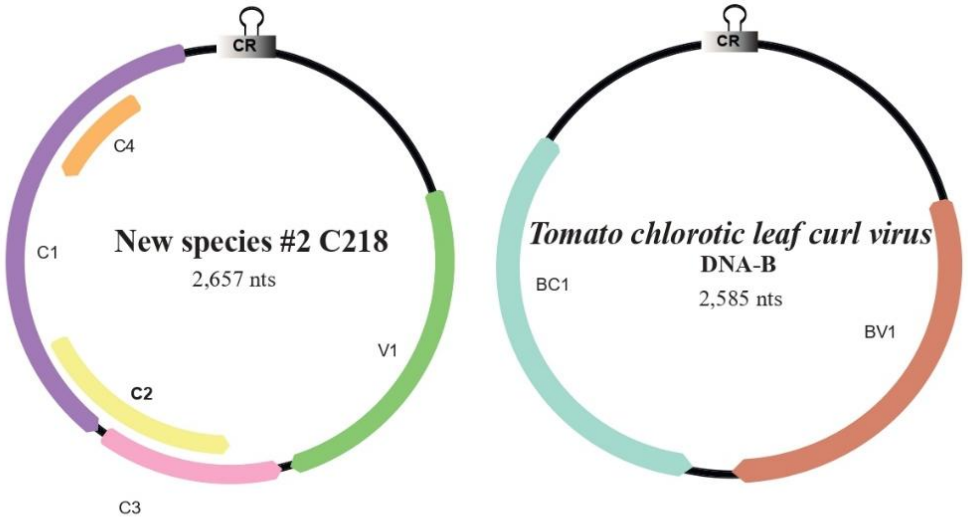

B)

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DNA-B GTGTCTCCAGATGAGTGTCTCCAATTGAGCTCCTCTCAAACTTGCGATATGTATTGGAGACTAGAGAC
ToCLCV AATATATAGTAGAGAAGTTCTCTAGGACCTCAGAACACGTGTCGGCCATCCGTTTAATATTACCG

DNA-B GTGTCTCCAGATGAGTGTCTCCAATTGAGCTCCTCTCAAACTTGCGATATGTATTGGAGACTAGAGAC
BGMV AATATATAGTATAGAAGTTCTCTAGGATCTCAGAACACGTGGCGGCCATCCGTTTAATATTACCG

A)

B)

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DNA-A GGTGCCAATATATAGTA-AGAAGTTCTTTAAGGATCTCTAGACACGTGGCGGCCATCCGTTATAATATTAC
CGGATGGCCGCGCGATTTTTG MPPKRFKIN

TGGCATACTTGTAAATAAGAGGGTGTACCCCGATTGAGCTCTCGTCTAAAAGTCTCTATGAATCGGTGTA
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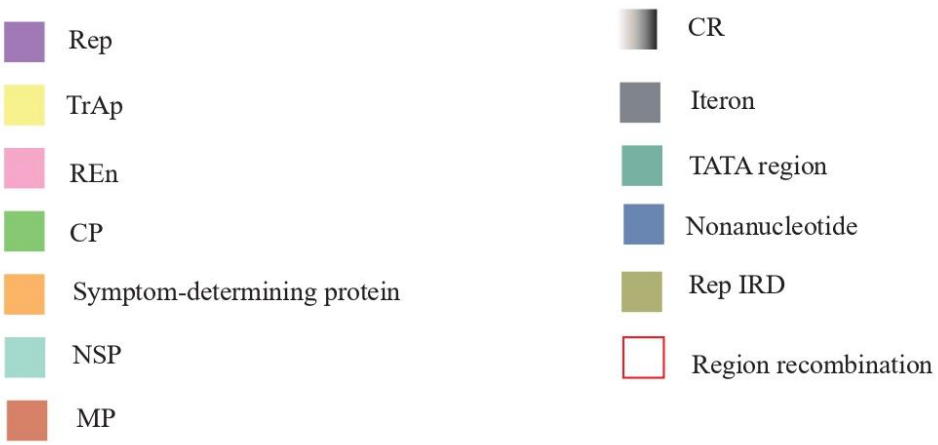

Figure 1. Genomic organization of the bipartite Begomovirus species that infects weeds. **Panel A**: Diagrammatic representation of the circular genomes of New species #1 a #4 and their respective open reading frames (ORFs). The ORFs V1, C1, C2, C3, and C4 are color-coded according to the putative function of their protein products. CP = capsid protein; Rep = replication-associated protein; TrAp = transactivator protein; Ren = replication enhancer; C4 = putative symptom determinant and silencing suppressor; IR = intergenic region, encompassing the hairpin. **Panel B**: A segment of the intergenic region showing iterons, TATA region, nonanucleotide, and at the end Rep = IRD (Rep Iteron-Related Domain).

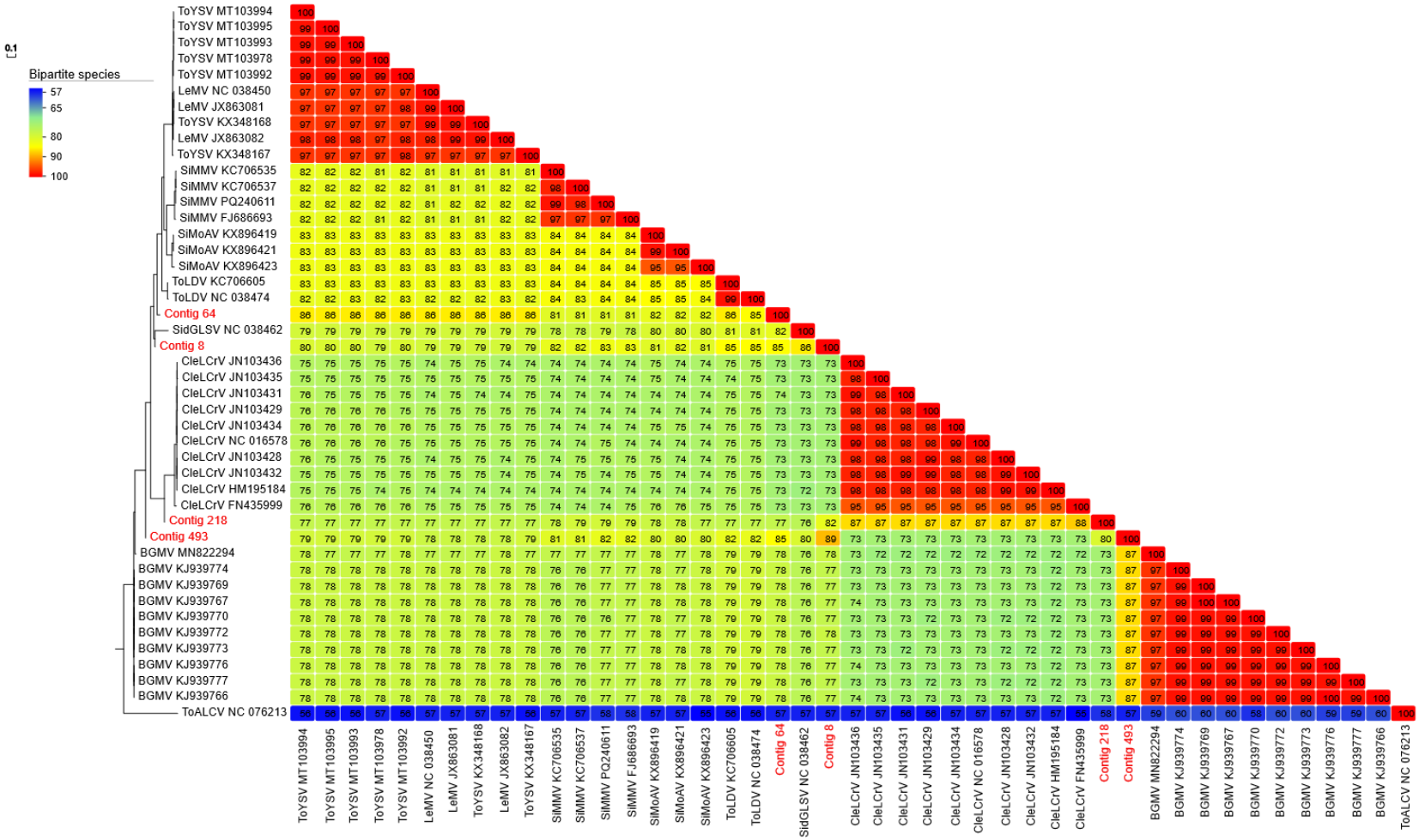

Figure 2. Pairwise identity in Sequence Demarcation Tool (SDT) analysis and phylogenetic reconstruction from DNA-A segment sequences of begomovirus associated tomato and weeds. Phylogenetic analysis showed that Contig 493 (NS#1) shares 79% to 80% identities with other begomoviruses, with the closest phylogenetic identity being 80% with NS#4 and 87% with BGMV (MN822294); Contig 218 (NS#2) is phylogenetically closest to Cleome leaf crumple virus - CleLCrV (FN4359999) with 88% identity; Contig 64 (NS#3) is phylogenetically closest to 86% with tomato leaf distortion virus - ToLDV (NC_038474), and Contig 08 (NS#4) has 86% identity and is phylogenetically closest to Sidastrum golden leaf spot virus – SidGLSV (NC_038462). Bean golden mosaic virus – BGMV, Cleome leaf crumple virus - CleLCrV, Leonurus mosaic virus – LeMV, Sidastrum golden leaf spot virus – SidGLSV, Sida micrantha mosaic virus – SiMMV, Sida mottle Alagoas virus – SiMoAV, tomato leaf distortion virus – ToLDV, tomato yellow spot virus – ToYSV. Outgroup: tomato apical leaf curl virus – ToALCV.

CHAPTER 5

First Report of *Mulberry crinkle leaf virus* Infecting *Morus alba* in Brazil

Work published in the Journal Plant Disease

First Report of Mulberry crinkle leaf virus Infecting *Morus alba* in Brazil

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Mulberry (*Morus alba* L. and *M. nigra* L.) cultivation is expanding worldwide (Giora et al. 2022). There is a major concern of the Brazilian agribusiness regarding the introduction of exotic viruses and their potential negative impacts on the domestic silk and mulberry fruit production. In China, geminiviruses have been identified in symptomatic *M. alba* plants (Lu et al. 2015; Lu et al. 2022), underlining the importance of monitoring the potential introduction of exotic viruses into new mulberry production areas. Surveys were carried out from September 2024 to February 2025, to analyze the association of geminiviruses in *M. alba* plants displaying mild mosaic and overall chlorosis in the apical leaves (~ 10% incidence) in orchards and individual trees in public areas of the Federal District (DF) region. A total of 17 symptomatic mulberry samples were collected. Total DNA from foliar tissues were extracted using a CTAB-based method (Boiteux et al. 1999) and used as a template for rolling circle amplification (RCA) (Inoue-Nagata et al. 2004). Equimolar amounts of the RCA samples were pooled for high-throughput sequencing (HTS) on an Illumina NovaSeq6000 platform (Agrega, Porto Alegre–RS, Brazil). Sequencing generated 19,575,404 reads (each of ≈ 150 nucleotides [nt]) that were processed and assembled in CLC Genomics Workbench 23.0.3 (Qiagen Bioinformatics, Denmark) and Geneious® 11.0.5 (Dotmatics, New Zealand). Virus-like contigs (n = 62,444) were detected corresponding to 103 species within five viral families. These contigs were compared with a local viral RefSeq database using BLASTn and BLASTx with Geneious® 11.0.5. One contig (C93) of 2,968 nucleotides (coverage of 8,869 reads) matched to a single-stranded circular DNA virus. Contig C93 (accession number PV277991) displayed a putative loop structure involving the nonanucleotide 5'–TAATATTAC–3', which is highly conserved among viruses of *Geminiviridae* family. BLASTn analysis of C93 returned 92–93% identity (95% query coverage) to isolates of mulberry crinkle leaf virus (MCLV; *Mulcrilevirus mori*) in GenBank. In pairwise comparisons using SDT v1.3 (Muhire et al. 2025), C93 shared 91.1 to

91.8% genome identity with several isolates of MCLV retrieved from GenBank and 60.9% identity with an isolate of paper mulberry leaf curl virus 1 (MN595125), the only other taxon in the genus *Mulcrilevirus*. The tentative species demarcation criterion for the genus *Mulcrilevirus* is 78% (Roumagnac et al. 2022), thus indicating that the sequence derived from C93 belongs to MCLV. To confirm the presence of MCLV across individual samples, a pair of abutting primers [F1 Mulcri (5'–GGG AAG TGT GAG TCG ATT GAG AGA AGG–3') and R1 Mulcri (5'–ACA CTT CCC ACT CCG CTC CAG A–3')] was designed based upon C93 and the RCA product was used as template for PCR (annealing temperature of 53 ºC). The expected full-genome fragment (≈ 2.9 kb) was amplified and MCLV was detected in a single sample (named as RDF–831). Sanger sequencing confirmed the viral identity. This is the first record of MCLV in Brazil and in South America. The leafhopper vector *Tautoneura mori* (Lu et al. 2022) was not detected thus far in Brazil, suggesting that MCLV was more likely introduced into the country via either contaminated tree seedlings or vegetative-propagated materials (cuttings). Therefore, the establishment of efficient detection systems for MCLV would be a recommended preemptive control strategy especially for new mulberry orchards.

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CHAPTER 6

A new Neotropical *Citlodavirus* species (*Geminiviridae*) associated with *Samanea tubulosa* (Fabaceae)

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Work for submission to the Archives of Virology

Abstract

High-Throughput Sequencing (HTS) is contributing to discovery of new viruses in a wide array of environmental samples and living organisms, including plants. Herein, HTS-based survey of plant species displaying single-stranded DNA virus-like symptoms allowed the recovery a new *Citlodavirus* species (*Geminiviridae*). Using PCR with species-specific primers, this new *Citlodavirus* was detected in *Samanea tubulosa* (family Fabaceae) in the Mato Grosso - MT (Central-West of Brazil). The genomic organization was typically of Citlodavirus genus and shares 72% identity with *Citlodavirus passiflorae*. The results reinforce the HTS-based technology is a valuable diagnostic tool for provide rapid information about plant viruses previously not detected. Additional studies are essential to determine the ecological relevance and putative pathogenic profile of this new citlodavirus in Neotropical agroecosystems.

Citlodavirus was recently established as one new genus with divergent genomes in the family *Geminiviridae* (Rougmanac et al. 2021; Rougmanac et al. 2022), and currently has six described species (ICTV 2025). This genus harbors viruses with monopartite genomes, ranging in size from 3.639 to 3.763 nucleotides (nts) (Roumagnac et al., 2022) and have the virion-strand origin of replication nonanucleotide motif 5'-TAATATTAC-3', conserved within the family (Fontenele et al., 2018). Members of the genus *Citlodavirus* have six open reading frames (ORFs) in their genomic organization. In the viral direction V1/CP (Coat Protein), V2/MP (Movement protein) and V3, and in the complementary direction C1/Rep and RepA (Replication-associated protein) and C2/TrAP (Transcriptional activator protein). In addition, *Citlodavirus* species also have the stem-loop, in the long intergenic region (RIL), containing the nonanucleotide (5'-TAATATTAC-3') (Rougmanac et al. 2021; Rougmanac et al. 2022). There no natural vector formally identified thus far. However, it has been postulated that the whitefly *Parabemisia myricae* is the vector of *Citlodavirus citri* (citrus chlorotic dwarf associated virus – CCDaV) (Loconsole et al., 2012). To corroborate this, phylogenetic analyses indicated that the amino acid and nucleotide sequences of *Citlodavirus* coat proteins are more closely related to members of the whitefly-transmitted *Begomovirus* genus (Rougmanac et al., 2022).

For the demarcation and identification of species within *Citlodavirus*, the criterion of 78% nucleotide sequence identity was adopted (Roumagnac et al., 2022; ICTV 2025). Like so, citlodaviruses whose genome sequences display less that 78% pairwise identity, coupled with

phylogenetic support, would be considered novel species (Roumagnac et al., 2022). Viruses of this genus have been detected in five botanical families: Theaceae, Rutaceae, Moraceae, Passifloraceae e Myricaceae. Among them, common camellia (*Camellia japonica*) (Zhang et al., 2018), citrus species (*Citrus* spp.) (Loconsole et al., 2012), paper mulberry (*Broussonetia papyrifera*) (Qiu et al., 2020), passion fruit (*Passiflora edulis*) (Fontenele et al., 2018), Chinese blueberry (*Myrica rubra*) (Gao et al., 2023), and the Jamaican honeybee (Bandoo et al., 2024). Only one of the six cataloged species of the genus is present in Brazil: *Citlodavirus passiflorae* (passion fruit chlorotic mottle virus – PCMov) (Fontenele et al., 2018). Four additional species were identified in Old World (China, Turkey, and Thailand), including *Citlodavirus citri* (Citrus chlorotic dwarf associated virus – CCDaV), *Citlodavirus camelliae* (Camellia chlorotic dwarf-associated virus – CaCDaV), *Citlodavirus broussonetiae* (Paper mulberry leaf curling associated virus 2 – PMLCV-2) and *Citlodavirus myricae* (Myrica rubra citlodavirus 1 – MRV1) (Guo et al., 2015, Yang et al., 2020; Yang et al., 2022; Zhang et al., 2018; Qiu et al., 2020; Gao et al., 2023). More recently, a new species *Citlodavirus apijamaicaense* (Apiscitlodal virus) was reported in Jamaica (Bandoo et al., 2024; Rubino et al., 2025). High-throughput sequencing (HTS) technologies have strongly contributed to expand our knowledge of viral and subviral diversity in different plant species (Maina et al. 2024; González-Pérez et al. 2024), including alphasatellites and ssDNA viruses from the *Geminiviridae* family (Queiroz-Ferreira et al., 2024; Oliveira et al. 2024; Reis et al. 2025a, Reis et al. 2025b; Reis et al. 2025c).

For this study, 114 symptomatic samples from 15 botanical families were collected between 2004 and 2022 in different Brazilian regions. For total DNA extraction, the modified CTAB 2x protocol with organic solvents was used (Boiteux et al., 1999) and submitted to enriched by RCA (Rolling Circle Amplification) (Inoue-Nagata et al., 2004) and the RCA products were pooled together and sequenced using Illumina NovaSeq-6000 platform 2 x 150 paired-end (Illumina) at Agrega (Rio Grande do Sul - Brazil). The HTS data were processed as follows: **(*i*)** low-quality reads were eliminated; **(*ii*)** sequences were reassembled using the CLC Genomics Workbench 23.0.3 program; and **(*iii*)** validation was performed using the BLASTn algorithm, comparing these sequences with the ssDNA Genbank virus database (https://www.ncbi.nlm.nih.gov/). The contigs were then mapped following the methodology proposed by Nery et al. (2020) and Reis et al. (2020), and the final contigs corresponding to the genomes of these viruses were obtained. The contigs were extended using the 'Map to reference' tool in Geneious 11.0.5 software, with a minimum overlap identity parameter of 90% to 99%, allowing mapping of the reads obtained in the HTS (Kearse et al., 2012).

The obtained reads (≈ 19,5 million) were trimmed and *de novo* assembled using CLC CLC Genomics Workbench 23.0.3 program, as described before. After this was possible to recover 62,444 contigs, which were classified using BLASTn against the viral RefSeq database (fev. 2023). Among these contigs, 103 sequences were correspondent to plant viruses. The contigs consist of previously characterized species of the genera *Begomovirus* (83), *Citlodavirus* (1), *Mulcrilevirus* (1), *Badnavirus* (11), *Caulimovirus* (1), *Soymovirus* (1), *Cyclovirus* (1), *Gemycircularvirus* (2), *Gemykolovirus* (1), and alphasatellite – *Clecrusatellite* (1).

One contig, with coverage of 3,707 nucleotides (nts) was identified and it was designated as the putative new viral species #6 C1998 (NS#6). The pairwise of nucleotide identity of NS#6 was below 78%. This result indicated that NS#6 correspond to a new *Citlodavirus* species, according to the current taxonomic criteria for the genus (Roumagnac et al., 2022). The ssDNA genome of NS#6 displayed 338 reads and 88% coverage, with 72.5% identity with *Citlodavirus passiflorae* (Passion fruit chlorotic mottle virus – PCMoV); accession NC_040706.1 (**Fig. 1**). A pair of specific primers was designed for this putative new species: **F1C1998** (26 nt): 5'-TTT TGA GGA AGG AAA GGA TGT ATT C-3' and **R1C1998** (25 nt): 5'-CCT CAA AAA TAA ACT CCA AGA ATA CGG-3'. The results of PCR assays revealed the presence of NS #6 in a Fabaceae sample (MT-012, *Samanea tubulosa*), collected in 2010 in Cuiabá, Mato Grosso State (North Region of Brazil). The complete sequence of the viral ssDNA was confirmed by dideoxy Sanger sequencing using specific primer pairs.

The genome NS #6 displays 3,707 nts with a ssDNA genomic organization typical of viruses of the genus *Citlodavirus* (Fig. 1A). NS #6 genome exhibits the ORF V1 (738 nts) that codes for the viral coat protein – CP; the ORF V2 (369 nts), which encodes the movement protein – MP; the ORF V3 (887 nts) that codes for a protein of yet unknown function, ORF C1 (960 nts), corresponding to the replicase protein – Rep and the ORF C1:C2 (843 nts), which encodes the replication-associated protein A – RepA (Qiu et al. 2020). In addition, it has an intergenic region (IR) that comprises the nucleotide sequence 5'–TAATATT/AC–3', conserved among members of the family *Geminiviridae*.

No significant recombination events were detected in the genome of NS #6, according to the parameters of the RDP5 program (Martin et al., 2020). The pairwise nucleotide sequence identities of this putative new *Citlodavirus* species as well other viruses in the genus were calculated using SDT (Muhire et al., 2014). This analysis showed that NS #6 shares 61–72% identities with other citlodaviruses, with the closest phylogenetic identity of 72% with

Citlodavirus passiflorae (Passion fruit chlorotic mottle virus – PCMoV), isolate of the United States (NC_040706) (**Fig. 2**).

The *Citlodavirus* constitute an emerging group within the *Geminiviridae* whose recent detection in cultivated plant species and alternative hosts suggests relevant sanitary implications. Currently, this group of viruses has been associated with diseases in passion fruit – *Passiflora edulis* (Fontenele et al. 2018), chinese blueberry – *Myrica rubra* (Gao et al., 2023), and species of *Citrus* (Loconsole et al., 2012). The citlodaviruses have been described as spillover events (Bandoo et al., 2024; Rubino et al., 2025), which indicates a complex maintenance and dispersal mechanisms in the agroecosystems. Molecular characterization and surveillance of these viruses are essential to understanding their diversity, identifying potential reservoir hosts, and developing effective management and quarantine measures, especially in tropical and subtropical regions, where plant diversity and agricultural intensification favor the emergence of epidemics.

In conclusion, the viruses identified herein as NS #6 is part of a unique group of *Citlodavirus*, representing the second record of a species of the genus in Brazil and the first report in a Fabaceae host, presenting enormous biological and molecular interest. These results expand our understanding of the distribution and evolution of *Citlodavirus* and highlight the role of new Fabaceae hosts as important components in the viral ecology in Neotropical agroecosystems.

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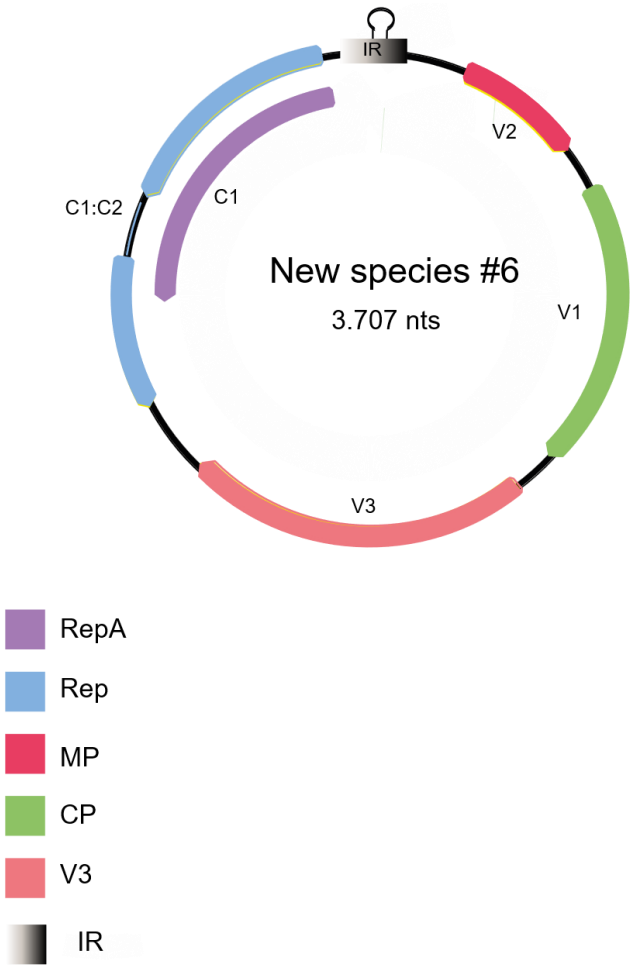
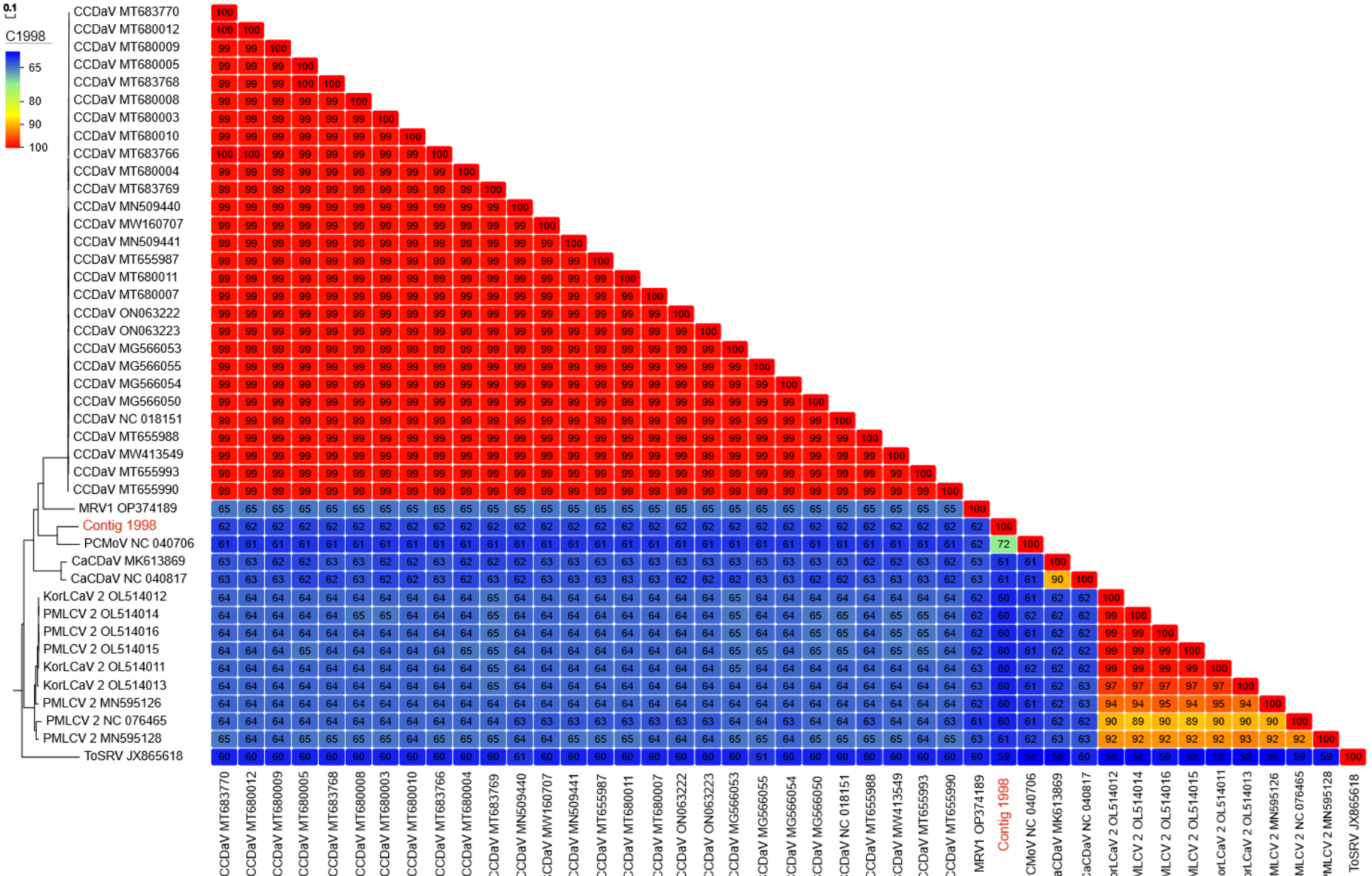


Figure 1. Ilustration of the genome organisation of citlodavirus from *Samanea tubulosa*. Diagrammatic representation of the circular genomes of the putative new species #6 and their respective open reading frames (ORFs). The ORFs V1, V2, V3, C1 and C1:C2 are color-coded according to the putative function of their protein products. CP = capsid protein; Rep = replication-associated protein; RepA = replication associated protein A; MP = movement protein; IR = intergenic region, encompassing the hairpin with TAATATTAC motif.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MRV1 OP374189
Contig 1998
PCMoV NC 040706
CaCDaV MK613869
CaCDaV NC 040817
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PMLCV 2 OL514014
PMLCV 2 OL514016
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KorLCaV 2 OL514011
KorLCaV 2 OL514013
PMLCV 2 MN595126
PMLCV 2 NC 076465
PMLCV 2 MN595128
ToSRV JX865618

Figure 2. Pairwise identity in Sequence Demarcation Tool (SDT) analysis and phylogenetic reconstruction from DNA segment sequences of *Citlodavirus* isolates associated tomato and weeds. Contig 1998 (NS#6) is most phylogenetically related to *Citlodavirus passiflorae* (Passion fruit chlorotic mottle virus – PCMoV), isolate NC_040706, sharing 72% pairwise identity, to which it is phylogenetically closest. Camellia chlorotic dwarf-associated virus – CaCDaV, Citrus chlorotic dwarf associated virus – CCDaV, Kozo leaf curling associated virus 2 – KorLCaV-2, Myrica rubra citlodavirus 1 – MRV1, passion fruit chlorotic mottle virus – PCMoV, Paper mulberry leaf curling associated virus 2 – PMLCV-2. Outgroup: tomato severe rugose virus – ToSRV.

CHAPTER 7

First Report of *Begomovirus cleomecrispi* in Paraguay

Work for submission to Plant Disease

First Report of *Begomovirus cleomecrispi* in Paraguay

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Weeds may contribute to the spread of plant virus epidemics by allowing to survive and acting as reservoirs of viruses, including begomoviruses and their vectors (*Bemisia tabaci*). In Brazil, one of this begomovirus comprises CLeLCrV (Cleome leaf crumple virus, genus *Begomovirus*, family *Geminiviridae*) initially identified in *Cleome affinis* (Wyant et al., 2012), and reported inducing symptoms in *Phaseolus* (P*haseolus lunatus* e *Phaseolus vulgaris*) (Wyant et al., 2012) and *Solanum lycopersicum* (tomato) plants (Reis et al., 2025), reinforcing the importance of monitoring the adaptation potential of this virus in new cultivar production areas. A total of 114 symptomatic samples exhibiting mild mosaic and chlorosis in apical leaves were collected between 2004 and 2022 across the five Brazilian regions (113) and Paraguay (2). Total DNA from leaf tissues was extracted using the CTAB with organic solvents (Boiteux et al. 1999), which then served as a template for rolling circle amplification (RCA) (Inoue-Nagata et al. 2004). The RCA samples, in equimolar quantities, were pooled for High-Throughput Sequencing (HTS) on the Illumina NovaSeq6000 platform (Agrega, Porto Alegre, RS, Brazil). A total of 19,575,404 reads (≈ 150 nucleotides [nt] each) were generated in the sequencing, which were processed and assembled in CLC Genomics Workbench 23.0.3 (Qiagen Bioinformatics, Denmark) and Geneious® 11.0.5 (Dotmatics, New Zealand). A total of 62,444 virus-like contigs were detected, equivalent to 103 contigs of viruses distributed in five viral families. These contigs were compared with a local viral RefSeq database using BLASTn and BLASTx with Geneious® 11.0.5. Two of these contigs was correspondent to a new isolate of CleLCrV. The DNA-A (C7119) with 2.730 nucleotides (nts) e coverage of 13,47 reads and the DNA-B (C156) with 2.662 nts and coverage of 12.613 reads were recovered by HTS and confirmed in one sample as described below. Both contigs contained the nonanucleotide 5'–TAATATTAC–3', highly conserved among viruses of the *Geminiviridae* family. BLASTn analysis of C7119 returned 94.33% identity (100% query coverage) with DNA-A of *Begomovirus cleomecrispi* (= *Cleome leaf crumple virus*) (Accession FN435999.1,

GenBank) and C156 showed 91.56% identity (100% query coverage) with the DNA-B isolate of the CleLCrV virus (Accession FN436000.1).

In pairwise comparisons using SDT v1.3 (Muhire et al. 2025), C7119 shared 92 to 94.33% genomic identity with several CleLCrV DNA-A isolates retrieved from GenBank, and C156 shared 88.29 to 91.56% identity with CleLCrV DNA-B isolates. The provisional species demarcation criterion for the genus *Begomovirus* is 91% pairwise identities across the entire DNA-A genome (Brown et al. 2015), thus indicating that the C7119-derived sequence belongs to CleLCrV. To confirm the presence of CleLCrV DNA-A and DNA-B in individual samples, a pair of primers for DNA-A [CleLCrV-A-For (5′– GAC TCG ACG TTC TGT GGT –3′) and CleLCrV-A-Rev (5′– TCC TAG TCG GGG CTC ACT –3′)] and DNA-B segment [CleLCrV-B-For (5′– TAG GAA AGC AAA ACG AGA ATG GAA –3′) and CleLCrV-B-Rev (5′– GCT TTC CTA AAT CGC AAT TGA TC –3′)] were designed and used in PCR assays. The RCA product was used as a template for PCR (annealing temperature of 51°C for DNA-A and 58°C for DNA-B). The complete genome for both segment (DNA-A and DNA-B) was recovered in *Cleome affinis*, (sample PAR-005 collected in Mayor Otaño, Paraguay, in 2010). Sanger sequencing confirmed the viral identity. This is the first record of CleLCrV in a sample from Paraguay. The *Bemisia tabaci* vector has already been detected in Paraguay, with recent reports of biotypes B and Q (Espinoza-Morel et al., 2024). The presence of the vector favors the introduction and circulation of new *Begomovirus*, increasing infections in crops and weeds near crops. Therefore, continuous monitoring of these viruses, especially in weeds, is essential to prevent viral spread in agricultural crops.

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CHAPTER 8

First Report of a *Gemycircularvirus mocha1* associated to mallow and cabbage in Brazil

Work for submission to the New Disease Reports

First Report of *Gemycircularvirus mocha1* in mallow and cabbage

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Additional key words: *Brassica oleracea*, *Abutilon theophrasti*s, viruses plant disease

Mallow (*Abutilon theophrasti*s) and cabbage (*Brassica oleraceae* var. *capitata*) are found in five Brazilian regions. Surveys were carried out from October of 2022, and two symptomatic samples of weeds and cultivated and, non-cultivated plants displaying mild mosaic and generalized chlorosis and mosaic (Fig. 1) were collected of weeds and non-cultivated plants were collected closed tomato crops in the Federal District of Urutaí, Goiás state. Total DNA from leaf tissues was extracted using the CTAB method with organic solvents (Boiteux et al., 1999) and used as a template for Rolling Circle Amplification (RCA) (Inoue-Nagata et al., 2004). The RCA samples were pooled after quantification and submitted to High-Throughput Sequencing (HTS) on the Illumina NovaSeq6000 platform (Agrega, Porto Alegre, RS, Brazil). As a result, a total of 19575404 reads (average read length of 150 bp) was obtained and processed and assemble using CLC CLC Genomics Workbench 23.0.3 (Qiagen Bioinformatics, Denmark) and Geneious® 11.0.5 (Dotmatics, New Zealand). Virus-like contigs were detected (62,444) and compared with a local viral RefSeq database using BLASTn and BLASTx with Geneious® 11.0.5.

One contig named as C1043 with 2,195-nucleotides and covering of 566 reads was completely recovered and after pairwise sequence comparisons using BLASTn analysis of sequence C1043 display 91.26% identity (99% of query coverage) with the species *Gemycircularvirus mocha1* (*Momordica charantia-associated gemycircularvirus*) [NC_075310.1]. The species demarcation classifications for the genus *Gemycircularvirus* is 78% identity (Varsani and Krupovic 2017), thus indicating that the sequence derived from C1043 belongs to the DNA of *Gemycircularvirus mocha1*. In order to confirm the presence of *Gemycircularvirus mocha1* in individual samples, a pair of adjacent primers for DNA detection [MomoF (5'– CCC ACC CGA AAA AGC TCT TACG –3') and MomoR (5'– GGG GTG AGG GAT TTT CGG GT – 3')] was designed based on C1043. The RCA product was used as a template for PCR (annealing temperature of 56°C). The expected genome for the DNA fragment (≈2.1 kb) was amplified, and Gemycircularvirus mocha1 was detected in two different samples: cabbage (*Brassica oleracea var. capitata*) from the Federal District region (isolate DF-824) and mallow (*Abutilon theophrasti* – RGO-834), both of them collected in state of Goiás. Sanger sequencing of partial genome confirmed viral identity. This is the first record of *Gemycircularvirus mocha1* in cabbage and mallow samples. Viruses of the genus *Gemycircularvirus* (family *Genomoviridae*) have been reported in various hosts, including cultivated hosts such as cassava (*Manihot esculenta*) (Onile-Ere et al., 2025) and tomato (*Solanum lycopersicum*) (Reis et al., 2022), and in weeds (de Rezende et al., 2018). This reflects their ability to infect different plant groups in agricultural and non-agricultural environments. Although there are no known vectors for viruses of the genus *Gemycircularvirus* (Varsani et al., 2017), a progressive expansion of their host range has been observed, including cultivated species and weeds. This indicates a process of diversification and adaptation that deserves attention. This scenario highlights the importance of ongoing studies on the epidemiology and potential impact of these viruses on agroecosystems.

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FIGURE 1

Leaf of *Abutilon theophrasti*s with widespread chlorosis and intense yellowing of the leaf blade.

CHAPTER 9

First Report of *Gemykolovirus heris*1 in *Digitaria catamarcensis* in Brazil

Work for submission to the New Disease Reports

First Report of *Gemykolovirus heris*1 in *Digitaria catamarcensis* in Brazil

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Additional key words: high-throughput sequencing, viruses plant disease

Weeds plant are an invasive species widespread and sub-tropical and tropical areas associated to a wide array of field and vegetable crops. In October 2022, *Digitaria catamarcensis* plants (occurring nearby to commercial tomato fields) were found exhibiting mild mosaic and overall chlorosis (Fig. 1-2). For analysis, total DNA was extracted from leaf tissues using the CTAB method with organic solvents (Boiteux et al., 1999). The purified total DNA was then employed as a template in Rolling Circle Amplification (RCA) assays (Inoue-Nagata et al., 2004). The RCA samples were sent for High-Throughput Sequencing (HTS) on the Illumina NovaSeq6000 platform (Agrega, Porto Alegre, RS, Brazil), after being pooled in equimolar amounts. The sequencing resulted in 19,575,404 reads (≈ 150 nucleotides each), which were processed and assembled using the CLC Genomics Workbench 23.0.3 (Qiagen Bioinformatics, Denmark) and Geneious® 11.0.5 (Dotmatics, New Zealand) software. A total of 62,444 viral contigs were detected after HTS analysis. A unique virus-like contig (named C5696) was identified after its comparison with a viral RefSeq database using BLASTn and BLASTx with Geneious® 11.0.5. The contig C5696 displayed 2,208 nucleotides with a coverage of 98 reads. BLASTn analysis of the C5696 fragment resulted in 88.44% identity and 56% match coverage with the DNA isolate of the species *Gemykolovirus heris*1 (*Gemykolovirus*) [NC_076273.1]. The provisional criterion for species demarcation in *Gemykolovirus* is 78% matched identity in the entire

genome (Varsani and Krupovic 2017). According to this criteria, the contig C5696 corresponds to a strain of the species *Gemykolovirus heris1* (= *Plant-associated Genomovirus* 7). A pair of adjacent primers [GemykF (5'– GCT CTT CGA ATA TCT CTT CCG –3') and GemykR (5'– GGT GAC TGT TCG GAC CAT TC –3')] was designed based on the C5696 sequence aiming to confirm the presence of this viral species in individual samples. For the PCR reaction (annealing temperature of 56°C), the RCA product was used as a template. The expected complete genomic fragment (≈2.2 kb) was amplified, and *Gemykolovirus heris*1 DNA was detected in only in one *Digitaria catamarcensis* (Poaceae) sample (RGO-833), which was collected in 2022 in Urutaí, Goiás State (Central Brazil). Sanger sequencing confirmed the viral identity. *Digitaria* is a pantropical genus, encompassing a large number weed species of economic importance (Touafchia et al., 2023). To our knowledge, this is the first worldwide record of *Gemykolovirus heris*1 in *D. catamarcensis*. The genus *Gemykolovirus* (family *Genomoviridae*) encompasses yet poorly characterized species, known mainly from metagenomic studies. Viruses of the genus *Gemykolovirus* have been identified in insects (Rosário et al., 2018) and in various plant species, including weeds (Nery et al., 2023), suggesting a possible ecological virus-arthropod interaction in nature as well as agroecosystems. Despite this, no vector was detected with involvement in its transmission thus far. Nevertheless, the increasing detection of these viruses in agricultural environments, reinforces the importance of continuous monitoring this viruses and their natural hosts, including weeds. Additional studies are essential to better understand the ecology and potential dissemination pathways of *Gemykolovirus*, contributing to the assessment of emerging phytosanitary risks and guiding management strategies.

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FIGURE 1

A. Leaf of *Digitaria catamarcensis* with mosaic and generalized chlorosis, with chlorotic areas following the direction of the parallel veins. **B.** Leaf of *Digitaria catamarcensis* with yellowing and necrosis.

CAPÍTULO 10

Conclusões e Perspectivas

Em síntese destacam-se algumas importantes informações e conclusões neste capítulo. As plantas daninhas estão intrinsecamente associadas aos sistemas de cultivo agrícola, ocorrendo de forma recorrente em áreas de produção, principalmente de tomateiro, uma hortaliça de grande importância econômica mundial e nacional. Esse fato impacta diretamente nos eventos de salto de hospedeiras, visto que as plantas daninhas funcionam como reservatórios virais e de vetores. Além disso, plantas daninhas são capazes de abrigar uma grande variedade viral, colaborando para a existência de infecções mistas e consequentemente favorecendo a ocorrência de variabilidade genética através dos processos de recombinação e pseudo-recombinação. Plantas daninhas apresentam características peculiares relacionadas à rusticidade, dormência e abundância no banco de sementes de solos. No contexto deste trabalho, embora o foco seja plantas com estas características, permitiu-se também coletas contemplando o conceito amplo usado muitas vezes como sinonímia como plantas alternativas e invasoras. Estas plantas têm sido relatadas como hospedeiras de diversos isolados de gêneros virais, com destaque para o gênero *Begomovirus* (família *Geminiviridae*). Begomovirus apresentam ssDNA em encapsidado separadamente em uma (espécies monopartidas) ou duas partículas (espécies bipartidas), e são transmitidos com grande eficiência pelo vetor aleirodídeo *Bemisia tabaci.*

Isolados de outros gêneros virais, tanto de RNA como de ssDNA vêm sendo recentemente identificados tanto em plantas daninhas quanto em plantas cultivadas que ocorrem espontaneamente no entorno das áreas de cultivo, ampliando a descoberta de novas espécies virais e aumentando a gama de hospedeiras de espécies já conhecidas. Neste contexto ferramentas como *Hight-Throughput Sequencing* (HTS) e softwares de bioinformática vem permitido grandes avanços na Ciência, incluindo a Virologia. O estudo do viroma em plantas tem crescido desde os primeiros usos do HTS (Kreuze et al. 2009; Al Rwahnih et al. 2009; Adams et al. 2009), e essa técnica vem sendo utilizada em vários estudos, permitindo a descoberta e a caracterização de uma grande diversidade de vírus. Associada a ela, a *Polymerase Chain Reaction* (PCR) e a *Rolling Circle Amplification* (RCA) contribuem para o enriquecimento e detecção de vírus, permitindo, além do sequenciamento, a detecção nas amostras individuais.

Contudo, observa-se uma lacuna significativa de estudos direcionados às plantas daninhas, nas quais o universo viral ainda permanece amplamente pouco explorado. Dessa forma, o objetivo do presente estudo foi realizar uma prospecção visando contribuição e levantamento referente a uma ampla variedade de plantas daninhas sintomáticas (114), pertencentes a 15 famílias botânicas, que foram coletadas nos arredores de plantações de

tomate, localizadas nos cinco estados brasileiros e no Paraguai. Sendo considerado o trabalho de viroma com maior número de famílias botânicas até o presente momento.

O resultado do HTS gerou 19,575.404 reads e 62,444 *contigs*, distribuídos em 10 gêneros: *Badnavirus* (2), *Begomovirus* (12), *Caulimovirus* (1), *Citlodavirus* (1), *Clecrusatellite* (1), *Cyclovirus* (1), *Gemycircularvirus* (1), *Gemykolovirus* (1), *Mulcrilevirus* (1), e *Soymovirus* (1). O gênero Begomovirus foi o mais expressivo, corroborando com os resultados de diversas pesquisas. Desse resultado, seis espécies novas foram identificadas, cinco delas pertencentes ao gênero *Begomovirus* e uma do gênero *Citlodavirus*, reforçando a ideia de que as plantas daninhas abrigam um universo viral ainda amplamente desconhecido.

Nesse sentido, a caracterização de cinco novas espécies de begomovírus evidencia a elevada diversidade e complexidade evolutiva desse grupo viral. Dentre essas espécies, uma delas apresenta genoma monopartido e quatro genomas bipartidos, sendo particularmente relevante o registro do begomovírus monopartido, uma organização genômica raramente observada no Novo Mundo. Esses resultados reforça a ocorrência de eventos evolutivos e de adaptação ainda pouco compreendidos nesse grupo viral. Paralelamente, a identificação de uma nova espécie de *Citolodavirus* amplia o escopo da diversidade viral descrita, indicando que o viroma associado a essas plantas permanece amplamente subexplorado.

Para a detecção das plantas hospedeiras que abrigam esses vírus, foram desenhados 15 pares de *primers* específicos neste estudo. Esses *primers* foram empregados em reações de PCR, possibilitando a amplificação de regiões genômicas alvo e resultando em um total de 53 detecções positivas. A utilização desses primers contribuiu para a confirmação da presença viral em diferentes amostras, evidenciando sua eficiência e especificidade na identificação dos vírus associados às plantas hospedeiras analisadas, confirmadas por sequenciamento *Sanger*.

Dentre as famílias botânicas das plantas coletadas (Asteraceae, Bignoniaceae, Cactaceae Brassicaceae, Capparaceae, Caricaceae, Cleomaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Moraceae, Poaceae, Ruscaceae e Solanaceae), a família Lamiaceae foi a que apresentou o maior número de vírus detectados, abrigando 10 detecções do vírus *Begomovirus leonuri* (*Leonurus mosaic virus*) em *Leonurus Sibiricus*. Além disso, as diversas detecções também nos permitiram a identificação de infecções mistas, reforçando a ideia de que as plantas daninhas são capazes de hospedar uma ampla gama de vírus, de diferentes gêneros em uma mesma amostra.

Os resultados obtidos também revelam avanços expressivos no que se refere à ampliação da distribuição geográfica e do espectro de hospedeiras de diferentes grupos virais. A detecção de *Mulcrilevirus* no Brasil representa um resultado expressivamente relevante, uma vez que,

até então, esse grupo havia sido relatado exclusivamente na China, configurando um importante registro de expansão geográfica. De forma semelhante, as detecções envolvendo *Gemycircularvirus* e *Gemykolovirus* constituem novidades relevantes, tanto pela identificação em novas localidades quanto pela associação com hospedeiras ainda não descritas para esses vírus. Adicionalmente, o novo relato envolvendo *Cleome leaf crumple virus (Begomovirus cleomecrispi)* no Paraguai, reforça a importância das plantas espontâneas e pouco estudadas como hospedeiras de vírus emergentes. Em conjunto, esses dados evidenciam a dinâmica e a plasticidade da distribuição viral, ressaltando que diferentes regiões e hospedeiras ainda permanecem subamostradas, o que reforça a relevância de estudos voltados à prospecção e caracterização da diversidade viral em distintos contextos geográficos.

Destarte, os resultados obtidos no presente trabalho demonstraram-se extremamente relevante ao ampliar de maneira significativa o conhecimento sobre a diversidade e a distribuição de vírus associados a plantas daninhas em agroecossistemas, corroborando de forma consistente evidências descritas na literatura, que apontam as plantas daninhas como importantes reservatórios virais nos agroecossistemas. A identificação de múltiplas espécies virais evidencia a elevada diversidade viral associada a essas plantas, fornecendo subsídios fundamentais para a compreensão do papel dessas plantas como reservatórios virais, além de contribuir para o aprimoramento de estratégias de monitoramento fitossanitário. Adicionalmente, as detecções reforçam não apenas a ampla ocorrência desses vírus em diferentes hospedeiras, mas também a eficiência das abordagens moleculares empregadas. Em conjunto, os resultados demonstram que as plantas daninhas não atuam apenas como hospedeiras ocasionais, mas desempenham um papel central na manutenção, diversificação e potencial disseminação de vírus, ressaltando sua relevância epidemiológica e a importância de estudos voltados à caracterização do viroma associado a essas espécies, que podem favorecer abordagens mais integradas de vigilância, manejo e prevenção de viroses em sistemas agrícolas. Dessa forma, este estudo não apenas preenche lacunas importantes do conhecimento científico, como também se consolida como uma referência para pesquisas voltadas ao monitoramento e à gestão de plantas daninhas em agroecossistemas.

Embora os resultados obtidos representem avanços relevantes, estes ainda abrem espaço para investigações futuras. No contexto do gênero *Badnavirus*, estudos adicionais envolvendo a detecção por PCR para a identificação da hospedeira do *Banana streak CA virus* são recomendados, assim como a verificação da possibilidade de infecção episomal devido os Elementos virais endógenos característicos da família *Caulimovirida*; e também a aplicação de abordagens de *primer walking* para a recuperação dos genomas completos de *Blueberry red*

ringspot virus (*Soymovirus*) e *Calfel virus* LSF31_cyc880 (*Soymovirus*). Além disso, ensaios biológicos com as seis novas espécies descritas poderão fornecer informações fundamentais sobre infectividade, patogenicidade e interação vírus–hospedeiro.

ANEXOS

Resumos publicados em anais de congressos

III Simpósio de Biologia Microbiana, “Biodiversidade microbiana: Interações e Aplicações” realizado na Universidade de Brasília, no período de 27 a 29 de novembro de 2023.

Metagenomics of begomoviruses associated with tropical and subtropical weeds from 15 botanic families

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Resumo: Begomoviruses, responsible for many emerging diseases, currently constituting one of the main biotic problems in several crops, especially in tomatoes (*Solanum lycopersicum*). In this sense, weeds have been studied as virus reservoirs. With the aim of identifying viral diversity in weeds associeated wih tomato, High-Throughput Sequencing (HTS) was used. A total of 114 samples of symptomatic weeds belonging to 15 botanical families were collected. These were subjected to DNA extraction, enriched by Rolling Circle Amplification (RCA) and sequenced, resulting in 19,575,404 reads and 62,444 contigs. In this way, 103 viral genomes were recovered, of which 83 contigs were associated with the *Begomovirus* genus. Of these, six genomes presented identity below 91%, being classified as putative new species. The contig 218 showed 90.44% identity with cleome leaf crumple virus (ClLCrV); whereas contig 493 displayed 89.38% identity with bean golden mosaic virus (BGMV); contig 5250 with 85.17% identity to tomato severe rugose virus (ToSRV); contig 12847 displayed 85.18% identity with tomato leaf distortion virus (ToLDV), contig 159 showed 89.43% identity with Leonurus mosaic virus (LeMV), and contig 12357 displayed 88.99% identity level with BGMV. The results indicate that weeds might function as potential reservoirs of virus, which can induce negative impacts in crops of economic importance.

Key words: *Begomovirus*; Tomato; Weeds; *High-Throughput Sequencing.*

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IV Simpósio de Biologia Microbiana, "Adaptações microbianas às mudanças climáticas", realizado na Universidade de Brasília, no período de 27 a 29 de novembro de 2024.

Plantas daninhas: vilãs ou vítimas em co-evolução com espécies de *Geminiviridae*

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Palavras-chave: Metagenoma; *High-Throughput Sequencing*; Begomovirus.

Resumo: Os vírus são responsáveis por muitas doenças emergentes, atualmente constituindo um dos principais problemas bióticos em várias culturas, incluindo o tomateiro (*Solanum*

lycopersicum). Nesse sentido, plantas daninhas associadas ao tomateiro vêm sendo apontadas como os principais reservatórios de patógenos virais. A utilização em viromas vegetais de plataformas de sequenciamento aliada com análises de bioinformática têm permitido (desde 2009) a descoberta e catalogação de novas espécies virais, especialmente dentro da família *Geminiviridae*. Neste contexto, o *High-Throughput Sequencing* (HTS) foi utilizado com o objetivo de identificar a diversidade viral presente em plantas daninhas associadas ao tomateiro. Foram coletadas 114 amostras de plantas daninhas sintomáticas em 15 famílias botânicas, distribuídas nas cinco macro-regiões do Brasil e no Paraguai, no espaço temporal de 2004 a 2022. As amostras foram submetidas a extração de DNA e enriquecidas para ssDNA por amplificação do círculo rolante (RCA), e em seguida sequenciadas na plataforma Illumina NovaSeq 6000. Foram recuperados 62.444 *contigs,* correspondendo a 103 genomas virais. As sequências se distribuíram em dez gêneros: *Badnavirus* (11), *Begomovirus* (83), *Caulimovirus* (1), *Citlodavirus* (1), *Clecrusatellite* (1), *Cyclovirus* (1), *Gemycircularvirus* (2), *Gemykolovirus* (1), *Mulcrilevirus* (1) e *Soymovirus* (1). A maioria das espécies foi classificada dentro do gênero *Begomovirus* (80,58% dos *contigs*). Em relação ao gênero *Begomovirus*, foram recuperadas 12 espécies conhecidas e cinco potenciais espécies novas. Os dados obtidos apontam que as plantas daninhas podem desempenhar o papel de reservatórios de vírus de ssDNA. Entretanto não se sabe se estes vírus, principalmente espécies novas, são originários de plantas daninhas ou alguma espécie cultivada. Desta forma, uma atenção maior deve ser dada as plantas daninhas, pois representam um potencial risco para culturas de alto valor econômico. O presente trabalho amplia a compreensão sobre as potenciais fontes de infecção, sugerindo que as interações entre culturas comerciais e espécies invasoras desempenham um papel crucial na disseminação viral e no estabelecimento de estratégias de controle efetivo dessas doenças.

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NEW BEGOMOVIRUS INFECTING LEONURUS JAPONICUS PLANTS IN TOMATO FIELDS

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Plant viruses are a major threat to crop production worldwide, including tomato (*Solanum lycopersicum*). Weeds infesting tomato fields might be important reservoirs of begomoviruses. In this study, we report a new *Begomovirus* species (*Geminiviridae*) infecting *Leonurus japonicus* (Lamiaceae). A total of 114 symptomatic weed samples (15 botanical families) were collected between 2004 and 2022 across five Brazilian regions. The samples were subjected to DNA extraction (CTAB method), used as a template in Rolling Circle Amplification (RCA), and submitted to High-Throughput Sequencing (HTS), using the Illumina NovaSeq 6000 platform. The data were assembled and analyzed using CLC Genomics Workbench v.8.0 (Qiagen), Geneious® 11.1.5 and BLASTn (GenBank RefSeq), resulting in a total of 19,575.404 reads and 62,444 contigs. After Muscle alignment and SDT, a contig (named as #329) displayed the highest nucleotide identity of 84% with *Begomovirus solanumseverugosi* (tomato severe rugose virus – ToSRV) (MW596573). This value is below the species demarcation limit established by the ICTV (91%), indicating the identification of a novel *Begomovirus* monopartite species. Specific primers (PC329F/PC329R) were designed, and PCR assays confirmed the presence of the novel begomovirus in one *L. sibiricus* sample (PR–134) collected in Paraná State (South Brazil). This result reinforces the role of weed species as reservoirs of viruses, favoring their maintenance and dissemination across distinct agroecosystems.

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LARGE-SCALE METAGENOMIC ANALYSIS OF THE DIVERSITY OF SINGLE-STRANDED DNA VIRUSES IN TOMATO-ASSOCIATED WEEDS

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Weeds are customary reservoirs and alternate hosts of plant pathogens, including single-stranded DNA (ssDNA) gemiviruses. In the present study, High-Throughput Sequencing (HTS) was used to catalog the viral diversity present in weeds frequently associated with the tomato crop. For this, a total of 114 leaf samples from weeds exhibiting typical begomovirus symptoms (mosaic and golden mosaic) were collected near and within tomato fields between 2004 and 2022. These samples were subjected to total DNA extraction (CTAB), enriched by Rolling Circle Amplification (RCA), and submitted to HTS using an Illumina NovaSeq 6000. A total of 19,575.404 reads and 62,444 contigs were recovered. The sequences were assembled using CLC Genomics Workbench v.8.0 (Qiagen) and Geneious® 11.1.5, followed by BLASTn analysis (GenBank RefSeq). One hundred and three (103) genomes were identified with correspondence to ssDNA viruses. A total of 22 distinct species were detected across ten (10) genera: *Badnavirus* (2), *Begomovirus* (12), *Caulimovirus* (1), *Citlodavirus* (1), *Clecrusatellite* (1), *Cyclovirus* (1), *Gemycircularvirus* (1), *Gemykolovirus* (1), *Mulcrilevirus* (1), and *Soymovirus* (1). Hence, our analyses revealed a remarkable diversity of viral genera present in weeds associated with the tomato crop under Brazilian conditions. Thus, the wide range of viruses detected highlights the major role of these weeds in the viral epidemiological dynamics, serving not only as reservoirs but also as potential sites for recombination and emergence of new viral variants.

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