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

ANÁLISE DA DIVERSIDADE DE VÍRUS DE DNA DE FITA-SIMPLES E AGENTES SUBVIRAIS EM PLANTAS DANINHAS DAS FAMÍLIAS FABACEAE, MALVACEAE E SOLANACEAE ASSOCIADAS AO TOMATEIRO

HENRIQUE DE SOUSA HONORATO

Brasília-DF
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Análise da diversidade de vírus de DNA de fita-simples e agentes subvirais em plantas daninhas das famílias Fabaceae, Malvaceae e Solanaceae associadas ao tomateiro

Tese apresentada à Universidade de Brasília como parte dos requisitos parciais para a obtenção do título de doutor em fitopatologia pelo programa de pós-graduação em Fitopatologia.

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Aos meus pais Maria Zelma e Francisco
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Análise da diversidade de vírus de DNA de fita-simples e agentes subvirais em plantas daninhas das famílias Fabaceae, Malvaceae e Solanaceae associadas ao tomateiro

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Honorato, Henrique de Sousa. **Análise da diversidade de vírus de DNA de fita-simples e agentes subvirais em plantas daninhas das famílias Fabaceae, Malvaceae e Solanaceae associadas ao tomateiro.** 2024. Número de páginas (142). Tese (Doutorado em Fitopatologia) – Universidade de Brasília, Brasília, Distrito Federal.

O tomateiro (*Solanum lycopersicum* L.) é a hortaliça de maior importância socioeconômica mundial. A movimentação do solo, a disponibilidade de adubos químicos e orgânicos, o uso de irrigação e os espaçamentos nos campos de tomateiro favorecem a emergência de plantas daninhas que competem por água, luz e nutrientes, além de atuarem como hospedeiras alternativas de pragas e patógenos. Dentre os patógenos está o gênero *Begomovirus* (família *Geminiviridae*), maior grupo de vírus vegetais, que possui DNA circular de fita simples e transmissão relacionada ao complexo de espécies crípticas de *Bemisia tabaci*. A alta diversidade desses vírus está relacionada aos mecanismos de mutação, recombinação e pseudorrecombinação. O uso de *High-Througput Sequencing* (HTS) constitui importante ferramenta no estudo de populações virais. Neste contexto, este trabalho buscou realizar (via HTS) o levantamento e caracterização de vírus de DNA de fita-simples e agentes subvirais em plantas daninhas presentes em cultivos de tomateiro. Foi estabelecido um pool de amostras composto por plantas das famílias Fabaceae (23 amostras) e Solanaceae (64 amostras), e outro pool composto por plantas da família Malvaceae (78 amostras), no qual foram realizadas detecções de EuYMV por meio de PCR (*Polymerase Chain Reaction*). As amostras enriquecidas através de *Rolling Circle Amplification* (RCA), e submetidas ao HTS em plataforma Illumina NovaSeq-6000, permitiram a obtenção de sequências que foram convertidas em *contigs* com o auxílio do *software* CLC Genomics 7.5, analisadas no Geneious R11.1 e comparadas com o banco de dados do GenBank via algoritmo BLASTn. Doze genomas virais foram recuperados, oito deles eram *Begomovirus*, um deles nova espécie. Dois isolados do gênero *Topilevirus* foram recuperados, assim como um isolado do gênero *Gemycircularvirus* e um agente subviral pertencente ao gênero *Alphasatellite*. O uso de *primers* espécie-específicos em PCR detectou 6 vírus em 37 amostras. Após a identificação botânica pelos genes *rbcL* (ribulose-1,5-bisfosfato) e *matK* (maturase K), duas novas hospedeiras de ToSRV, oito novas hospedeiras EuYMV, três novas hospedeiras de SimMV e oito novas hospedeiras de ToMoLCV, evidenciando a diversidade viral existente entre plantas daninhas e a atuação destas plantas como reservatórios virais em áreas próximas aos cultivos de tomateiro. Os resultados reforçam a importância epidemiológica de plantas daninhas para os begomovírus, aponta a presença de EuYMV em hospedeiras alternativas em novas áreas, e o predomínio de ToSRV e ToMoLCV no Brasil.

Palavras chaves: *Geminiviridae*, *High-Throughput Sequencing*, *Topilevirus*, *Genomoviridae*

GENERAL ABSTRACT

Honorato, Henrique de Sousa. **Analysis of the diversity of single-stranded DNA viruses and subviral agents in weeds of the families Fabaceae, Malvaceae and Solanaceae associated with tomato.** 2024. Number of pages (142). Thesis (PhD in Phytopathology) – University of Brasília, Brasília, Distrito Federal.

The tomato plant (*Solanum lycopersicum* L.) is the vegetable of greatest socioeconomic importance in the world. Soil movement, the availability of chemical and organic fertilizers, the use of irrigation and spacing in tomato fields favor the emergence of weeds that compete for water, light and nutrients, in addition to acting as alternative hosts for pests and pathogens. Among the pathogens is the genus *Begomovirus* (family *Geminiviridae*), the largest group of plant viruses, which has single-stranded circular DNA and transmission related to the cryptic species complex of *Bemisia tabaci*. The high diversity of these viruses is related to the mechanisms of mutation, recombination and pseudorecombination. The use of High-Throughput Sequencing (HTS) is an important tool in the study of viral populations. In this context, this study sought to carry out (via HTS) the survey and characterization of single-stranded DNA viruses and subviral agents in weeds present in tomato crops. A pool of samples composed of plants from the Fabaceae (23 samples) and Solanaceae (64 samples) families was established, and another pool composed of plants from the Malvaceae family (78 samples), in which EuYMV detections were performed by means of PCR (Polymerase Chain Reaction). The samples enriched by Rolling Circle Amplification (RCA) and submitted to HTS on the Illumina NovaSeq-6000 platform allowed the obtaining of sequences that were converted into contigs with the aid of the CLC Genomics 7.5 software, analyzed in Geneious R11.1 and compared with the GenBank database via the BLASTn algorithm. Twelve viral genomes were recovered, eight of which were Begomovirus, one of them a new species. Two isolates of the genus *Topilevirus* were recovered, as well as one isolate of the genus *Gemycircularvirus* and one subviral agent belonging to the genus *Alphasatellite*. The use of species-specific primers in PCR detected 6 viruses in 37 samples. After botanical identification by the rbcL (ribulose-1,5-bisphosphate) and matK (maturase K) genes, two new ToSRV hosts, eight new EuYMV hosts, three new SimMV hosts and eight new ToMoLCV hosts, evidencing the viral diversity existing among weeds and the action of these plants as viral reservoirs in areas close to tomato crops. The results reinforce the epidemiological importance of weeds for begomoviruses, indicate the presence of EuYMV in alternative hosts in new areas, and the predominance of ToSRV and ToMoLCV in Brazil.

Keywords: *Geminiviridae*, High-Throughput Sequencing, *Topilevirus*, *Genomoviridae*

GENERAL INTRODUCTION

The tomato (*Solanum lycopersicum* L.), whose center of origin is in the Andean regions, belongs to the genus *Solanum*, the largest within the Solanaceae family containing around 1500 species distributed throughout the New Word area (Blanca et al. 2012; Pereira et al. 2016). It is the vegetable crop with the largest production on a global scale, with China being the largest producer with 68,241,810.69 tons (t), followed by India (20,694,000 t), Turkey (13,000,000 t) and the United States (10,199,753 t) (FAOSTAT 2024). Brazil ranks eighth among the largest tomato producers in the world, with a total cultivated area of around 55,707 hectares (ha) and production that exceeds 4.1 million tons (IBGE 2024). The states with the highest tomato production are Goiás (1,307,458 t), São Paulo (1,039,696 t) and Minas Gerais (531,169 t) (IBGE 2024).

Weeds are common in tomato fields and they negatively interfere with the development of the crop due to the competition for nutrients, water, light and the release of substances that inhibit the development of the crop. In addition, weeds may function as hosts for pests and pathogens, including many viruses (Talamini and Nunes 2018; Kitajima 2020). Among the main weeds reported in tomato cultivation are the solanaceae joá-bravo (*Solanum sisymbriifolium*), joá-de-capote (*Nicandra physalodes*), Maria-pretinha (*Solanum americanum*); the malvaceae, guanxuma (*Sida rhombifolia*) and the fabaceae, fedegoso (*Senna obitusifolia*) (Silva et al. 2006).

The genus *Begomovirus* (family *Geminiviridae*) constitutes an important group of plant pathogens, with a number of 445 species, the largest among plant-infecting viruses (ICTV 2024). These viruses are classified as monopartite (only with the DNA-A component) or bipartite, with two similarly sized DNA components (DNA-A and DNA-B) (Navas-Castillo 2023; ICTV 2024). There is also a correlation between the genomic composition of begomoviruses and their geographic distribution that allows the division between begomoviruses from the Old World (Africa, Asia and Europe) and the New World (Americas) (Navas-Castillo & Fiallo-Olivé 2020).

The transmission of begomoviruses is associated with the complex of cryptic species of *Bemisia tabaci*. The predominant species of this complex are *B. tabaci Middle East-Asia Minor1* – MEAM 1, previously called biotype B, and *B. tabaci Mediterranean* – MED, former as biotype Q (Rosen et al. 2015). In the last 30 years, both biotypes have invaded many countries around the world and displaced some of the native cryptic biotypes. In Brazil, there is thus far a predominance of *B. tabaci* MEAM1, a highly polyphagous species, with more than 1000 hosts

The presence of *B. tabaci* MED was detected for the first time in Brazil in the municipality of Barra do Quaraí in Rio Grande do Sul, in *C. annuum* L. plants in a greenhouse and in *Ipomoea batata* L. under field conditions. (Barbosa et al. 2015). In greenhouse tomato plantations, *B.tabaci* MED is already predominant in the state of São Paulo, even though it is associated with inefficiency in the transmission of begomovirus (Nogueira et al. 2024).

Begomoviruses have three basic mechanisms of genetic variability: mutation, recombination, and pseudorecombination (Duffy and Holmes 2009; Rojas et al. 2018). Weeds, native flora, and spontaneously occurring plants might function as virus reservoirs (Kitajima 2020) and as suitable hosts for these mechanisms, intensifying the emergence of new viral strains and species. The high diversity and variability of begomoviruses is a factor that demands more sustainable management procedures, among which the adoption of cultivars with resistance/tolerance genes has been considered the simplest and most efficient strategy, reducing the impacts of viral infection on the tomato crop. (Boiteux et al. 2012).

The study of viral populations or communities of viruses, whether of known species or not, has become faster and less expensive with the advent of High-Throughput Sequencing (HTS) (Barba and Hadidi 2015; Adams and Fox 2016; Villamor et al. 2019). The adoption of this technology has overcome the challenges of the plant virus detection process and has significantly contributed to provide very useful ecological and epidemiological information across distinct group of viruses (Massart et al. 2019). Analyzes performed with the combination of metagenomics and HTS for the characterization of viruses and viroids are known as “virome” (Barba et al. 2014; Villamor et al. 2019). Therefore, this work seeks to expand analyzes like these, carrying out metagenomic analysis of the viral diversity of *Geminiviridae* present in samples collected near tomato crops around Brazil; reporting new interactions between begomoviruses and hosts from the Fabaceae, Malvaceae and Solanaceae families and molecularly characterizing new species.

The information described herein will help to understand the geographic distribution of viral species from the *Geminiviridae* family, the genetic diversity of this group of viruses in weeds and tomatoes, giving support to the development of more efficient genetic improvement strategies.

HYPOTHESES

- Begomoviruses ToSRV and ToMoCLV are prevalent among weeds and minor crops adjacent to tomato crops in Brazil
- The number of host species for begomoviruses has increased among weeds of the families Fabaceae, Malvaceae and Solanaceae
- The presence of weeds from different botanical families has favored the increase in genetic variability and the diversity of viral species from the *Geminiviridae* family in tomato crops in Brazil.
- The distribution of a specific set of begomoviruses may be conditioned by climatic and environmental factors, contributing to the regionalization of species, which make their occurrence endemic (restricted to certain areas and hosts).
- The number of reports available in the literature does not reflect the real diversity of species of the *Geminiviridae* family, with many novel and undescribed viruses existing in the tomato-weed system and across distinct geographic regions in Brazil.

GENERAL OBJECTIVE

To prospect and catalog the diversity of begomoviruses in weed and spontaneous plants present in tomato fields and elucidate aspects of the epidemiological role of these plants in the evolution and genetic structure of tomato-infecting begomovirus populations from Brazil.

SPECIFIC OBJECTIVES

- To carry out surveys of viral species of the *Geminiviridae* family associated with common Fabaceae and Solanaceae weeds of the tomato crop in a large chronological (2002-2020) and geographic (all five Brazilian macroregions) scale;
- To characterize the genetic diversity of begomovirus populations occurring in leaf samples of weeds collected in association (in or around) tomato crops;
- To compare the genetic structure of begomovirus populations occurring in weeds and tomato;
- To provide the genomic characterization of a subset of potential new species of *Begomovirus* associated with weeds.
- To identify novel weed hosts for previously described tomato-infecting begomoviruses.

CHAPTER 1

LITERATURE REVIEW

LITERATURE REVIEW

1. The tomato crop

The tomato plant (*Solanum lycopersicum* L.) belongs to the phylum Magnoliophyta, class Magnoliopsida, order Solanales and genus *Solanum* (Naturdata 2020). One hundred and six (106) genera and more than 2300 species are classified within the Solanaceae family (Oliveira et al. 2020). The genus *Solanum* is the largest within the Solanaceae family, containing about 1500 species distributed throughout South, Meso, and North Americas. In Brazil, ≈ 350 species of the genus *Solanum* have been identified, many of which are endemic (Pereira et al. 2016).

The tomato is considered a cosmopolitan plant due to its wide distribution across different regions of the globe. South America is the center of origin of tomatoes and other members of the *Solanum* (section *Lycopersicon*), more precisely the Andean region and the Galápagos Islands (Peralta et al. 2005). In turn, Mexico, especially the cities of Puebla and Vera Cruz, has been considered the main center of domestication of this vegetable (Blanca et al. 2012). The agricultural use of this species in Brazil began in the late 19th century with the arrival of European immigrants (Ayenan et al. 2019).

The natural architecture of the tomato plant is composed of abundant lateral branching. However, one of the characteristics selected during the domestication process was precisely the plant architecture (with determinate versus indeterminate growh habits) that serves to different types of cultivation. Cultivars with determinate growth habit are used for the processing industry, while cultivars with indeterminate growth habit are used for fresh-market (*in natura* consumption) (Zhang et al. 2019; González-Arcos et al. 2019).

Among the vegetable crops produced on a global scale, tomatoes have the greatest socioeconomic importance, constituting a source of employment and income for both large producers and family farmers (Lin et al. 2019). China is the largest producer with 68,241,810.69 tonnes (t), followed by India (20,694,000 t), Turkey (13,000,000 t) and, the United States (10,199,753 t) (FAOSTAT 2024). Brazil is in 8th position, among the largest producers in the world, with a total cultivated area of around 55,707 hectares (ha) and production that exceeds 4.1 million tons (IBGE 2024). The states with the highest tomato production are Goiás (1,307,458 t), São Paulo (1,039,696 t) and, Minas Gerais (531,169 t) (IBGE 2024). In the Distrito Federal, 27,742 tons of tomatoes are produced in an area of approximately 350 ha (IBGE 2024). An average of 106,000 direct jobs are estimated in tomato cultivation, from soil preparation to the harvesting stage (Socoloski 2017).

2. Weeds in tomato crop

Weeds negatively interfere with tomato growth and development via competition for nutrients, water, light, and the release of substances that inhibit crop development (Talamini and Nunes 2018). The constant movement of the soil with the use of machinery in successive crops; the use of high levels of chemical and organic fertilizers; daily irrigation; spacing between plants and the slower crop development in the first weeks after planting are the major vulnerability factors that favor the development of weed populations in tomato fields (Ronchi et al. 2010).

In addition to the direct yield reduction caused by competition for light and nutrients, weeds can act as hosts for pests and pathogens that affect the tomato plant (Silva 2009). Weeds, plants of the native flora, and spontaneously occurring species have been the targets of several studies since they can act as reservoirs of several begomoviruses, serving as suitable hosts for the recombination and emergence of new viral species (Kitajima 2020). As many of these plants are perennial or semi-perennial, they can maintain viral populations between growing seasons, allowing the perpetuation and/or fixation of viral isolates under natural conditions (Fernandes 2015).

Among the main weeds reported in tomato cultivation are the solanaceous plants as joá-bravo (*Solanum sisymbriifolium* Lam.), joá-de-capote (*Nicandra physalodes* (L.) Gaertn.), black night shade or maria-pretinha (*Solanum americanum* Mill.); malvaceous plants: guanxuma (*Sida* species L.) and fabaceous – fedegoso (*Senna obtusifolia* L.) (Silva et al. 2006).

3. Weed management

Over the past few decades, the development of numerous agricultural technologies has allowed productivity to be increased and ensured global food security. Among these technologies are herbicides, which industrial agriculture heavily relies on to maintain food and animal feed production, and associates their use with the development of genetically modified tolerant crops (Ofosu et al. 2023; Ehrlich et al. 2015). The size of the global pesticide market reached an approximate value of R$84.5 billion in 2019, with 51.9% corresponding to the herbicide market, according to the report by The Business Research Company (2023).

The potential of weeds to evolve, epigenetic capacity, hybridization, herbicide resistance, herbicide tolerance, vulnerability of cropping systems, coevolution of weeds with human management, and the ability of weeds to cope with climate change contribute to these plants crossing harvests and posing a major challenge to agricultural production (Clements et

al. 2021). In the United States of America (USA), Europe, Australia, Canada, Brazil, and China, where agricultural systems are industrialized, there are a greater number of records of herbicide-resistant weeds. Weeds have developed resistance to 21 of the 31 known herbicide modes of action and to 165 different herbicides (Heap 2023). Point mutations that confer resistance are recurrent in weed populations as part of their genetic variation and, when added to the selection pressure of herbicides, facilitate the proliferation of these biotypes (Délye et al. 2013). The indiscriminate application of herbicides can generate environmental imbalances by eliminating bees, butterflies, spiders and other beneficial organisms necessary for pollination and crop protection (Kughur et al. 2012).

Preventive and cultural weed control measures aim to increase the competitiveness of crops against weeds. Soil preparation, irrigation, planting time, sowing methods, planting density, phytosanitary conditions of fields, mulch and spacing are important factors in establishing management strategies (Gu et al. 2021). Biological weed control depends on natural mechanisms such as predation and parasitism (Den Breeyen et al. 2022). Bioherbicides are therefore based on the use of allelochemicals, natural by-products, plant extracts, microorganisms and insects as control mechanisms, capable of interrupting photosynthesis, nutrient absorption and other vital functions of weeds (Tanaka et al. 2017; Müller-Schärer et al. 2020).

4. Viruses in weeds

The peculiar biological and phenological aspects of wild plants and weeds make them potential reservoirs for viruses that can spread into cultivated areas, leading to disease outbreaks and the potential emergence of new viruses (Power and Mitchell 2004; Ma et al. 2020). Mixed viral infections are common in weeds, allowing for a wide range of begomovirus-begomovirus interactions within host plant cells. These interactions facilitate the occurrence of natural mechanisms that generate variability, such as recombination and pseudo-recombination, which can cause alterations in the genetic structure of the original viral population, as well as favor the emergence of new variants (Roossinck et al. 1997; Roossinck et al. 2012).

Although there is a significant number of genomic studies in weeds and wild/native plants, it is believed that there are many viruses yet to be identified and described. The information derived from genomic studies can be a very important tool to understand the prevalence and composition of the viral community (Bernardo et al. 2018). In addition, a wide range of viruses and subviral pathogens has been described in weeds, wild/native plants, or in

minor crops in the Malvaceae (42), Solanaceae (39) and Fabaceae (16) families (**Figure 1**). The distribution of viruses within these three botanical families is irregular. For instance, 35 potyviruses have already been reported in Solanaceae and 24 in Fabaceae, whereas only five potyviruses have been reported in Malvaceae. Some of these Malvaceae-infecting potyviruses are Bidens mottle virus (Huang and Jan 2011), Malva vein clearing virus (Wang et al. 2020), papaya ringspot virus (Biswas et al. 2014), watermelon mosaic virus (Niu et al. 2019), and zucchini yellow mosaic virus (Choi et al. 2002).

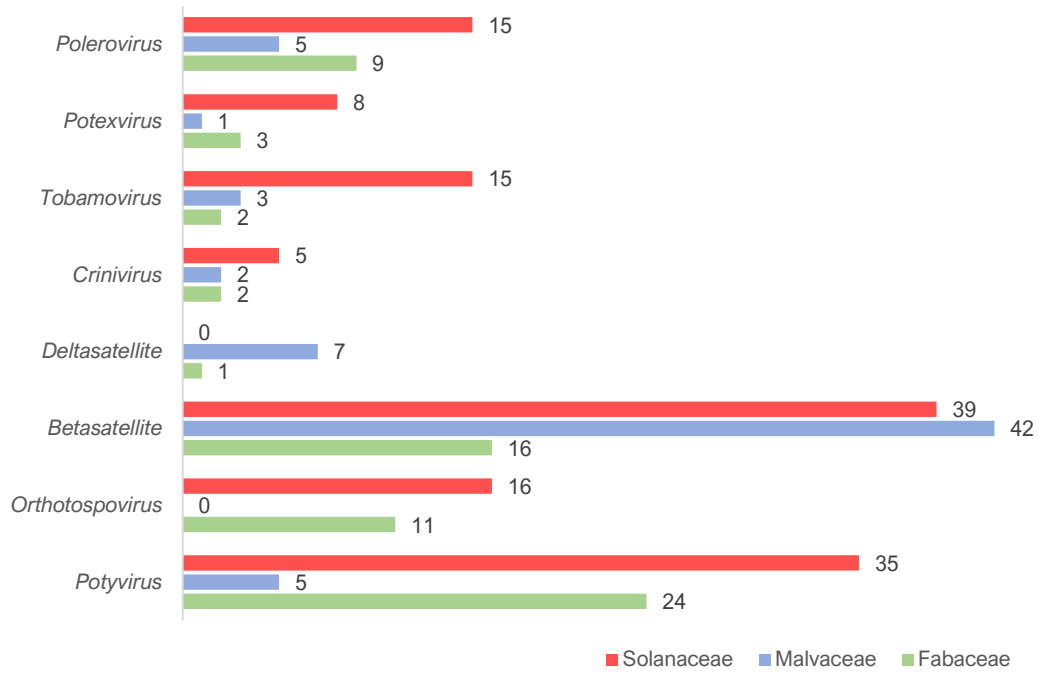

Figure 1. Number of viruses by genus in weeds and minor crops of the Fabaceae, Malvaceae and Solanaceae families worldwide, according to GenBank (2024), Host DataBase (2024) and Kitajima (2020).

Outbreaks in Brazil are related to the natural occurrence of begomovirus in malvaceous species of the genus *Sida* and *Sidastrum*, which display wide geographical and ecological distributions in Brazil and Latin America. Different viruses classified in the *Geminiviridae* family were reported infecting *Sida* species (Jovel et al. 2007). Beside this, species of the Fabaceae family, such as common bean (*P. vulgaris* L.), *Crotalaria juncea* (L.), and *Pachyrhizus erosus* (L.) are reported as natural and/or experimental hosts of tomato severe rugose virus – ToSRV (Macedo et al. 2017; Pereira-Silva et al. 2022). There are also reports of infection by ToSRV in *S. betaceum* (Cav.), *S. torvum* (Sw.) (Solanaceae family) as well as *Oxalis latifolia* (Kunth.) (Oxalidaceae family) in leaf samples collected near tomato crops (Pereira-Silva et al. 2022).

Weeds of the botanical families Malvaceae (Ambrozevicius et al. 2002; Barreto et al. 2013), Sterculiaceae (Assunção et al. 2006), Capparaceae (Assunção et al. 2006), Fabaceae (Ambrozevicius et al. 2002; Castillo-Urquiza et al. 2008), Euphorbiaceae (Santos et al. 2003; Barreto et al. 2013), and Solanaceae (Castillo-Urquiza et al. 2008) have been reported as natural and/or experimental hosts of *Begomovirus* species across several producing regions and /or geographic areas. These plant taxa are, therefore, potential sources of begomovirus inoculum that can be transmitted by *B. tabaci* to tomato and other crops. Examples of viruses already reported in plants from the botanical families Fabaceae, Malvaceae, and Solanaceae will be presented below.

The countries with the highest numbers of begomoviruses reported in either weed species or minor crops from the Fabaceae, Malvaceae and Solanaceae families are India (86), Brazil (40), Mexico (28), and China (27) (**Figure 2**). Reports of begomoviruses infecting members of the three botanical families are distributed among 56 countries (**Table 1)**.

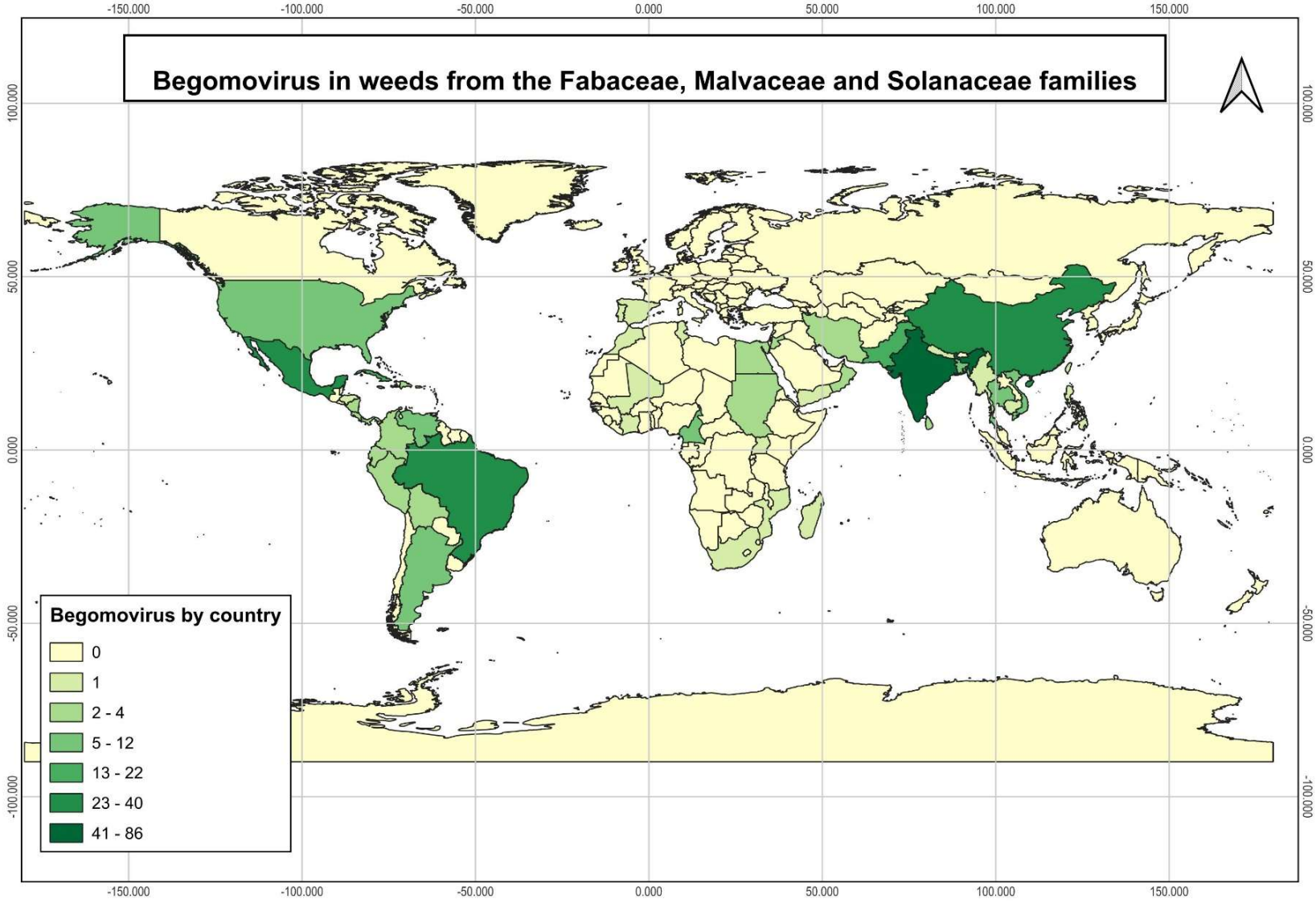

Figure 2. Global distribution of begomoviruses associated with weed species from the Fabaceae, Malvaceae and Solanaceae families based on extensive literature search as well as the viral species reported in the GenBank (2024), Host Database (2024) and Kitajima et al. (2020). Reports of begomovirus in members of the three botanical families are distributed across 56 countries.

Table 1. Global occurrence of begomoviruses associated with weed species from the Fabaceae, Malvaceae and Solanaceae families based on extensive literature search as well as the viral species reported in the GenBank (2024), Host Database (2024), and Kitajima et al. (2020). Reports of begomovirus in members of the three botanical families are distributed across 56 countries (countries and begomoviruses occurring in each country are listed in alphabetic order within each column).

Argentina
- Bean bushy stunt virus
- Pepper blistering leaf virus
- Sida Brazil virus
- Sida golden mosaic Brazil virus
- Soybean blistering mosaic virus
- Tomato dwarf leaf virus
- Tomato mottle wrinkle virus
- Tomato yellow spot virus
- Tomato yellow vein streak virus

Bangladesh
- Bhendi yellow vein mosaic virus
- Bitter gourd yellow vein virus
- Chilli leaf curl virus
- Corchorus golden mosaic virus
- Croton yellow vein mosaic virus
- Mesta yellow vein mosaic virus
- Squash leaf curl China virus
- Synedrella leaf curl virus
- Tomato leaf curl Bangladesh virus
- Yellow vein mosaic virus

Barbados
- Bean mosaic crinkle Barbados virus

Belize
- Merremia mosaic virus
- Tomato mottle virus

Benin

Egypt
- Hollyhock leaf crumple virus
- Okra curl leaf virus

Guadeloupe
- Potato yellow mosaic virus

Honduras
- Rhynchosia golden mosaic virus

India
- Ageratum enation virus
- Ageratum leaf curl virus
- Ageratum yellow vein virus
- Bhendi yellow vein Bhubhaneswar virus
- Bhendi yellow vein Delhi virus
- Bhendi yellow vein Haryana virus
- Bhendi yellow vein India virus
- Bhendi yellow vein mosaic Maharashtra virus
- Bhendi yellow vein mosaic virus
- Cajanus cajan yellow mosaic virus
- Chili leaf curl Bhatinda virus
- Chilli leaf curl Ahmedabad virus
- Chilli leaf curl Ahmedabad virus-India [India/Ahmedabad/2014]
- Chilli leaf curl Bhavanisagar virus
- Chilli leaf curl Bijnour virus-India
- Chilli leaf curl Coochbehar virus
- Chilli leaf curl Gonda virus
- Chilli leaf curl India virus
- Chilli leaf curl Kanpur virus
- Chilli leaf curl Multan virus-India [India/Guntur/2009]
- Chilli leaf curl Salem virus-India [India/Salem/2008]

Myanmar
- Tomato yellow leaf curl Thailand virus

Nepal
- Pea leaf distortion virus
- Tomato leaf curl Karnataka virus

Nicaragua
- Pepper golden mosaic virus
- Tomato leaf curl Sinaloa virus
- Tomato mosaic Havana virus
- Tomato severe leaf curl virus

Nigeria
- African cassava mosaic virus
- Chayote yellow mosaic virus
- East African cassava mosaic Cameroon virus
- Soybean chlorotic blotch virus
- Soybean mild mottle virus
- Soybean mottle mosaic virus

Oman
- Okra leaf curl Oman virus
- Tomato leaf curl Oman virus

Pakistan
- African cassava mosaic virus
- Chili leaf curl Pakistan virus
- Cotton leaf curl Alabad virus
- Cotton leaf curl Burewala virus
- Cotton leaf curl Khokran virus
- Cotton leaf curl Multan virus
- Cotton leaf curl Rajasthan virus
- Cotton leaf curl Shahdadpur virus

Cotton yellow mosaic virus

Bolivia

Abutilon mosaic Bolivia virus
Solanum mosaic Bolivia virus

Brazil

Abutilon Brazil virus
Abutilon mosaic Brazil virus
Bean golden mosaic virus
Blainvillea yellow spot virus
Centrosema yellow spot virus
Cleome leaf crumple virus
Corchorus mottle virus
Cotton chlorotic spot virus
Cowpea bright yellow mosaic virus
Euphorbia yellow mosaic virus
Hibiscus golden 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
Malvaviscus yellow mosaic virus
Melochia mosaic virus
Melochia yellow mosaic virus
Okra mottle virus
Pavonia mosaic virus
Pavonia yellow mosaic virus
Sida angular mosaic virus
Sida chlorotic vein virus
Sida common mosaic virus
Sida golden mosaic virus
Sida micrantha mosaic virus
Sida mottle Alagoas virus
Sida yellow blotch virus
Sida yellow leaf curl virus

Chilli leaf curl Vellanad virus
Chilli leaf curl virus
Corchorus yellow vein mosaic virus
Cotton leaf curl Alabad virus
Cotton leaf curl Bangalore virus
Cowpea golden mosaic virus
Crotalaria juncea begomovirus
Croton yellow vein mosaic virus
Cyamopsis tetragonoloba leaf curl Sikar virus
Cyamopsis tetragonoloba leaf curl virus
Datura leaf curl virus
Dolichos yellow mosaic virus
Eclipta yellow vein virus
French bean leaf curl Madikeri virus
French bean leaf curl virus
Hibiscus leaf curl virus
Hollyhock leaf curl virus
Hollyhock yellow vein mosaic virus
Hollyhock yellow vein virus
Horsegram yellow mosaic virus
Indian cassava mosaic virus
Jatropha yellow mosaic India virus
Jute yellow mosaic virus
Kenaf leaf curl virus
Malachra yellow mosaic virus
Malachra yellow vein mosaic virus – India
Mesta yellow vein mosaic Bahraich virus
Mesta yellow vein mosaic virus
Mimosa yellow vein virus
Mungbean yellow mosaic India virus
Mungbean yellow mosaic virus
Okra enation leaf curl virus
Okra leaf curl India virus
Okra leaf curl virus
Okra yellow vein mosaic virus
Papaya leaf crumple virus
Pepper leaf curl Bangladesh virus
Pepper leaf curl Lahore virus

Cotton leaf curl virus
Croton yellow vein virus
Euphorbia yellow leaf curl virus
Gossypium punctatum mild leaf curl virus
Hollyhock leaf curl virus
Hollyhock yellow vein mosaic virus
Malvastrum yellow vein Lahore virus
Papaya leaf curl virus
Pedilanthus leaf curl virus
Pepper leaf curl Lahore virus
Rhynchosia yellow mosaic virus
Tomato leaf curl Kerala virus

Panama

Macroptilium golden mosaic virus
Potato yellow mosaic Panama virus
Potato yellow mosaic virus

Peru

Pepper leafroll virus
Tomato leaf deformation virus

Philippines

Malvastrum leaf curl Philippines virus

Portugal

Tomato yellow leaf curl Axarquia virus

South Africa

Abutilon mosaic virus

Spain

Tomato yellow leaf curl Sardinia virus

Sri Lanka

Sida yellow mosaic Alagoas virus
Sidastrum golden leaf spot virus
Soybean chlorotic spot virus
Tomato crinkle leaf yellows virus
Tomato mild mosaic virus
Tomato severe rugose virus
Tomato yellow spot virus
Tomato yellow vein streak virus
Wissadula yellow mosaic virus

Cambodia

Malvastrum yellow vein Cambodia virus

Cameroon

Okra leaf curl Cameroon virus
Okra leaf curl virus
Pepper yellow vein Mali virus
Soybean chlorotic blotch virus
Tomato leaf curl Cameroon virus
Tomato yellow leaf curl Mali virus

China

Ageratum yellow vein China virus
Ageratum yellow vein virus
Bidens pilosa leaf crumple virus
Chili leaf curl Pakistan virus
China bean begomovirus
Crassocephalum yellow vein virus
Malvastrum leaf curl Guangdong virus
Malvastrum leaf curl virus
Malvastrum yellow mosaic virus
Malvastrum yellow vein Honghe virus
Malvastrum yellow vein virus
Malvastrum yellow vein Yunnan virus
Papaya leaf curl China virus
Papaya leaf curl Guandong virus
Pepper leaf curl Yunnan virus
Pepper yellow vein Mali virus
Pepper leaf curl Varanasi virus
Pepper yellow leaf curl Aceh virus
Pepper yellow leaf curl Indonesia virus
Pepper yellow leaf curl Indonesia virus 2
Potato apical leaf curl virus
Radish leaf curl virus
Rhynchosia mosaic virus
Rhynchosia yellow mosaic India virus
Rhynchosia yellow mosaic virus
Senna leaf curl virus
Sida yellow vein virus
Solanum leaf curl Lakshmangarh virus
Squash leaf curl China virus
Sri Lankan cassava mosaic virus
Sunn hemp leaf distortion virus
Tobacco leaf curl Indonesia virus
Tobacco leaf curl Pusa virus
Tomato leaf curl Gujarat virus
Tomato leaf curl Java virus
Tomato leaf curl Joydebpur virus
Tomato leaf curl Karnataka 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 Sulawesi virus
Tomato leaf curl virus
Velvet bean severe mosaic virus
Whitefly-transmitted Indian begomovirus

Indonesia

Ageratum yellow vein virus
Pepper yellow leaf curl Aceh virus
Pepper yellow leaf curl Indonesia virus
Pepper yellow leaf curl Indonesia virus 2
Pepper yellow leaf curl virus
Tobacco leaf curl Indonesia virus
Chilli leaf curl Sri Lanka virus
Okra yellow vein mosaic virus
Sri Lankan cassava mosaic virus

Sudan

Cotton leaf curl Gezira virus
Datura leaf curl virus
Tomato leaf curl Sudan virus

Taiwan

Hibiscus vein enation virus

Thailand

Eggplant golden mosaic virus
Foetid cassia leaf curl virus-[Thailand]
Malvastrum coromandelianum yellow vein virus
Pepper yellow leaf curl Thailand virus
Pepper yellow leaf curl Thailand virus
Soybean crinkle leaf virus
Tobacco leaf curl Thailand virus
Tomato yellow leaf curl Thailand virus

Trinidad and Tobago

Rhynchosia minima Trinidad virus
Malachra alceifolia begomovirus – Trinidad
Sida rhombifolia Trinidad virus

Tunisia

Tomato leaf curl New Delhi virus

Uganda

Desmodium mottle virus

USA

Macroptilium mosaic Puerto Rico virus
Macroptilium mosaic virus
Malvastrum bright yellow mosaic virus

Sida yellow mosaic China virus
Tobacco curly shoot virus
Tobacco leaf curl Puer virus
Tobacco leaf curl virus
Tobacco leaf curl Yunnan virus
Tomato geminivirus
Tomato leaf curl China virus
Tomato leaf curl Hsinchu virus
Tomato yellow leaf curl China virus
Tomato yellow leaf curl Kanchanaburi virus
Tomato yellow leaf curl Shuangbai virus

Colombia

Bean leaf crumple virus
Passionfruit leaf distortion virus
Pepper rugose mosaic virus
Rhynchosia golden mosaic Colombia virus

Comoros

Tobacco leaf curl Comoros virus
Tobacco leaf curl Zimbabwe virus
Tomato leaf curl Namakely virus

Costa Rica

Calopogonium golden mosaic virus
Squash yellow mild mottle virus

Cote d'Ivoire

Okra yellow crinkle virus

Cuba

Capsicum begomovirus Cuba/2007
Common bean mottle virus
Common bean severe mosaic virus
Corchorus yellow vein Cuba virus
Corchorus yellow vein virus
Tomato leaf curl Java virus
Tomato leaf curl Sulawesi virus

Iran

Cotton leaf curl Gezira virus
Tomato leaf curl Palampur virus
Watermelon chlorotic stunt virus

Jamaica

Macroptilium golden mosaic virus
Macroptilium yellow mosaic virus
Malvastrum yellow mosaic Helshire virus
Malvastrum yellow mosaic Jamaica virus
Sida golden buckup virus
Tobacco leaf curl Cuba virus
Wissadula golden mosaic virus

Jordan

Squash leaf curl virus
Tomato yellow leaf curl Sardinia virus

Madagascar

Bean leaf curl Madagascar virus

Malawi

East African cassava mosaic Malawi virus

Mali

Okra yellow crinkle virus

Mauritius

Tomato yellow leaf curl virus

Mexico

Abutilon golden mosaic virus
Anoda geminivirus Yucatan
Bean golden yellow mosaic virus
Okra yellow mosaic Mexico virus
Rhynchosia mild mosaic virus
Rhynchosia mosaic virus
Sida golden mosaic virus
Sida golden yellow vein virus
Squash leaf curl virus
Watermelon chlorotic stunt virus

Venezuela

Bean chlorosis virus
Bean chlorotic mosaic virus
Bean leaf crumple virus
Bean white chlorosis mosaic virus
Cabbage leaf curl virus
Datura leaf distortion virus
Desmodium mosaic virus
Desmodium yellow spot virus
Macroptilium mottle virus
Rhynchosia mottle virus
Sida ciliaris golden mosaic virus
Tomato chlorotic leaf distortion virus

Vietnam

Ageratum leaf curl virus
Corchorus golden mosaic virus
Corchorus yellow vein virus
Kudzu mosaic virus
Ludwigia yellow vein virus
Mimosa yellow leaf curl virus
Pouzolzia golden mosaic virus
Sida leaf curl virus
Tomato leaf curl Hanoi virus
Tomato yellow leaf curl Kanchanaburi virus

Yemen

Tomato leaf curl Sudan virus

Desmodium leaf distortion virus
Euphorbia mosaic virus
Rhynchosia golden mosaic Havana virus
Rhynchosia golden mosaic Yucatan virus
Rhynchosia rugose golden mosaic virus
Sida golden mosaic Florida virus
Sida golden mosaic Liguanea virus
Sida golden yellow vein virus
Sida yellow mottle virus
Tobacco begomovirus - [Cuba:Granma:2007]
Tobacco yellow crinkle virus
Tomato mottle Taino virus
Tomato yellow leaf curl virus
Tomato yellow leaf distortion virus

Democratic Republic of the Congo

East African cassava mosaic virus

Dominican Republic

Macroptilium golden yellow mosaic virus
Tobacco leaf curl Cuba virus
Tobacco leaf curl Dominican Republic virus

Ecuador

Abutilon mosaic virus
Cabbage leaf curl virus
Bean latent virus
Bean yellow mosaic Mexico virus
Begomovirus isolate bean Iguala
Chino del tomate virus
Corchorus yellow spot virus
Cotton leaf crumple virus
Desmodium leaf distortion virus
Euphorbia mosaic virus
Hibiscus variegation virus
Jamaica yellow mosaic virus
Okra yellow mosaic Mexico virus
Pepper golden mosaic virus
Pepper huasteco yellow vein virus
Rhynchosia golden mosaic Sinaloa virus
Rhynchosia golden mosaic virus
Rhynchosia golden mosaic Yucatan virus
Sida golden mosaic Honduras virus
Sida mosaic Sinaloa virus
Sida yellow mosaic Yucatan virus
Tobacco apical stunt virus
Tomato chino La Paz virus
Tomato golden mottle virus
Tomato severe leaf curl virus
Vigna yellow mosaic virus
Whitefly-associated begomovirus 3

Morocco

Tomato yellow leaf curl Malaga virus

Mozambique

Begomovirus mozlegume

4.1. Begomovirus in genera of weeds, minor crops, and major crops of the Fabaceae family

Sixteen (16) genera of weeds or wild/native plants of the Fabaceae family have been reported, on a global scale, as hosts for 82 begomoviruses. Nineteen (19) of these begomoviruses have been already reported in Brazil (**Figure 3**) and are listed below with their respective references:

1. Bean golden mosaic virus – BGMV in *Macroptilium lathyroides* (L.) (Xavier et al. 2021),
2. Centrosema yellow spot virus – CenYSV in *Centrosema brasilianum* (L.) (Silva et al. 2012),
3. Cleome leaf crumple virus – ClLCrV in *Phaseolus lunatus* (L.) and *P. vulgaris* (L.) (Wyant et al. 2012),
4. Cowpea bright yellow mosaic virus – CoBYMV in *Vigna unguiculata* (L.) (Naito et al. 2021),
5. Cowpea golden mosaic virus – CGMV in *Vigna unguiculata* (L.) (Rodrigues et al. 2012),
6. Desmodium yellow spot virus – DesYSV in *Desmodium scorpiurus* (Sw.) (Kitajima 2020),
7. Euphorbia yellow mosaic virus – EuYMV in *Crotalaria* spp. (Barreto et al. 2013),
8. Macroptilium bright mosaic virus – MacBMV in *Macroptilium lathyroides* (L.) (Silva et al. 2012),
9. Macroptilium bright yellow interveinal virus – MaBYIV in *Macroptilium erythroloma* (Benth.) (Batista et al. 2022),
10. Macroptilium common mosaic virus – MaCMV in *Macroptilium lathyroides* (L.) (Silva et al. 2012),
11. Macroptilium yellow net virus – MaYNV in *Macroptilium lathyroides* (L.) (Silva et al. 2012),
12. Macroptilium yellow spot virus – MaYSV in *Canavalia* spp. (L.) (Silva et al. 2012),
13. Macroptilium yellow vein virus – MaYVV in *Macroptilium lathyroides* (L.) (Silva et al. 2012),
14. Okra mottle virus – OmoV in *Glycine max* (L.) Merr. (Fernandes et al. 2009),

15. Sida micrantha mosaic virus – SimMV in *Glycine max* (L.) Merr. (Alves-Freitas et al. 2019),
16. Sida mottle virus – SiMoV in *Glycine max* (L.) Merr. (Fernandes et al. 2009),
17. Sida yellow blotch virus – SiYBV in *Phaseolus lunatus* (L.) (Tavares et al. 2012),
18. Soybean chlorotic spot virus – SoCSV in *Macroptilium lathyroides* (L.) (Sobrinho et al. 2014) and
19. Tomato crinkle leaf yellow virus – TCrYLV in *Macroptilium atropurpureum* (DC.) Urb. (Silva et al. 2012).

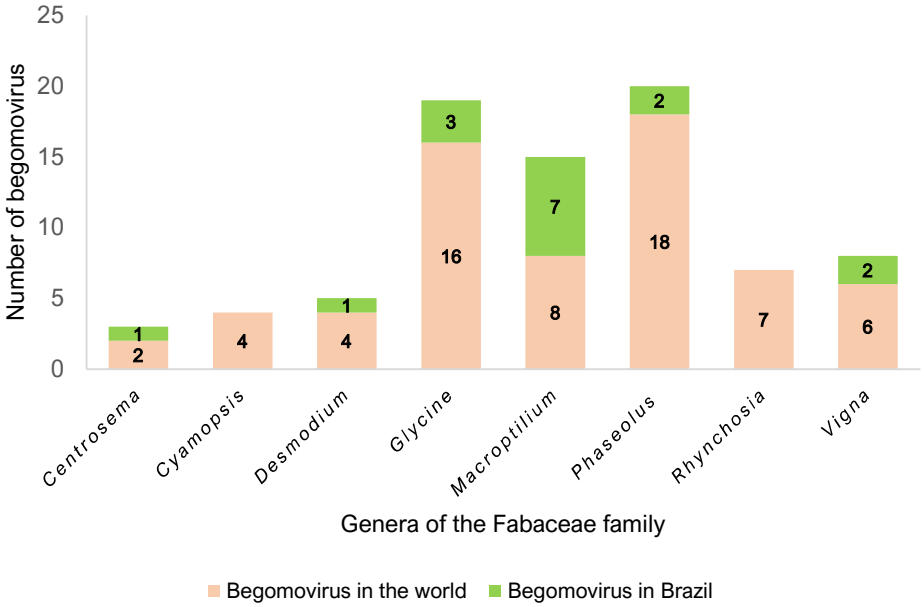

Figure 3. Number of begomoviruses reported in genera of the Fabaceae family based on extensive literature search as well as the viral species reported in the GenBank (2024), Host Database (2024), and Kitajima et al. (2020). The genus *Phaseolus* displays the highest number of begomoviruses (20), two of which are present in Brazil. Next, the largest number of reports comes from the genus *Glycine* with 19 begomoviruses in total, three of which are present in Brazil. In the genus *Macroptilium*, 15 begomoviruses have been reported in total, seven of which are present in Brazil. The genus *Vigna* has two reports of begomoviruses (both occurring in Brazil), whereas the genera *Cyamopsis* and *Rhynchosia* that have four and seven begomoviruses already described, respectively, all absent from Brazil.

4.2. Begomovirus in genera of weeds, minor crops, and major crops of the Malvaceae family

The number of begomoviruses reported infecting weeds, wild or minor crops in the Malvaceae family is 113 worldwide. The genera for which there is a greater number of reports of begomoviruses are *Abelmoschus*, *Gossypium*, *Hibiscus*, *Malvastrum*, and *Sida*. Of these 113 begomoviruses, 23 have already been reported in Brazil (**Figure 4**), including four viruses reported exclusively in Brazil:

1. Malvaviscus yellow mosaic virus in *Malvaviscus arboreus* (Cav.) (Lima et al. 2021),
2. Melochia mosaic virus in *Melochia* spp. (L.) (Fiallo-Olivé et al. 2015),
3. Melochia yellow mosaic virus in *Melochia* spp. (L.) (Fiallo-Olivé et al. 2015),
4. Pavonia mosaic virus in *Pavonia* spp. (L.) (Pinto et al. 2018) and
5. Pavonia yellow mosaic virus in *Pavonia* spp. (L.) (Pinto et al. 2018).

Altogether 24 begomoviruses have been reported in infecting species classified in the genus *Sida*, of which 16 are present in Brazil:

1. Sida angular mosaic virus – SiAMV in *Sida* spp. (L.) (Passos et al. 2016),
2. Sida bright yellow mosaic virus – SiBYMV in *Sida* spp. (L.) (Ferro et al. 2017),
3. Sida chlorotic mottle virus – SiCMoV in *Sida* spp. (L.) (Ferro et al. 2017),
4. Sida chlorotic vein virus – SiCVV in *Sida urens* (L.) (Passos et al. 2016),
5. Sida common mosaic virus – SiCMV in *Sida micrantha* (Schr.) (Almeida et al. 2013),
6. Sida ciliaris golden mosaic virus – SiCGMV in *Sida* spp. (L.) (Passos et al. 2016),
7. Sida golden mosaic Brazil virus – SiGMBV in *Sida* spp. (L.) (Kitajima 2020),
8. Sida golden mosaic virus – SiGMV in *Sida rhombifolia* (L.) (Lima et al. 2002),
9. Sida golden mosaic yellow virus – SiGMYV in *Sida* spp. (L.) (Kitajima 2020),
10. Sida mosaic Alagoas virus – SiMAV in *Sida* spp. (L.) (Kitajima 2020),
11. Sida mottle Alagoas virus – SiMoAV in *Sida* spp. (L.) (Tavares et al. 2013),
12. Sida mottle virus – SiMoV in *Sida* spp. (L.) (Tavares et al. 2013),
13. Sida yellow blotch virus – SiYBV in *Sida* spp. (L.) (Tavares et al. 2013),
14. Sida yellow leaf curl virus – SiYLCV in *Sida* spp. (L.) (Castillo-Urquiza et al. 2008),
15. Sida yellow mosaic Alagoas virus – SiYMAV in *Sida* spp. (L.) (Tavares et al. 2013) and

16. Sida yellow spot virus – SiYSV in *Sida* spp. (L.) (Xavier 2015).

Begomoviruses in the genera *Abutilon*, *Alcea*, *Anoda*, *Malachra*, *Malva* and *Malvastrum* have not been reported so far in Brazil (**Figure 4**).

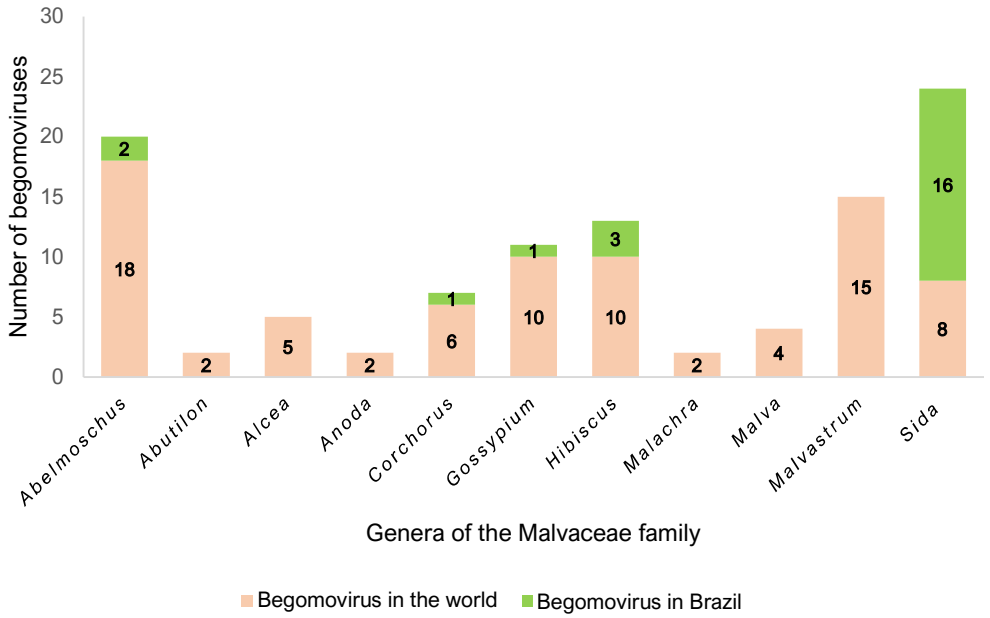

Figure 4. Number of begomoviruses reported in genera of the Malvaceae family based on extensive literature search as well as the viral species reported in the GenBank (2024), Host Database (2024), and Kitajima et al. (2020). The genus *Sida* is associated with the highest number of begomoviruses, 24 in total with 16 of them present in Brazil, followed by the genus *Abelmoschus* associated with 20 begomoviruses with two presents in Brazil. The genera *Abutilon*, *Alcea*, *Anoda*, *Malachra*, *Malva* and *Malvastrum* are not related to any begomovirus in Brazil.

4.3. Begomovirus in genera of weeds and minor crops of the Solanaceae family other than tomato and potato

In all 337 begomoviruses have already been reported in five genera of weeds, wild/native, minor, and major crops (**Figure 5**). The tomato crop alone was reported to be infected by 221 begomoviruses with 26 of them being present in Brazil (Oliveira 2022). Seven begomoviruses present in Brazil were reported infecting 12 weeds, wild/native or other solanaceous plants (other than tomato and potato), are listed below:

1. Blainvillea yellow spot virus in *Physalis* spp. (L.) (Rocha et al. 2013),
2. Euphorbia yellow mosaic virus in *Capsicum chinense* (Jacq.) (Catarino et al. 2020),
3. Physalis yellow spot virus in *Physalis* spp. (L.) (Kitajima 2020),
4. Tomato rugose mosaic virus in *Solanum melongena* (L.) (Kitajima 2020),

5. Tomato severe rugose virus in *Capsicum annuum* (L.) (Rocha et al. 2012),
6. Tomato severe rugose virus in *Capsicum baccatum* (L.) (Bezerra-Agasie et al. 2006),
7. Tomato severe rugose virus in *Solanum betaceum* (Cav.) (Pereira-Silva et al. 2022),
8. Tomato yellow spot virus in *Capsicum annuum* (L.) (Calegario et al. 2007),
9. Tomato yellow spot virus in *Nicandra physalodes* (L.) Gaertn. (Andrade et al. 2006),
10. Tomato yellow spot virus in *Nicotiana tabacum* (L.) (Kitajima 2020),
11. Tomato yellow spot virus in *Solanum commersoniii* (Dun.) (Kitajima 2020) e
12. Tomato yellow vein streak virus in *Capsicum annuum* (L.) (Firmino et al. 2009).

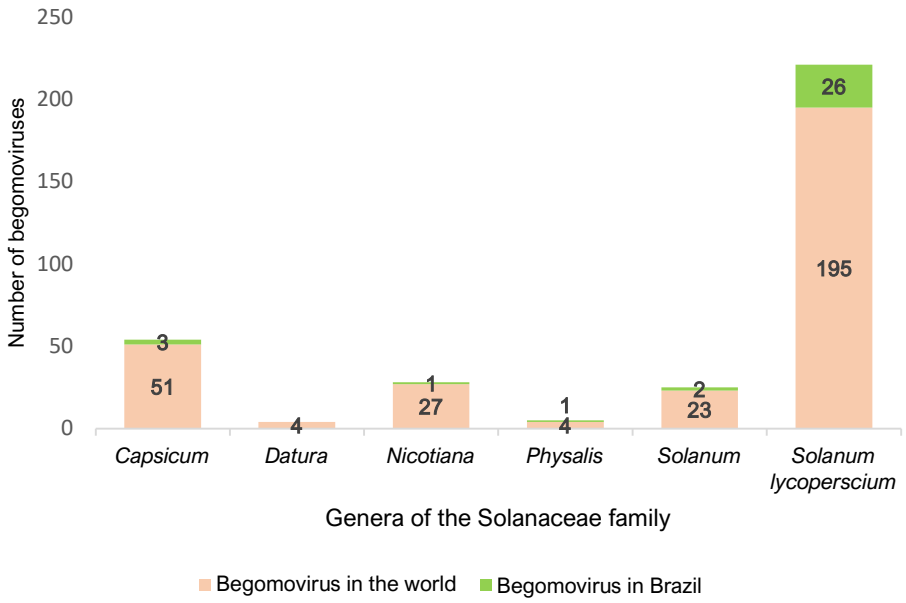

Figure 5. Number of begomoviruses reported in genera of the Solanaceae family based on extensive literature search as well as the viral species reported in the GenBank (2024), Host Database (2024), and Kitajima et al. (2020). The largest number of reported begomoviruses is concentrated in the *Solanum lycopersicum* species, the main crop of the Solanaceae family, with 221 species in the world, with 26 present in Brazil. Among weeds, wild plants, and crops of lesser expression, the largest number of begomoviruses is related to the genus *Capsicum*, 54 in total with three begomoviruses present in Brazil, followed by the genera *Nicotiana* (28), *Solanum* (except *S. lycopersicum* and *S. tuberosum*) (25), *Physalis* (5), and *Datura* (4), the latter genus without reports of a host in Brazil.

Several of the weed species mentioned above are commonly found in tomato fields or associated with tomato crops and function as viral reservoirs (Ma et al. 2020; Kitajima 2020). A brief description of tomato diseases will be presented below, with a focus on diseases caused by viruses and begomoviruses.

5. Main viruses in tomato cultivation

Viruses that affect tomato plants vary according to the variety group, season, and region of cultivation. It is common to observe symptomatic plants of minor crops of the Solanaceae family in areas where tomatoes are grown nearby. The main tomato-infecting viruses are classified within the genera *Orthotospovirus*, *Potyvirus*, *Cucumovirus*, *Tobamovirus*, *Crinivirus*, and *Begomovirus* (Inoue-Nagata et al. 2016; ICTV 2024). In the past, there have been reports of isolates of *Tobravirus* (Cupertino et al. 1991) and *Polerovirus* (Kitajima 2020). In recent years, isolates from the genera *Amalgavirus* (Martins 2016) and *Tymovirus* (Oliveira et al. 2013) have been characterized.

At least seven viruses have the most significant impact on tomato cultivation in Brazil, besides the begomovirus:

1. **Tomato chlorosis virus – ToCV** (genus *Crinivirus*, family *Closteroviridae*), detected for the first time in the country in the state of São Paulo, and transmitted by *Bemisia tabaci* (Barbosa et al. 2008; Vargas-Asencio et al. 2013; Abreu et al. 2012),
2. **Tomato mosaic virus – ToMV** (genus *Tobamovirus,* family *Virgaviridae*), initially detected in tomatoes of the cultivar 'Santa Clara' in the state of São Paulo (Moreira et al. 2013; Rangel et al. 2011),
3. **Potato virus Y – PVY** (genus *Potyvirus*, family *Potyviridae*) which, with its entry into the country, caused major losses in tomato production, but which lost importance with the development of resistant varieties (Lourenção et al. 2005; Esquivel-Fariña et al. 2022, ICTV 2024),
4. **Pepper yellow mosaic virus – PepYMV** (genus *Potyvirus*, family *Potyviridae*), found in the Midwest, Southeast regions, primarily infecting peppers (Inoue-Nagata et al. 2002; Esquivel-Fariña et al. 2022, ICTV 2024),
5. **Tomato spotted wilt virus – TSWV** (genus *Orthotospovirus,* family *Tospoviridae*), the first reports are from the 1930s causing the disease known as 'vira cabeça' (Lovato 2004; ICTV 2024),
6. **Tomato chlorotic spot virus – TCSV** (genus *Orthotospovirus,* family *Tospoviridae*) through analyzes of the host range, serology and amino acid divergence of the capsid protein, new species were known within the genus *Orthotospovirus* (Colariccio et al. 2004; ICTV 2024). TCSV is one of the causal agents of the "vira-cabeça" disease (Fonseca and Boiteux 2021),

7. **Groundnut ringspot virus – GRSV** (genus *Orthotospovirus,* family *Tospoviridae*) identified through the nucleotide sequence of the capsid protein, it is present in important tomato-producing states such as São Paulo (Camelo-García et al. 2014, ICTV 2024). GRSV is currently the most important causal agents of the "vira-cabeça" disease (Fonseca and Boiteux 2021; Jorge et al. 2023).

Other emergent viruses on a global scale (and which have not yet been reported in Brazil) are listed on the list of quarantine pests by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA 2022): tomato black ring virus - TBRV and tomato ringspot virus – ToRSV (genus *Nepovirus*, family *Secoviridae*), cucumber mosaic virus – CMV (genus *Cucumovirus*, family *Bromoviridae*); Pelargonium zonate spot virus – PZSV (genus *Anulavirus*, family *Bromoviridae*), tomato brown rugose fruit virus – ToBRFV (genus *Tobamovirus*, family *Virgaviridae*) and the viral complex related to tomato yellow leaf curl virus – TYLCV (genus *Begomovirus,* family *Geminiviridae*).

Viruses classified in the genus *Begomovirus* (family *Geminiviridae*) occupy a prominent place because they are more frequently associated with severe symptoms, mainly a consequence of the high population density of the whitefly (*B. tabaci*) in producing regions of the country and the wide range of alternative hosts both the virus and the vector (Breves et al. 2023 et al; Reis et al. 2020).

6. Family *Geminiviridae*

The *Geminiviridae* family is the largest among the plant-infecting families and is currently composed of 520 species allocated in 15 genera: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus*, *Turncurtovirus*, and *Welwivirus* (**Table 2**). The criteria that allow the separation of these genera include the host range, vectors, genome organization, and genomic sequence identity supported by phylogenetic analyses (Zerbini et al. 2017; Roumagnac et al. 2022; ICTV 2024). Some diseases caused by representatives members of this family are responsible for inducing yield and quality losses in crops of economic importance in tropical and subtropical regions, including tomato (*Solanum lycopersicum*) (Reis et al. 2020), sweet pepper (*Capsicum annuum*) (Inoue-Nagata et al. 2004), potato (*S. tuberosum* L.), soybean (*Glycine max*) (Inoue-Nagata et al. 2016), cassava (*Manihot esculenta*) (Kuon et al. 2019), bean (*Phaseolus vulgaris*) (Souza et al. 2018), cotton (*Gossypium hirstum*) (Zaidi et

al. 2020), okra (*Abelmoschus esculentus*) (Biswas et al. 2018), and hot peppers (*Capsicum* spp.) (Inoue-Nagata et al. 2016). Viruses of the *Geminiviridae* family are characterized by twinned particles with an icosahedral shape (18–20 *x* 30–32 nm) and a circular single-stranded DNA genome (ssDNA, single strand DNA), circular (2.5–3.0 kb) that they can be called monopartite (a single DNA-A component) or bipartite (two DNA components: DNA-A and DNA-B) (Varsani et al. 2017; Zerbini et al. 2017). Each component has about 2600 nucleotides (nts) and there is no sequence homology between them, except in the so-called common region (RC) of ≈ 200 nts where motifs related to viral replication are contained (Varsani et al. 2014; Brown et al. 2015; Rojas et al. 2018).

Table 2. Characteristics of genera of the *Geminiviridae* family with information on genome features, host species, vectors, and genomic organization.

Genome	Host	Genus (number of species)	Vector	Genomic organization (N/IR/ORFs)[1]	References
Bipartite /Monopartite	Dicotyledoneous	*Begomovirus* (445)	*Bemisia tabaci*	• 5'-TAATATTAC-3' • An intergenic region (LIR) and a common region (CR) with DNA-BORF's: AV1, AV2, AC1, AC2, AC3, AC4, AC5, AC6, AC7, BV1 and BV2	Hanley-Bowdoin et al. (2000); Rojas et al. (2018); Fiallo-Olivé et al. (2021); He et al. 2020; Li et al. (2021); ICTV (2024)
Monopartite	Dicotyledoneous	*Becurtovirus* (3)	*Circulifer haematoceps*	• 5'-TAAGATTCC-3' • Two intergenic regions (LIR and SIR) • ORF's: V1, V2, V3, C1 and C2	Yazid et al. (2008); Varsani et al. (2014); ICTV (2024)
		Capulavirus (4)	*Dysaphis plantaginea*	• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, V3, V4, C1, C2 and C3	Bernardo et al. (2016); Varsani et al. (2017); ICTV (2024)
		Citlodavirus (4)		• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, V3, V4, C1 and C2	Fontenele et al. (2018); ICTV (2024)
		Curtovirus (3)	*Curculifer tenellus*	• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, V3, C1, C2, C3 and C4	Varsani et al. (2014); Hanley-Bowdoin et al. (2013); ICTV (2024)
		Grablovirus (3)	*Spissistilus festinus*	• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, V3, C1, C2 and C3	Krenz et al. (2014); Bahder et al. (2016); Sudarshana et al. 2015; ICTV (2024)
		Mulcrilevirus (2)	*Tautoneura mori*	• 5'-TAATATTAC-3' • An intergenic region (IR) • ORF's: V1, V2, V3, V4, C1 and C2	Qiu et al. (2020); ICTV (2024)
		Opunvirus (1)	*Dactylopius* sp	• 5'-TAATATTAC-3'	Fontenele et al. (2020); ICTV (2024)

				• An intergenic region (IR) • ORF's: V1, V2, C1, C2, C3 and C4	
		Topilevirus (2)		• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, V3, C1, C2 and C3	Vaghi Medina et al. (2018); ICTV (2024)
		Topocuvirus (1)	*Micrutalis malleifera*	• 5'-TAATATTAC-3' • Two intergenic regions (LIR e SIR) • ORF's: V1, V2, C1, C2, C3 and C4	Briddon et al. (1996); ICTV (2024)
		Turncurtovirus (3)	*Curculifer tenellus*	• 5'-TAATATTAC-3' • An intergenic region (IR) • ORF's: V1, V2, C1, C2, C3 and C4	Razavinejad et al. 201; Kamali et al. 2016; Hasanvand et al. 2018; ICTV (2024)
	Dicotyledoneous /Monocotyledoneus	*Maldovirus* (3)		• 5'-TAATATTAC-3' • An intergenic region (IR) • ORF's: V1, V2, C1, C2, C3 and C4	Claverie et al. (2018); Liang et al. (2015); Al Rwahnih et al. (2017); ICTV (2024)
		Mastrevirus (45)	*Cicadulina mbila*	• 5'-TAATATTAC-3' • Two intergenic regions (LIR and SIR) • ORF's: V1, V2, C1 e C2	Cao et al. (2017); Souza et al. (2018); ICTV (2024)
	Monocotyledoneus	*Eragrovirus* (1)		• 5'-TAAGATTCC-3' • Two intergenic regions (IR-1 and IR-2) • ORF's: V1, V2, C1 e C2	Varsani et al. (2014); ICTV (2024)
	Gymnosperms	*Welwivirus (2)*		• 5'-TAATATTAC-3' • Two intergenic regions (LIR and SIR) • ORF's: V1, V2, V3, C1 e C2	Debat and Bejerman (2023); ICTV (2024)

* N – nonanucleotide; IR – intergenic region;
* ORFs (*open reading frames*) are indicated as being encoded in the viral sense = V or complementary sense = C; IR - intergenic region; LIR - long intergenic region; SIR - short intergenic region
* V1 = *Coat Protein*; V2 = *Movement Protein*; V3 = *Regulatory gene*; C1 = *Replication association protein*; C2 = *Trans-acting protein*; C3 = *Replication enhancer protein*; C4 = *symptom-determining protein*.

7. Genus *Begomovirus*: Genomic organization and host plants

Begomoviruses constitute an important group of plant pathogens responsible for severe diseases and losses in several crops of economic importance around the world (Rojas et al. 2018). This genus is the most numerous among plant-infecting viruses and represents 88% of the members of the *Geminiviridae* family (Zerbini et al. 2017; ICTV 2024). These viruses can be classified as monopartite (one component of genomic DNA), with a size between 2.5–3.1 kb, or bipartite, with two similarly sized DNA components (DNA-A and DNA-B) with ≈ 2.6 kb each (Hanley-Bowdoin et al. 2000; Fiallo-Olivé and Navas-Castillo 2023). The two genomic components of bipartite begomoviruses do not share a significant sequence, except for a fragment found in the intergenic region (IR) called the common region (RC), which ranges from 140 to 200 nucleotides (nts) in length (Lazarowitz et al. 1992; Cantú-Iris et al. 2019). This region comprises the origin of replication (*ori*), a stem-loop structure conserved with the conserved 'TAATATTAC' nonanucleotide, in which the cleavage site of the REP protein responsible for viral replication is located, and repeated sequences (iterons) (Heyraud et al. 1993, Argüello-Astorga and Ruiz-Medrano 2001, Argüello-Astorga et al 2004, Briddon et al 2010). This region is vital for preserving the integrity of the bipartite genome, allowing both components to be replicated by REP (Argüello-Astorga et al. 1994). The iterons usually present in this region differ in sequence between closely related viral species or even strains of the same species and are considered the main determinant of virus-specific replication. Thus, the geminivirus REP protein contains an iteron-specific recognition domain that has been mapped to the N-terminal region adjacent to distinct conserved RCR (Rolling Circle Replication) motifs (Choi and Stenger 1995; Gladfelter et al. 1997; Orozco et al. 1997; Chatterji et al. 1999; Argüelo-Astorga and Ruiz-Medrano 2001).

The species demarcation criteria currently used to identify new *Begomovirus* species are based on the identity of the sequences (complete DNA-A) aligned, so that a new monopartite or bipartite species must display identity levels lower than 91% when compared with all previously known species. If these identity levels do not exceed 94%, the begomovirus is considered a new strain (Brown et al. 2015). There is a correlation between the type of begomovirus and its geographic distribution. In Australia, Africa, Asia, and Europe (= Old World) there is a predominance of species with monopartite genomes, while in the Americas (= New World) viral species with bipartite genomes predominate (Rybicki 1994; King et al. 2011). The exceptions found in America are the tomato leaf deformation virus – ToLDeV, in Ecuador and Peru (Melgarejo et al. 2013; Sánchez-Campos et al. 2013), tomato twisted leaf

virus – ToTLV in Venezuela (Romay et al. 2019), and tomato mottle leaf curl virus – ToMoLCV (Vu et al. 2015; Souza et al. 2022), tomato leaf curl purple vein virus – ToLCPVV (Macedo et al. 2018), tomato golden net virus – ToGNV (Reis et al. 2023), and tomato yellow net virus - ToYNV (Reis et al. 2023).

Monopartite begomoviruses and the DNA-A component of bipartite begomoviruses have six to nine ORFs (Open Reading Frames), with the following arrangement: in the viral sense, V1 and V2; and in the complementary sense, C1, C2, C3, and C4 (the C5, C6, and C7 ORFs were recently characterized and are present in some begomoviruses). V1 encodes CP (coat protein), which is the only structural protein and is related not only to the encapsidation of genomic components but to transmission specificity (Ghanim et al. 2014). V2 increases efficiency in viral movement, in addition to suppressing post-transcriptional gene silencing (Roshan et al. 2018). In the complementary sense, C1 encodes REP (replication-associated protein), an essential protein for viral replication (Etessami et al. 1991). REP is not a polymerase; however, it acts as an endonuclease by cleaving the viral DNA at the 5'-TAAGATCC-3' sequence and initiating rolling circle replication (Ruhel and Chakraborty 2019). The C2 encodes TrAP (transcription-activating protein), a multifunctional protein that acts as an activator of viral transcription and is necessary for the expression of genes AV1 (CP – coat protein) and BV1 (NSP – nuclear shuttle protein), present in DNA-A and DNA-B (in the case of bipartite begomoviruses), respectively (Sunter et al. 1990; Sunter and Bisaro 1991). NSP may also act to suppress host gene silencing and is associated with viral pathogenicity (Vanitharani et al. 2004). ORF C3 encodes REn (replication enhancer protein) which enhances viral DNA replication, but is not essential for replication like REP (Hanley-Bowdoin et al. 2000). ORF C4, present in some begomoviruses, is related to the severity of symptoms or systemic movement in the plant (Jupin et al. 1994; Teng et al. 2010). The DNA-A component of some bipartite and monopartite begomoviruses encodes the AC5 protein in the complementary strand direction. The proteins identified as AC5 have about 100 amino acids and are encoded from the ORF located downstream of the AC3 ORF. Most of the AC5 ORF overlaps the 3’ half of the AV1 ORF and may have different functions as can be seen from the species where it is described. In the mungbean yellow mosaic India virus – MYMIV, the AC5 protein has 83 amino acid residues and acts in the viral infection, in addition to exerting RNA silencing suppression activity (Li et al. 2015). In tomato chlorotic mottle virus – ToCMoV, the protein has 250 amino acid residues and it does not play a key role in virus infection, as indicated by the mutagenesis analysis (Fontenelle et al. 2007). In watermelon chlorotic stunt virus – WmCSV, the AC5 protein has 255 amino acid residues and is also not necessary for

infectivity (Kheyr-Pour et al. 2000). The monopartite begomovirus Ageratum leaf curl Sichuan virus – ALCScV, described infecting *Ageratum conyzoides* plants in China encodes an AC5 protein. According to mutational analysis, AC5 contributes to the virus infection process (Li et al. 2021). The AC6 protein was recently characterized in tomato leaf curl China virus detected in samples of *Nicotiana benthamiana*, and with an approximate size of 97 amino acids. AC6 has high specificity with the mitochondria. AC6 is considered evolutionarily conserved, with results from sequence analyzes indicating its presence in approximately 36% of begomoviruses (Wang et al. 2022). The AC7 ORF, also recently characterized via mass spectrometry in tomato yellow leaf curl virus. AC7 is approximately 27 amino acids in size, exhibits interaction with the protein AC2 in the nucleus and with AV2 in the cytoplasm. When AC7 was experimentally blocked, the onset of viral infection was delayed, resulting milder symptoms and less accumulation of viral DNA (Liu et al. 2023).

In bipartite begomoviruses, a subset of DNA-A ORFs is homologous to the genomic DNA of monopartite species (*viz*. AC1/Rep, AC2/TrAP, AC3/REn, AC4, AV2, and AV1/CP), and the DNA-B component encodes two additional proteins: BV1/NSP (nuclear shuttle protein) – nuclear transport protein that acts in the viral movement from the nucleus, where replication occurs, to the cytoplasm (Ward and Lazarowitz 1999); and BC1/MP (movement protein) – a movement protein (encoded by V2 in monopartite species) that is responsible for cell-to-cell viral movement, possibly in interaction with MP (Gutierrez 2002). It is conceptually accepted that NSP facilitates the nuclear egress of newly replicated ss-vDNA through nuclear pores (Martins et al. 2020).

Viruses classified as *Begomovirus* are associated with a wide range of cultivated and non-cultivated dicot hosts, as well as ornamentals. Among the cultivated host species infected by begomovirus are beans (Souza et al. 2018), cotton (Zaidi et al. 2020), cucurbits (Aguiar et al. 2018), okra (Biswas et al. 2018), sweet pepper (Inoue-Nagata et al. al. 2004) and tomato (Reis et al. 2020). Among weeds, *Sida* spp. (Passos et al. 2016), *Macroptilium* spp. (Batista et al. 2022), *Malva* spp. (Shery et al. 2017), and *Jatropha* spp. (Kashina et al. 2013). Ornamental plants like *Abutilon* spp. (Paprotka et al. 2010) and *Lonicera* spp. (Ali et al. 2014) are also begomovirus hosts. Evolution at the local level has resulted in begomoviruses that infect a particular crop or weed species and cause similar symptoms, for example, in tomato crops, there are more than 90 species, and in weeds of the genus *Sida*, there are more than 30 species (Rojas et al. 2018). The main symptoms of begomovirus infections include yellowing, leaf curling, and plant stunting, which leads to significant yield loss (Sohrab 2020; Reis et al 2020).

8. *Begomovirus* replication

With the introduction of the virus by the vector insect into the host cell, the infection process begins, and the genetic material of the virus is transported to the nucleus, with the single-stranded circular viral DNA converted into double-stranded circular DNA and packaged in the host nucleosomes in the form of extrachromosomal minichromosomes. The entire process of synthesis of the complementary strand occurs with the help of the host enzymatic machinery (Stanley 1985). The replication of begomoviruses occurs in the cell nucleus through a mechanism called "rolling circle replication" (RCR) that generates single-stranded circular viral DNA, although recombination-dependent replication (RDR) has also been reported (Gutierrez 1999; Hanley-Bowdoin et al. 2000; Jeske et al. 2001; Gutierrez et al. 2004). Replication initiator protein (REP) is a sequence-specific DNA-binding protein. During the RCR process, REP recognizes and binds at a specific point in the viral sense by introducing a cleavage in the conserved nonanucleotide region within the hairpin. The free 3'-OH end then acts as a primer for host DNA polymerase to initiate DNA synthesis (Lazarowitz et al. 1992; Gutierrez 1999; Hanley-Bowdoin et al. 2000).

Within the nucleus, replication occurs in three distinct stages. The first stage of replication is the conversion of single-stranded DNA (ssDNA) to double-stranded DNA (dsDNA), known as the replicative form (RF). This intermediate form will serve as the template for viral transcription and for the synthesis of new ssDNA strands via the rolling circle mechanism (Stanley 1985; Reis 2020). This process starts with connecting the REP to a specific sequence in the CR composed of two specific repeated sequences called "iterons". After REP binding, it cleaves the ssDNA strand initiating the viral replication cycle. In the second step, using the rolling circle mechanism, dsDNA is used as a template for ssDNA amplification. And the third stage of replication is characterized by the synthesis of ssDNA from dsDNA that occurs at the end of the replication cycle, with the accumulation of viral genomes for encapsidation (Gutierrez 2002; Monsalve-Fonnegra et al. 2002; Yadava et al. 2010; Pradhan et al. 2017).

8.1. Transmission of begomovirus

Begomoviruses are transmitted by members of the *Bemisia tabaci* cryptic species complex. The *B. tabaci* complex is subdivided into 11 groups, harboring 44 cryptic species classified based on the sequence analysis of the mtCOI gene (*mitochondrial cytochrome oxidase* I) and the comparison of sequences described for different species (Liu et al. 2012).

Divergence levels above 3.5% are the main criterion adopted for defining a new member of the complex (Dinsdale et al. 2010). The type of relationship established between the virus and vector is of the non-propagative circulatory type (Czosnek and Ghanim 2012). The predominant vector species are those of the genetic group *B. tabaci* MEAM1 (*Middle East-Asia Minor* 1 = biotype B), and *B. tabaci* MED (*Mediterranean* = biotype Q) (Rosen et al. 2015).

Biological properties such as host plants, resistance to insecticides, ability to transmit different viral species, and their ability to induce physiological disturbances in a given set of hosts can also differentiate species of the *B. tabaci* complex (Rosen et al. 2015). Over the past 30 years, biotypes B and Q were prevalent across many countries, being able to displace some native cryptic biotypes. In Brazil, there is a predominance of *B. tabaci* MEAM1, a highly polyphagous species, with more than 1000 hosts. The presence of *B. tabaci* MED was detected in the municipality of Barra do Quaraí in Rio Grande do Sul, in plants of *C. annuum* L. in a greenhouse and in *Ipomoea batatas* L. under field conditions (Barbosa et al. 2015). In greenhouse tomato plantations, *B.tabaci* MED is already predominant in the state of São Paulo, even though it is associated with inefficiency in the transmission of begomovirus (Nogueira et al. 2024).

8.2. Evolution and variability of the Begomovirus

Begomoviruses have three basic mechanisms of genetic variability: mutation, recombination, and pseudo-recombination (Duffy and Holmes 2009; Rojas et al. 2018).

The **mutation mechanism** is induced by the replication cycle, generation time, and natural selection. Inefficient or non-existent repair by host polymerases is assumed as the main factor associated with high mutation rates of the begomoviruses since these enzymes play such a role in the cell genome (Shackelton and Holmes 2006).

The **recombination mechanism** consists of exchanging fragments of genetic segments during replication, being an important mechanism, and source of genetic variation, for the maintenance and repair of DNA or RNA molecules (Klein et al. 2019). The frequency of these events contributes to increasing the genetic diversity of begomoviruses, resulting in the emergence of new species increasing their evolutionary potential and adaptation to new environmental conditions (Díaz-Pendón et al. 2019). It is the main mechanism of genetic variability that, together with factors such as the high incidence of mixed infections, high levels of viral replication, and the *B. tabaci* vector, especially the B biotype, contribute to the occurrence of more recombination events (Castillo-Urquiza et al 2008; Marwall et al. 2014). In the *Geminiviridae* family, this mechanism leads to the emergence of new species and even new

genera, such as the genus *Becurtovirus*, which may have originated from viral species of the genera *Curtovirus* and *Mastrevirus*, since the ORF's V1, V2 and V3 are analogous to the genus *Curtovirus* and the ORF's C1 and C2 to species of the genus *Mastrevirus*; (Yazdi et al. 2008; Varsani et al. 2014).

In the **reassortment mechanism**, is when the exchange of DNA-A and DNA-B components from different viruses occurs during multiple viral infections. It is a process that is only possible if the interaction of the REP protein with the region at the origin of replication displays high specificity (Bull et al. 2007).

9. Satellite DNAs associated with Begomovirus

Recent studies have indicated that in addition to the DNA-A and DNA-B genomic components, a subset of satellite DNAs is associated with monopartite and bipartite begomoviruses. Three types of satellite DNA have been described in association with begomoviruses: alphasatellites (Zhou et al. 2013), betasatellites (Kumar et al. 2017), and deltassatellites (Lozano et al. 2016).

Alphasatellites are associated with members of the *Geminiviridae*, *Nanoviridae*, and *Metaxyviridae* families. Alphasatellites (formerly known as DNA-1) are single-stranded circular DNA molecules (≈1-1.4 kb) that encode in the viral sense a single replication-associated protein (REP), being dependent on the helper virus for movement, encapsidation, and transmission by the vector (Palukaitis et al. 2008; Briddon et al. 2018; Varsani et al. 2021). Alphasatellites are also characterized by the presence of an adenine-rich region and a hairpin structure, the TAGTATT/AC nonanucleotide (Iqbal et al. 2021). There is no specific function attributed to the alphasatellites and they are not shown to be necessary for infection or the development of symptoms. On the other hand, alphasatellites attenuate symptoms in plants infected with begomovirus-betasatellite complexes by regulating viral and/or betasatellite DNA titers (Briddon et al. 2004; Wu and Zhou 2005). Recent studies demonstrate that the REP protein encoded by some alphasatellites can suppress the host plant gene silencing mechanism at the post-transcriptional level (Abbas et al. 2019; Zhao et al. 2022). The association of alphasatellites with begomoviruses occurs more frequently with monopartite begomoviruses than with bipartite ones, however, there are recent reports of association with mastreviruses (Rosario et al. 2013; Hamza et al. 2018). The discovery of alphasatellites occurred before betasatellites in the Old World. Similarly, alphasatellites have been identified in the New World associated with bipartite begomoviruses (Paprotka et al. 2010; Romay et al. 2010). The origin

of alphasatellites may be linked to nanoviruses, as they exhibit a lot of structural similarity with these viruses. It is believed that the alphasatellites were acquired by a begomovirus during mixed infection with a nanovirus (Mansoor et al. 2003; Mansoor et al. 1999; Wu and Zhou, 2005). Alphasatellites belong to the *Alphasatellitidae* family, established in 2017, with two subfamilies, 11 genera, and 71 species. The demarcation of species within the subfamily *Geminialphasatellitinae* indicates identity levels lower than 88% for new species. The genera in association with viruses of the *Geminiviridae* family are, *Ageyesisatellite*, *Clercusatellite*, *Colecusatellite*, and *Gosmusatellite* (Briddon et al. 2018; ICTV 2022).

Betasatellites are small circular subviral components associated with geminiviruses and have been reported to occur mainly in the Old World. Its discovery begins with the identification of a satellite molecule associated with the tomato leaf curl virus (ToLCV) in Australia (now classified in the genus *Deltasatellite* of the family *Tolecusatellitidae*) (Nawaz-ul-Rehman et al. 2020). Betasatellites have small genomes (≈1360 nts) and do not share nucleotide identity with their helper viruses. The genomic organization of betasatellites is highly conserved and includes an adenosine-rich region (A-rich), a satellite-conserved region (SCR), and a small open reading frame that encodes a protein called bC1 (Briddon et al. 2003; Briddon et al. 2008). Initially, betasatellites were associated with monopartite begomoviruses, but now they are routinely identified in association with bipartite begomoviruses as well. So far, betasatellites are limited to the Old World, however, a recent discovery suggests that a defective form of a betasatellite of African origin (cotton leaf curl Gezira betasatellite – lacking Bc1) has been found in the United States (Briddon et al. 2003; Villegas et al. 2019). Classification as betasatellite or deltassatellite is based on the genomic nucleic acid sequence, and the species demarcation criterion for both genera has a limit of 91% nucleotide sequence identity. 119 species of betasatellites are accepted by the ICTV (Nawaz-ul-Rehman et al. 2021; ICTV 2022).

The family *Tolecusatellitidae* was recently created to encompass the genera *Betasatellite* and *Deltasatellite*. The family name originates from the first satellite identified in Australia (tomato leaf curl virus satellite). This first species is now classified as a member of the genus *Deltasatellite*, being renamed tomato leaf curl deltasatellite (ToLCD). Deltasatellites are characterized by a genome of ≈0.7 kb and currently correspond to 11 species, phylogenetically distinct. (Dry et al. 1997; Nawaz-ul-Rehman et al. 2021). They were initially found in the Old World, but later there were reports of association with bipartite New World begomoviruses (Fiallo Olivé et al. 2012; Lozano et al. 2016). Unlike alphasatellites and betasatellites, deltassatellites do not encode any proteins. Deltasatellites are totally dependent

on the helper virus for replication, and movement in plants, as well as for transmission via the *B. tabaci* vector. The presence of deltasatellites in some host-virus combinations results in reduced begomovirus accumulation and/or attenuated symptom expression (Fiallo-Olivé et al. 2016; Hassan et al. 2016).

10. The use of High-Throughput Sequencing (HTS) in plant virology

The study of viral populations or virus communities, whether of known species or not, has become faster and less costly since the insertion of High-Throughput Sequencing (HTS) together with improved sequencing technologies, including Illumina, 454, Pacific Biosciences, Ion Torrent, and Nanopore (Barba and Hadidi 2015; Adams and Fox 2016; Villamor et al. 2019).

The adoption of this technology overcame challenges in the plant virus detection process and contributed with very useful ecological and epidemiological information for a better understanding of virus species. The first studies carried out with the use of HTS in plant virology were completed a decade ago (Adams et al. 2009; Al Rwahnih et al. 2009; Donaire et al. 2009; Kreuze et al. 2009). Currently, the use of HTS allows the discovery of many new species, having consolidated itself as a classic approach in research laboratories and diagnosis of plant virology (Massart et al. 2019).

Analyzes performed through the combination of metagenomics and HTS for the characterization of viruses and viroids are collectively called “viromes” (Barba et al. 2014; Villamor et al. 2019). Studies on the diversity of plant viruses have reported that the samples were submitted to procedures of enrichment of viral particles prior to sequencing through semi-purification protocols followed by transmission of nucleic acid (DNA or RNA), as well as acquired from dsRNA and sRNAs (= small RNAs produced as a result of plant defense control against viruses, such as gene silencing) (Roosinck et al. 2015). For DNA virus enrichment, a protocol employing Rolling Circle Amplification – RCA is performed (Idris et al. 2014; Kathurima et al. 2016; Massart et al. 2019).

In recent work carried out in Mexico, 132 non-cultivated plant species belonging to 34 families were subjected to metagenomic analysis combined with HTS. These analyses indicated a great diversity of *Begomovirus* in non-cultivated plants of the families Brassicaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, and Solanaceae. Fourteen begomoviruses with monopartite genomes and five with bipartite genomes were

detected, in addition to species of the genus *Curtovirus*, BCTV, and TPCTV (Rodríguez-Negrerte et al. 2019).

In tomato crops in Brazil, samples with typical symptoms of viral infections were collected in Brazlândia (DF), Campinas (SP), and Araguari (MG). The results obtained from the collected materials showed a great viral diversity of natural occurrence in the Brazilian production fields of GRSV and TSWV; pepper ringspot virus – PepRSV (*Tobravirus*); ToCV (*Crinivirus*); Sida micrantha mosaic virus – SiMMV and ToSRV (*Begomovirus*); tomato blistering mosaic virus – ToBMV (*Tymovirus*); pepper yellow mosaic virus – PepYMV and potato virus Y – PVY (*Potyvirus*); and pepper mild mottle virus – PMMoV (*Tobamovirus*). This work is also the first report of southern tomato virus – STV (genus *Amalgavirus*) in the country and report of a probable new species of *Ilavirus* due to its low nucleotide identity with the species Ageratum latent virus – ALV and Parietaria mottle virus – PmoV (Martins et al. 2016). A gemycircularvirus (*Genomoviridae* family), a new alphasatellite, and two new species of begomovirus were identified in tomatoes without the *Ty*-1 gene using HTS, which allowed the evaluation of the diversity of ssDNA viruses in tomato samples with or without the gene *Ty*-1 (Reis et al. 2020) as well as *Ty*-3 (Oliveira et al. 2024).

Thus, HTS technology, supported by different bioinformatics tools, is used to recover information from small RNA sequences, derived from nucleic acids of plants, infected by begomovirus. This type of analysis increases dramatically the knowledge about viral diversity and helps to precocious detection of emerging viruses that affect crops across different agroclimatic regions (Vilamor et al. 2019; Pandey et al. 2021).

Table 3. Properties of High-Throughput Sequencing (HTS) sequencing platforms.

Sequencing Platform	Classification (Generation)	Operation principle	Read size	Error rate (%)	Advantages	Drawbacks
Illumina	Second generation	Technology based on "sequencing by synthesis" with a reversible terminator technology with fluorescent label.	200 - 600 bp	0.1	- Multiplexing reduces cost per sample. - Many computational tools dedicated to short-read data mining.	- Long runtime. - Difficulties with *de novo assembly*. - Haplotype phasing. - Identification of transcription isoforms and structural variants.
Ion Torrent		Method based on Ion Sphere particles in microwell via emulsion PCR.	200 – 600 bp	<0.1	- Low error rate - Many computational tools dedicated to short-read data mining.	- Difficulty in sequencing through homopolymeric regions.
Pacific Biosciences (PacBio)	Third generation	Also known as SMRT (Single Molecule Real Time), it is generated by ligating hairpin adapters (SMRTbell adapters) to both ends of the DNA template molecule.	Up to 300 kb	~14	- Very long readings. - Phasing polymorphic genes. - Data collected in real time. - Does not require DNA amplification.	- High error rate. - High investment. - Big instruments.
Nanopore		Based on the passage of single-stranded nucleic acid (DNA or RNA) through a pore of the staphylococcal protein α-hemolysin (αHL).	Up to 4 Mb	2–15	- Better phasing of polymorphic genes. - Detection and adequate characterization of structural rearrangements. - Real-time data collection and faster response time. - Portable and lower-cost instruments. - Field application.	- High error rate. - Subject to signal-to-noise restrictions.

Adapted from Hu (2021).

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CHAPTER 2

Metagenomics of ssDNA viruses and subviral satellites associated with weeds of the Fabaceae and Solanaceae families.

ABSTRACT

The invasion of *Bemisia tabaci Middle East Asian Minor* 1 (MEAM 1) (= Biotype B) in the 1990s is the most relevant historical event associated with the dramatic increase in outbreaks of tomato-infecting begomoviruses in Brazil. This polyphagous vector is able to colonize a wide range of hosts, spreading begomoviruses across the country. Weeds from the Fabaceae and Solanaceae families are quite common around and within commercial fields and they may function as alternative hosts of one or more tomato-infecting begomoviruses. High-Throughput Sequencing (HTS) is a powerful tool for large-scale detection of begomoviruses in plants, helping to elucidate crucial epidemiological aspects of this group of pathogens. Herein, we employed a HTS-based strategy (Illumina NovaSeq 6000 platform) to characterize a pool of leaf samples displaying begomovirus-like symptoms from weed species of the Fabaceae family (23 samples) and from the Solanaceae family (64 samples). Bioinformatics analyzes combined with the use of PCR with species-specific primers allowed the recovery of eight begomovirus: tomato severe rugose mosaic virus – ToSRV, bean golden mosaic virus – BGMV, tomato golden vein virus – TGVV, tomato mottle leaf curl virus – ToMoLCV, tomato rugose mosaic virus – ToRMV, tomato chlorotic mottle virus – ToCMoV, Sida micrantha mosaic virus – SimMV, and a putative new species phylogenetically related to SimMV. Two viruses of the genus *Topilevirus* were detected in samples collected in the Distrito Federal and Goiás. A virus of the genus *Gemycircularvirus* (family *Genomoviridae*) not yet reported in plants was also found. One of the contigs, named as new species #1, displayed 90.6% nucleotide identity with the reference genome of SiMMV (KU852503.1), suggesting that it is a new begomovirus according to the current criteria for species demarcation. ToSRV was found to be predominant among Fabaceae and Solanaceae weeds. PCR assays with species-specific primers allowed the identification of two new hosts of ToSRV. Four new hosts of Euphorbia yellow mosaic virus (EuYMV) and three new solanaceous hosts of SimMV were also detected in these assays. Eight new hosts of tomato mottle leaf curl virus (ToMoLCV) were detected across the Northeast, Midwest, and Southeast regions. These results highlight the viral diversity in weeds and emphasize their role as reservoirs of tomato-infecting begomoviruses across major producing regions.

Keywords: high-throughput sequencing, host range, *Geminiviridae*, *Topilevirus*, *Genomoviridae*

1. Introduction

Viruses of the *Geminiviridae* family have single-stranded circular DNA (ssDNA) genomes individually encapsidated in twinned, nearly icosahedral particles measuring 22 x 38 nm composed of 110 capsid protein subunits organized as 22 pentameric capsomers (Hipp et al. 2017; Hesketh et al. al. 2018). Viruses classified in this family are either monopartites (with a single DNA molecule of ~2600-3600 nucleotides – nts) or bipartites (with two DNA molecules: DNA-A and DNA-B, each with ~2600 nts) (Zerbini et al. 2017; ICTV 2024). *Geminiviridae* is the largest family of plant viruses comprising 520 species distributed in 15 genera. Criteria used for differentiation of genera include host range, vectors, genomic organization, and genomic sequence identity combined with phylogenetic analysis (Zerbini et al. 2017; Roumagnac et al. 2022; ICTV 2024).

The genus *Begomovirus* is the most numerous in the *Geminiviridae* family, comprising around 445 species. Six to nine ORFs (open reading frames) are present in the genome of bipartite begomoviruses, according to the viral species. Two ORFs are in the viral direction (AV1 and AV2) and four to seven in the complementary strand (AC1, AC2, AC3, AC4, AC5, AC6 and AC7). The AV1 gene encodes the coat protein (CP), essential for genome encapsidation, viral movement, and vector transmission (Ghanim et al. 2014). The AV2 gene codes for a putative pathogenicity factor and has functions related to viral movement, symptom development, and it may also act as a gene-silencing suppressor (Roshan et al. 2018). In the complementary sense, AC1 (replication initiator protein, REP) is essential in viral DNA replication (Ruhel and Chakraborty 2019). The AC2 gene encodes the transcriptional activating protein (TrAP) that interferes with transcriptional and post-transcriptional gene silencing (also named as transcriptional gene silencing – TGS and post-transcriptional gene silencing – PTGS), and CP expression (Fiallo-Olivé et al. 2021). The gene encoding the AC3 protein (replication enhancer protein, REn) increases viral DNA accumulation and is involved in interaction with host retinoblastoma-related proteins (RBR) (Hanley-Bowdoin et al. 2000). The AC4 protein neutralizes PTGS by inhibiting siRNA accumulation and is considered an important symptom determinant (Fiallo-Olivé et al. 2021). The DNA-A component of some begomoviruses encodes the AC5 protein (with ~100 amino acids). This protein may exert different functions such as RNA silencing suppression activity or contribute to the infection process (Li et al. al. 2015). Recently, two new viral ORFs (positioned in the complementary direction) were characterized in begomoviruses. The novel AC6 protein (with ~ 97 amino acids) was inicially characterized in tomato leaf curl China virus, being located specifically in the mitochondria. AC6 protein is

considered evolutionarily conserved, being present in ~36% of begomoviruses (Wang et al. 2022). Using mass spectrometry, the novel AC7 protein (with ~ 27 amino acids) was identified in tomato yellow leaf curl virus, exhibiting interaction with the AC2 proteins (in the nucleus) and AV2 (in the cytoplasm). Experimental assays blocking the AC7 protein caused a delay in the onset of viral infection, milder symptoms, and less accumulation of viral DNA and proteins (Liu et al. 2023). In monopartite begomoviruses, the genomic DNA is homologous to the bipartite DNA-A with the following correspondence of ORFs: AC1/Rep, AC2/TrAP, AC3/REn, AC4, AV2 and AV1/CP (Ward and Lazarowitz 1999). In bipartite begomoviruses, two additional non-overlapping ORFs are found in the DNA-B component, BC1, a movement protein (MP), and BV1, the nuclear transport protein (NSP) (Martín-Hernández and Pagán 2022).

Crops of economic importance classified in the botanical families Fabaceae and Solanaceae show significant yield and quality losses caused by viruses of the *Geminiviridae* family. These crops include tomato (*Solanum lycopersicum* L.) (Reis et al. 2020), sweet pepper (*Capsicum annuum* L.) (Inoue-Nagata et al. 2004), potato (*S. tuberosum* L.), soybean (*Glycine max* L.) (Inoue-Nagata et al. 2016), cowpea [*Vigna unguiculata* (L.) Walp.] (Naito et al., 2019), and common bean (*Phaseolus vulgaris* L.) (Souza et al. 2018). The first report of begomovirus in tomato crops in the world was made in the state of São Paulo, in 1960 (Flores et al. 1960). However, the first report of begomovirus in the country had been made ten years earlier, in a weed of *Euphorbia heterophyla* (Costa and Bennett 1950). In beans, the most relevant crop of the Fabaceae family, the first begomovirus described was bean golden mosaic virus (BGMV) causing great damage to plantations during the dry period in the 1970s in the South, Southeast, and Midwest regions of Brazil (Costa, 1975). In the 1990s, BGMV was also detected in soybean crops in Brazil (Gilbertson et al. 1991). In the early 1990s, the presence of a new biotype of the vector *B. tabaci Middle East-Asia Minor* 1 – MEAM1 (= biotype B) was reported in Brazil, initially in São Paulo, and later in several other states (França et al. 1996). *Bemisia tabaci* MEAM-1 has a wider host range than the endemic *B. tabaci* biotype A, easily colonizing a wide range of Solanaceae hosts (Bedford et al. 1994; Ambrozevicius et al. 2002; Hashmi et al. 2018) and Fabaceae (Freitas-Vanzo et al. 2021). The polyphagy of *B. tabaci* MEAM1 favors the occurrence of mixed infections, increasing the likelihood of recombination events between begomoviruses and the emergence of new viruses (Pita et al. 2001; Reis et al. 2020).

The consistent presence of weeds and wild/native plants growing side by side with commercial crops is quite often observed in tropical and subtropical agrosystems. Many of the cultivated Solanaceae species grow alongside weed species of the same family (Singh et al.

2016). Besides competing for resources such as light, water, and nutrients, weeds can function as potential reservoirs of crop-infecting viruses. These viruses can be transmitted to cultivated plants via polyphagous insect vectors, leading to either disease outbreaks or the emergence of new viral species (Power et al. 2011; Dille et al. al. 2016). An example of a weed favoring variability-generating events concerns the emergence of adapted recombinants of tomato yellow leaf curl virus – TYLCV from mixed infections occurring in the black nightshade (*Solanum nigrum*) (García-Andrés et al. 2006). There are indications that the viruses emerging in solanaceous plants can also infect plants of the Fabaceae family, such as tomato rugose mosaic virus – ToRMV, which was detected in *Nicandra physalodes* and *Phaseolus vulgaris* growing close to tomato fields (Fernandes et al. 2006).

The potential impact of the global viral genomic population (virome) on the emergence of diseases has been underestimated for a long time. However, the role of non-cultivated plants as reservoirs and evolution vessels of crop-adapted begomoviruses is now under more intense investigation (Guevara-Rivera et al. 2022; García-Arenal and Zerbini 2019). In this regard, High-Throughput Sequencing (HTS) has been a powerful tool to identify both new virus-host interactions and new viruses using metagenomics and ecogenomics approaches (Barba et al. 2014; Massart et al. 2019).

Given this scenario, we performed metagenomic analyzes using HTS tools in order to characterize of the virome of weed and invasive species from the Fabaceae and Solanaceae families. This set of analyses will allow the elucidation of the genetic structure and epidemiological dynamics of begomoviruses in these hosts as well as their potencial role as reservoirs of crop-infecting viral species. For this, a representative set of Solanaceae and Fabaceae samples were collected across all five Brazilian macroregions from 2001 to 2020.

2. Material and methods

2.1. Foliar samples of weeds in tomato crops and DNA extraction

Leaf samples showing symptoms similar to those of begomovirus (apical and interveinal chlorosis, yellowing, golden mosaic, severe mosaic, leaf deformation, epinasty, roughness and dwarfism) were collected between 2001 and 2020 in nine Brazilian states, in addition to the Distrito Federal, thus covering representatives of the five regions of the country, in a total of 87 individual samples distributed in the two botanical families of interest: Fabaceae, with 23 samples and Solanaceae, with 64 samples (**Table 1**). DNA extraction was carried out using a modified protocol of 2X CTAB + organic solvents (Boiteux et al. 1999). The total DNA from

each sample was stored at -20°C. In addition to the botanical family of the host (including Fabaceae, Solanaceae), another criterion for sample selection was choose representatives samples from different states within the collection regions. Thus, the samples were selected and organized by botanical family, region, city, and year of collection.

2.2. Enrichment via Rolling Circle Amplification (RCA) of circular DNA molecules in each sample

Circular DNA molecules obtained in the extraction process were selectively enriched by rolling circle amplification (RCA) assays (Inoue-Nagata et al. 2004). The next step consisted of analysis in agarose gel and via NanoVue Plus®. Concentrations were adjusted and then used to comprise the pool for sequencing. (**Table 1**).

Table 1. Information about the samples used in this work and organization of the pool according to the botanical family and region of origin of the collection (city and year) and isolate code according to the Begomovirus Collection of the CNPH Laboratory in Brasília-DF, Brazil.

Botanical Family	Region	City and year	Corresponding sample code
Solanaceae	North	Manacapuru (2016); Gurupi (2007); Guaraí (2007); Araguaina (2008); Aragominas (2008); Gurupi (2008); and Gurupi (2008)	AM–046; TO–051; TO–065; TO–096; TO–097; TO–191, and TO–195
	North East	Guaraciaba do Norte (2005) and Serra do Ibiabapa (2011)	CE–020 and CE–051
	Midwest	Gama (2003); Gama (2003); Ponte Alta Norte (2003); Gama (2004); Gama (2004); Gama (2004); Gama (2009); Gama (2009); Gama (2009); Pipiripau (2010); Gama (2011); Gama (2011);Taquara (2011); Rajadinha (2012); Rajadinha (2013); Rajadinha (2013); Tabatinga (2013); Tabatinga (2013); Gama (2013); Taquara (2014); Gama (2016); Gama (2019); Gama (2019); Gama (2020); Gama (2020); Gama (2020); Gama (2020); Goianápolis (2003); Goianápolis (2003); Orizona (2003); Morrinhos (2003); Goianápolis (2003); Leopoldo de Bulhões (2004); Leopoldo de Bulhões (2004); Leopoldo de Bulhões (2004); Leopoldo de Bulhões (2004); Orizona (2008); Goianápolis (2010); Bonfinópolis (2010); Goiânia (2012); Bonfinópolis (2015); Padre Bernardo (2019); and Teresópolis de Goiás (2020)	DF–020; DF–033; DF–061; DF–077; DF–078; DF–079; DF–267; DF–268; DF–300; DF–345; DF–401; DF–413; DF–445; DF–486; DF–508; DF–509; DF–533; DF–558; DF–561; DF–587; DF–623; DF–715; DF–737; DF–744; DF–757; DF–764; DF–776; GO–041; GO–073; GO–203; GO–215; GO–253; GO–312; GO–318; GO–327; GO–329; GO–408; GO–459; GO–466; GO–521; GO–598; GO–624; and GO–639
	Southeast	Venda Nova (2011); Venda Nova (2011); Venda Nova (2013); Araguari (2012); Araguari (2012); Araguari (2012); Capão Bonito (2011); Tupã (2012); Tupã (2012); Bragança Paulista (2013); Bragança Paulista (2013); and Sumaré (2020)	ES–045; ES–049; ES–110; MG–336; MG–337; MG–355; SP–100; SP–127; SP–130; SP–150; SP–152, and SP–283
	South	Feliz (2013)	RS–085
TOTAL			**64 samples**
Fabaceae	North	Palmas (2008); Palmas (2008); Lagoa da Confusão (2008); Lagoa da Confusão (2008); and Aragominas (2008)	TO–104; TO–118; TO–137; TO–140, and TO–215
	North East	Jequié (2011); Jequié (2011); and Quixeré (2017)	BA–104; BA–105, and CE–076

	Midwest	Taquara (2005); Gama (2005); Gama (2008); Gama (2008); Gama (2008); UnB (2011); Lago Norte (2019); Lago Norte (2019); CNPH (2020); Gama (2020); Gama (2020); Goiânia (2003); and Padre Bernardo (2019)	DF–161; DF–199; DF–253; DF–254; DF–257; DF–368; DF–709; DF–710; DF–750; DF–753; DF–763; GO–050; GO–051; and GO–624
	Southeast	Araguari (2012)	MG–352
	South	-	-
TOTAL			**23 samples**

2.3. High Throughput Sequencing (HTS) of weeds of the Fabaceae and Solanaceae families

The pool comprised of 87 samples: 23 from the Fabaceae and 64 Solanaceae species. The pool was then subjected to HTS on a platform with the Illumina NovaSeq 6000 system. This pool was named as PFS.

2.4. Analysis of sequences obtained in High-Throughput Sequencing (HTS)

The reads generated in the sequencing were analyzed according to the following workflow: **(1)** elimination of low-quality readings; **(2)** sequence reassembly using the CLC Genomics Workbench 10 program; and **(3)** validation of the contigs via the BLASTn algorithm comparing with the GenBank ssDNA virus database (https://www.ncbi.nlm.nih.gov/). Following the methodology proposed by Nery et al. (2020) and Reis et al. (2020), the next step consisted of mapping the contigs of potential viruses in order to obtain the final sequences corresponding to the genomes of distinct viruses. These contigs were extended using the 'Map to reference' tool available in the Geneious 11.0.5 software (parameter 90 to 99% of minimum overlap identity) that allows mapping reads obtained in the HTS that are related to the contig (Kearse et al. 2012). After assembly, the contigs were analyzed according to the RefSeq viral database (NCBI) comparison under very high stringency conditions (minimum match percentage = 98%) via the BLASTn algorithm. For ORF annotation based on the GenBank reference genome, sequences were subjected to the MUSCLE alignment option of the 'Pairwise/ Multiple align' tool (Geneious 11.0.5 software). Using the '*De Novo* Assemble' tool, the contigs from the PFS pool were submitted to a taxonomic prediction analysis using the Kaiju web server (https://kaiju.binf.ku.dk/server), with default classification parameters (Tran and Phan 2020). This analysis indicated sequences of putative viral origin. Then, the largest sequences were selected, assembled, and subsequently aligned to the reference genomes with greater identity using the MUSCLE alignment option of the 'Pairwise/ Multiple align' tool for ORF annotation. In addition, analyses were carried out with the intergenic region (in monopartite viruses) and the common region (in bipartites) of all putative new species. In the common region, the nonanucleotide and iteron motifs were characterized, as well as the REP-IRD domains (Rep Iteron-Related Domains) that allow confirming that DNA-A and DNA-B components are cognate (Argüello-Astorga and Ruiz-Medrano 2001; Argüello-Astorga et al., 2004). For comparison across isolates and viral species, the sequences were subjected to

multiple MUSCLE pair-by-pair alignments with the aid of the SDT program (Muhire et al. 2014).

2.5. Use of species-specific primers for detection PCR in individual samples

Based on the contig assembly results, PCR analyzes were performed to detect viruses in the samples. For some of the viruses recovered in HTS, specific primers previously designed and available in the library of the Laboratory of Plant Virology – LVV – Departament of Phytopathology – UnB allowed the detection step. Initially, the row and column system (LxC) were used, in which a matrix was determined with the number of cells closest to the number of samples, with the distribution of samples being done horizontally in alphabetical order of the collection regions (Midwest, Northeast, North, Southeast and South) (**Table 1**). As a positive control, aliquots from the pool and samples of isolates previously characterized by Reis et al. (2020) and Batista (2020), were used. Detection on individual samples was performed with species-specific primers by using a L (Line) *x* C (column) system. PCR were performed to a total volume of 12.5 µL containing 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 (Forward and Reverse) (10 µM); 0.1 µL of *Taq* DNA Polymerase enzyme (5U/ µL, Invitrogen); 8 µL of MilliQ water and 2 µL of RCA (diluted in MilliQ water 1:10). PCR amplification reactions (from RCA) consisted of the following steps: **1. Denaturation** (94º C for 2 minutes, followed by 35 cycles each of 94º C for 30 seconds); **2. Annealing** (at specific temperature for each primer used as shown in **Table 2**, for 45 seconds) and **3. Extension** (72º C for 3 minutes). The amplicons were visualized on a 1% agarose gel stained with ethidium bromide.

2.6. Sequence analysis

According to the criteria established by Brown et al. (2015), the complete DNA-A genome of a putative novel begomovirus was used in Sequence Demarcation Tool (SDT) analyses in which another 39 DNA-A sequences of begomoviruses infecting tomatoes and weeds in Brazil (data obtained in March 2024) were subjected to MUSCLE alignment (Edgar 2004).

2.7. Botanical identification of hosts using *rbcL* e *matK* genes

The precise botanical identification of the host species of the pool was done through DNA barcode (Hebert et al. 2003). The regions of the *rbcL* gene (encoding the protein ribulose-1,5-bisphosphate or Rubisco) are useful since they are highly conserved (Hollingsworth et al. 2009). The gene *matK* encodes the protein maturase K, which evolves rapidly but has a strong phylogenetic signal, making its use relevant for taxonomic purposes (Müller et al. 2006).

Table 2. Informations about specific PCR primer pairs used for detection of begomovirus and topilevirus, with specific primer name, sequence and recognition temperature for each primer. Information extracted from Reis et al. (2020) and Batista (2019).

Viral species - Components	Primer name	(Foward) 5'-3'/ (Reverse) 3'- 5'	Annealing temperature °C
***Tomato severe rugose virus* DNA-A**	ToSRV-For5.1	AGCGTCGTTAGCTGTCTGGCA	58
	ToSRV-Rev5	TGCCGCAGAAGCCTTGAACGCACCT	
***Euphorbia yellow mosaic virus* DNA-A**	EuYMV-A-R-For	GGGGTTCCAAGTCCAATAAAGATGA	52
	EuYMV-A-R-Rev	CAGACACCTTATATTTGCCGGATTC	
***Tomato mottle leaf curl virus* DNA-A**	ToMoLCV-For	TGTGGTCCAGTCAATAAATG	47
	ToMoLCV-Rev	TGACTGGACCACATAGTAAA	
***Sida micrantha mosaic virus* DNA-A**	SiMMV-For	GATCTCGCTCCCCCCTCT	58
	SiMMV-Rev	AGATCGCACGACAACCAG	
Tomato apical leaf curl virus	Cap1PstI-F	CTGCAGAYTTGCGCGGATCGATTAAT	68
	Cap1PstI-R	CTGCAGAAATGCGTTGTAACTTCTCGGATAT	
Tomato associated geminivirus 1	Cap2KpnI-F	GGTACCCCCCTTGGAAATGTAGTCTGCAAC	66
	Cap2KpnI-R	GGTACCTTTGAGGAGAGAGGTATACTTCG	

3. Results and Discussion

The HTS sequencing carried out on the Illumina NovaSeq 6000 platform generated 7,477,430 reads, 38,575 contigs that corresponded to 109 segments of viral genomes (in the analysis carried out via BLASTn) present in the raw data of the PFS pool. This pool was composed of 87 foliar samples of Fabaceae and Solanaceae plants exhibiting begomovirus-like symptoms (**Table 3**). The Illumina NovaSeq 6000 platform has the advantages of high output (1.2 – 6,000 Gb), high precision, minimum accuracy of 75% at a running cost of ~ 10 USD/GB (Kumar et al. 2019; Hu et al. 2021).

Table 3. Raw sequencing data from the PFS pool (composed of 87 foliar samples of Fabaceae and Solanaceae species displaying begomovirus-like symptoms) obtained by High-Throughput Sequencing with the Illumina NovaSeq 6000 platform.

Botanical Family	Reads	Contigs	BLAST
Fabaceae (23) and Solanaceae (64)	7.477.430	38.575	109 virus sequences

3.1. Viral diversity of the sample pool of the Fabaceae and Solanaceae families

The genomic information obtained by HTS and contig assembly allowed the recovery of 12 single-stranded (ss) viral DNA genomes: eight begomovirus-like, two *Topilevirus*, one *Alphasatellite* and one *Gemycircularvirus* (contig 18119), a genus that is related to Sclerotinia gemycirculavirus 1 (Krupovic et al. 2016). (**Table 4**).

Most viruses recovered via HTS were previously reported in hosts from the Fabaceae and/or Solanaceae families, except: **(1)** The Euphorbia yellow mosaic alphasatellite – EuYMA that was previously detected in association with the begomovirus Euphorbia yellow mosaic virus – EuYMV occurring on *Euphorbia heterophylla* (Euphorbiaceae) and *Cleome affinis* (Cleomaceae) in Brazil (Paprotka et al. 2010), and (**2)** *Gemycircularvirus* (family *Genomoviridae*): Pacific flying fox faeces associated Gemycircularvirus-2, which was initially characterized in samples of bat feces from the archipelago of Tonga located in the southern portion of the Pacific Ocean (Male et al. 2016). Studies indicate that EuYMA negatively affects the transmission of EuYMV by *B. tabaci* MEAM1, in addition to modulating symptoms and viral accumulation (Sattoriva et al. 2014; Mar et al. 2017). The *Genomoviridae* family comprises plant viruses such as Momordica charantia associated virus – MoaGmV and Euphorbia heterophylla associated gemycircularvirus – EuaGmV, which are associated with *M. charantia* and *E.*

heterophylla, respectively. Species demarcation criterion in the *Genomoviridae* family is nucleotide identity less than 78% in alignments with already known genomoviruses (Rezende et al. 2018).

Among the 12 viral genomes recovered are two members of the *Topilevirus* genus, viz: ToALCV (contig 18) and TAG1 (contig 9186) (**Table 4**). According to the criteria for demarcating species of the genus *Topilevirus*, isolates with nucleotide identity below 78% can be classified as new species (Vaghi-Medina et al. 2018).

The genomes of seven previously reported *Begomovirus* species were recovered here: **(1)** tomato mottle leaf curl virus – ToMoLCV (contig 35310), **(2)** tomato severe rugose virus – ToSRV (contig 2433), **(3)** bean golden mosaic virus – BGMV (contig 106), **(4)** tomato golden vein virus – TGVV (contig obtained from taxonomic prediction analysis using the Kaiju web server), **(5)** tomato rugose mosaic virus – ToRMV (contig obtained from taxonomic prediction analysis using the Kaiju web server), **(6)** tomato chlorotic mottle virus – ToCMoV (contig obtained in taxonomic prediction analysis using the Kaiju web server), and **(7)** Sida micrantha mosaic virus – SiMMV. The contig 26029 was a divergent begomovirus genome which was tentatively named as new species #1 (**NS#1**). This sequence displayed 90.6% identity with Sida micrantha mosaic virus, corresponding to a potential new species according to current taxonomic criteria (Brown et al. 2015).

Among the begomoviruses recovered ToSRV was one of the most prevalent. ToSRV is a bipartite begomovirus that infects tomato plants and is widely disseminated in two important Brazilian biomes, namely the Cerrado and the Atlantic Forest, with characteristics such as high adaptive capacity and prevalence in field conditions, especially in Central Brazil (Reis et al. 2020; Duarte et al. 2021b). The genome recovered from monopartite begomovirus ToMoLCV (Souza et al. 2022), with 2630 nucleotides and coverage of 100 reads, represents the only monopartite begomovirus found in the pool subjected to HTS. BGMV, another begomovirus recovered in the genome assembly stage, is associated with bean crops and other hosts from the Fabaceae family such as *Phaseolus lunatus*, *Canavalia ensiformis*, *Vigna unguiculata*, *Macroptilium atropurpureum* and *Macroptilium erythroloma* (Freitas-Vanzo et al. 2021; Batista et al. 2020). The sequence named as contig 6 displayed 92.82% identity with BGMV, which would place it as a potential new strain of this begomovirus (Brown et al. 2015). For the seven bipartite begomoviruses with DNA-A recovered, the cognate DNA-B components were also detected (**Table 5**). The cognate analysis, carried out according to Arguello-

Astorga and Ruiz Medrano (2001), indicated the following relationships: ToSRV DNA-A (contig 213) and ToSRV DNA-B (contig 180), with the iteron GGTAGT; BGMV DNA-A (contig 106) and BGMV DNA-B (contig 210), with the iteron GGTGT; ToRMV (contig obtained in taxonomic prediction analysis using the Kaiju web server) and ToRMV (contig 13), with the iteron GGTAGT; ToCMoV DNA-A (contig obtained from taxonomic prediction analysis using the Kaiju web server) and ToCMoV DNA-B (contig 6436), with the iteron GGGGT; TGVV DNA-A (contig obtained in the taxonomic prediction analysis using the Kaiju web server) and TGVV DNA-B (contig 47), with the iteron GGGGT; SimMV DNA-A (contig 5362) and SimMV DNA-B (contig 29), with the iteron GGTGT. The potential new species related to Sida micrantha mosaic virus referring to contig 26029 presented contig 381 as a cognate, with a distance of 97.87% and with the iteron GGTGT.

Table 4. Contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of assembled viral genomes, E-value, the corresponding viral description, and GenBank accession for DNA-A segments of begomovirus-like contigs obtained by High-Throughput Sequencing (HTS). The pool of samples PFS comprised 23 symptomatic foliar samples from Fabaceae species and 64 foliar samples from Solanaceae species. The contig highlighted in gray is from a putative cognate DNA-A segment of a new bipartite begomovirus.

Contig	Read coverage	Assembled genome size (nt)	Coverage (BLAST) (%)	Identity (%)	E value	Description	GenBank Accession
213	58,403	2592	100	99.61	0	Tomato severe rugose virus DNA-A	MT733811.1
106	49,435	2617	100	99.58	0	Bean golden mosaic virus DNA-A	MN822294.1
179	20,329	2570	100	99.57	0	Tomato severe rugose virus DNA-A	MT215007.1
18	87,136	2874	100	99.34	0	Tomato apical leaf curl virus	MH539677.1
Kaiju	140	2561	100	99.22	0	Tomato golden vein virus DNA-A	KC706652.1
35310	100	2630	100	99.16	0	Tomato mottle leaf curl virus DNA-A	MT733813.1
Kaiju	24,399	2596	99	98.91	0	Tomato rugose mosaic virus DNA-A	MT215006.1
Kaiju	2937	2636	99	98.74	0	Tomato chlorotic mottle virus DNA-A	MT214086.1
5362	23,282	2676	100	98.24	0	Sida micrantha mosaic virus DNA-A	KC706535.1
6	34,721	2608	100	92.82	0	Bean golden mosaic virus DNA-A	KJ939843.1
4697	336	1339	100	92.47	0	Euphorbia yellow mosaic alphasatellite	KY559642.1
9186	2323	2611	100	91.42	0	Tomato associated geminivirus 1	MF072689.1
26029	35,822	2678	100	90.60	0	Sida micrantha mosaic virus DNA-A	KU852503.1
18119	85	2263	68	81.12	0	Pacific flying fox faeces associated gemycircularvirus-2	NC_038485.1

Table 5. Contigs, read coverage, assembled genome size, BLASTn coverage, sequence identity of assembled viral genomes, E-value, the corresponding viral description, and GenBank accession for DNA-B segments of begomovirus-like contigs obtained by High-Throughput Sequencing (HTS). The pool of samples PFS comprised 23 symptomatic foliar samples from Fabaceae species and 64 foliar samples from Solanaceae species. The contig highlighted in gray is from a putative cognate DNA-B segment of a new bipartite begomovirus.

Contig	Read coverage	Assembled genome size (nt)	Coverage (BLAST) (%)	Identity (%)	E value	Description	Access	Cognate DNA A contig	Distance to DNA-A (%)	Iteron
180	47,728	2570	100	99.57	0	Tomato severe rugose virus DNA-B	EF534708.1	213	93,413	GGTAGT
210	62,494	2592	100	99.42	0	Bean golden mosaic virus DNA-B	MT626897.1	106	98,844	GGTGT
13	47,855	2570	100	99.26	0	Tomato rugose mosaic virus DNA-B	MT215007.1	Kaiju	79,500	GGTAGT
6436	163	2597	100	98.96	0	Tomato chlorotic mottle virus DNA-B	MT214087.1	Kaiju	96,175	GGGGT
47	1348	2531	100	98.03	0	Tomato golden vein virus DNA-B	MT733807.1	Kaiju	91,071	GGGGT
29	21,444	2657	100	94.40	0	Sida micrantha mosaic virus DNA-B	KU852504.1	5362	95,294	GGTGT
339	13,328	2646	93	91.39	0	Sida micrantha mosaic virus DNA-B	EU908734.1	-	-	-
7761	14,299	2532	85	89.68	0	Sida micrantha mosaic virus DNA-B	KC706533.1	-	-	-
381	10,891	2647	100	87.47	0	Sida micrantha mosaic virus DNA-B	EU908734.1	26029	97,872	GGTGT

The DNA-A component of the putative NS#1 (contig 26029) displayed 90.60% identity with SimMV (KU852503.1). The entire DNA-A component of NS#1 displayed 2678 nucleotides with five ORFs *viz*: in the viral sense, AV1 (coat protein – CP), 756 nucleotides; and in the complementary direction AC1 (replication-associated protein – REP), 1077 nucleotides; AC2 (transcription activator – TrAP), 390 nucleotides; AC3 (replication enhancer – REn), 399 nucleotides and AC4 (symptoms determinant), 258 nucleotides (**Figure 1A**). The cognate analysis indicated the putative NS#1 as a bipartite displaying 97.87% identity between the DNA-A component (contig 26029) and the DNA-B component (contig 381) with a common region (CR) of 188 nucleotides. DNA-B displayed two ORFs annotated with sizes of: in the viral sense, BV1 (nuclear shuttle protein - NSP), 771 nucleotides; and in the complementary direction, BC1 (movement protein – MP), 882 nucleotides (**Figure 1B**). The sequence GGTGT was identified as the iteron for contig 26029 and the RepIRD (Replication iteron-related domain) was the amino acid sequence MPPPKRFKIS, which is peculiar to the group of New World begomoviruses as proposed by Arguello-Astorga and Ruiz Medrano (2001).

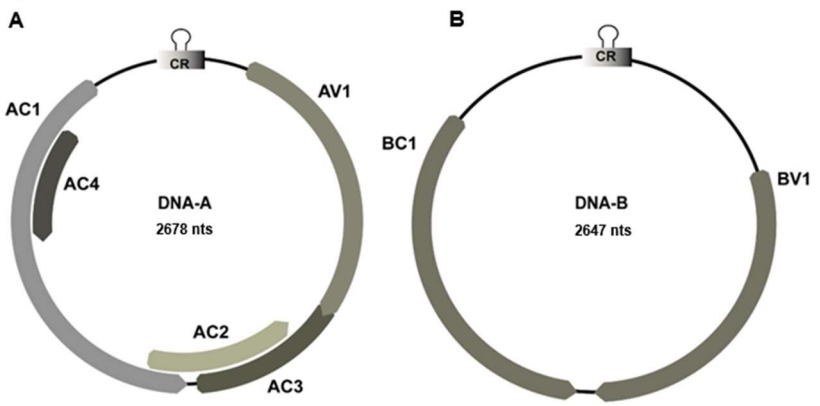

Figure 1A. Genomic organization of DNA-A of the putative new *Begomovirus* species 1 (NS#1; contig 26029). Five ORFs (open reading frames) were annotated in the DNA-A component: AV1 (CP), in the viral sense and AC1 (Rep), AC2 (TrAP), AC3 (Ren), AC4 (symptoms determinant). **1B.** Two ORFs (open reading frames) were annotated in the DNA-B component (contig 381): BV1 (NSP), in the viral sense and BC1 (MP), in the complementary sense.

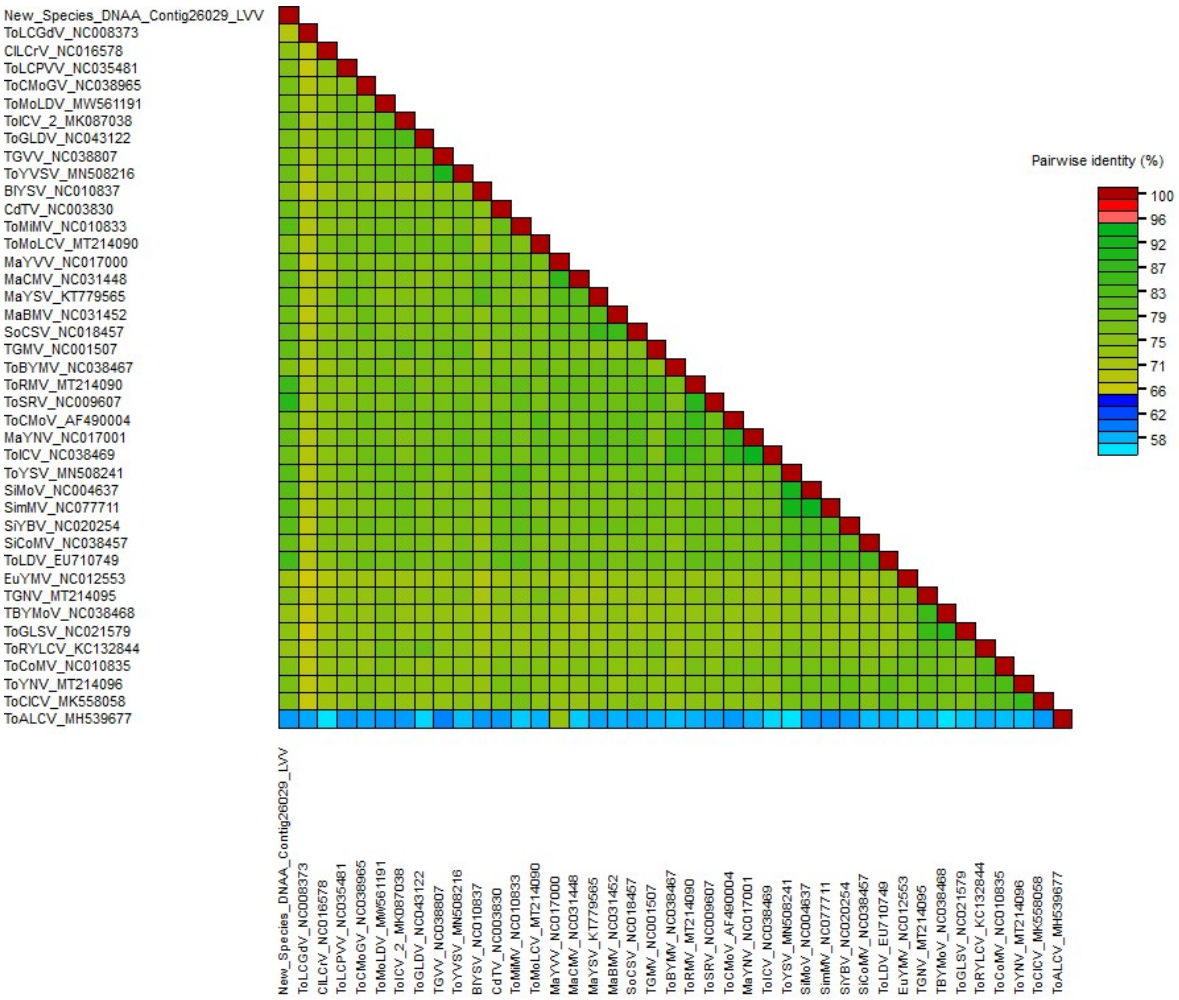

Figure 2. SDT (Sequence Demarcation Tool) of complete DNA-A sequences with identities and distances between the DNA-A component of the putative new *Begomovirus* species 1 (contig 26029), begomovirus isolates that infect tomato and weeds families Fabaceae and Solanaceae in Brazil, and the *Topilevirus* tomato apical leaf curl virus (isolate MH539677). The GenBank accession numbers corresponding to each viral isolate are associated with the respective acronyms. Nucleotide identity among isolates ranged from 56 to 100%. Blainvillea yellow spot virus – BlYSV (NC010837), Chino del tomate virus – CdTV (NC003830), Cleome leaf crumple virus – ClLCrV (NC016578), Euphorbia yellow mosaic virus - EuYMV (NC012553), Macroptilium bright mosaic virus – MaBMV (NC031452), Macroptilium common mosaic virus – MaCMV (NC031448), Macroptilium yellow net virus – MaYNV (NC017001), Macroptilium yellow spot virus – MaYSV (KT779565), Macroptilium yellow vein virus – MaYVV (NC017000), Sida common mosaic virus – SiCoMV (NC038457), Sida micrantha mosaic virus – SimMV (NC077711), Sida mottle virus – SiMoV (NC004637), Sida yellow blotch virus – SiYBV (NC020254), soybean chlorotic spot virus – SoCSV (NC018457), Tomato apical leaf curl virus – ToALCV (MH539677), tomato bright yellow mottle virus – TBYMoV (NC038468), tomato bright yellow mosaic virus – ToBYMV (NC038467), tomato chlorotic leaf curl virus – ToClLCV (MK558058), tomato chlorotic mottle Guyane virus - ToCMoGV (NC038965), tchlorotic mottle virus – ToCMoV (AF490004), tomato common mosaic virus – ToCoMV (NC010835), tomato golden leaf distortion virus – ToGLDV (NC043122), tomato golden leaf spot virus – ToGLSV (NC021579), tomato golden mosaic virus – TGMV (NC001507), tomato golden net virus - TGNV (MT214095), Tomato golden

vein virus – TGVV (NC038807), tomato interveinal chlorosis virus – ToICV (NC038469), tomato interveinal chlorosis virus-2 - ToICV 2 (MK087038), tomato leaf curl Guangdong virus - ToLCGdV (NC008373), tomato leaf curl purple vein virus – ToLCPVV (NC035481), tomato leaf distortion virus – ToLDV (EU710749), tomato mild mosaic virus – ToMiMV (NC010833), tomato mottle leaf curl virus – ToMoLCV (MT214090), tomato mottle leaf distortion virus - ToMoLDV (MN561191), tomato severe rugose virus -ToSRV (NC009607), tomato yellow net virus – ToYNV (MT214096), tomato yellow spot virus – ToYSV (MN508241), tomato yellow vein streak virus – ToYVSV (MN508216).

3.2. Host identification via barcoding of the *matK* and *rbcL* genes and etection via PCR with virus species-specific primers in individual samples of the pool from the Fabaceae and Solanaceae.

In the pool of Fabaceae and Solanaceae species, seven viral genomes related to begomoviruses were recovered through HTS and contig assembly, using six previously designed primers (specifications in **Table 2**). The following begomoviruses were detected: EuYMV, SimMV, ToSRV and ToMoLCV, in addition to two viruses belonging to the *Topilevirus* genus, tomato apical leaf curl virus – ToALCV and tomato associated geminivirus 1 – TAG1.

Thus far, the following species have been reported as hosts of **ToSRV**: *Capsicum frutescens* (Bezerra-Agasie et al. 2006), *C. baccatum* (Bezerra-Agasie et al. 2006), *S. lycopersicum* (Bezerra-Agasie et al. 2006), *N. physaloides* (Barbosa et al. 2009), *Pachyrhizus erosus* (Pereira-Silva et al. 2022), *S. betaceum* (Pereira-Silva et al. 2022), *S. torvum* (Pereira-Silva et al. 2022), and *Oxalis latifolia* (Pereira-Silva et al. 2022). Herein, the use of species-specific primers allowed the detection of two new **ToSRV** hosts (both collected in the Federal District): a sample (DF–509) corresponding to the Solanaceae species *S. aethiopicum* var *gilo* and another sample (DF–368) corresponding to *Desmodium incanum*, a Fabaceae species. (**Figure 3**). The species-specific primer for ToSRV also allowed the detection of the virus in another ten samples of *Nicandra physalodes* distributed across the Midwest and Southeast regions of Brazil (DF–345, DF–486, DF-587, GO–203, GO–253, GO–312, GO–521, MG–336, MG–337, and MG–355) as well as in two *Capsicum frutescens* samples from the Federal District (DF–445 and DF–558) and one in the state of Ceará (CE–051) (**Figure 3**). The host and geographical expansion of ToSRV observed herein corroborates work carried out in Brazil, especially in the Midwest region, which points to the high adaptation capacity of this begomovirus (Pereira-Silva et al. 2022). The frequent presence of weeds in the vicinity of cultivated areas, linked to the wide geographic distribution of the *B. tabaci* MEAM1 vector, favors the sharing of begomoviruses among Fabaceae and Solanaceae species and might be one of the factors that accentuate the epidemiological importance of ToSRV especially for the Midwest region. In this highland area, grain and vegetable crops are planted in large scale, including processing tomatoes and soybeans (*Glycine max*). In no-choice transmission tests with *B. tabaci* MEAM1 under greenhouse conditions, the infection rates with ToSRV in *N. physalodes*, soybean and tomato were respectively 64.2%, 50% and 71.4%, and the transmission efficiency 20%, 43% and 33%. Soybean plants, even with a lower vector

preference, demonstrated significant potential as an asymptomatic source of inoculum, and could play a relevant role as an amplifying host in the epidemiology of the disease caused by ToSRV either in simultaneous or the subsequent tomato fields (Favara et al. 2023).

Using the **EuYMV** species-specific primer, four new hosts were detected, one in a *C. frutescens* sample from the state of Amazonas (AM–046) and three other samples collected in the Federal District corresponding *Physalis angulata* (DF–020), eggplant (*S. melongena*; DF–079) and *N. physalodes* (DF–776) (**Figure 3**). The presence of EuYMV in the state of Amazonas had already been reported in molecular detection work in samples of *C. chinense* in which pairwise sequence comparisons and phylogenetic analyzes based on the complete sequence of the DNA-A component indicated a low degree of genetic variability for this begomovirus (Catarino et al. 2020). From samples collected in the state of Goiás and the Distrito Federal, Fernandes et al (2011) reported four of the currently known hosts of EuYMV, namely: *C. annum*, *Datura stramonium*, *Nicotiana benthamiana* and *E. heterophylla*. Kitajima (2020) also listed *G. max*, *Macroptilium atropurpureum* and *Phaseolus vulgaris* as host of EuYMV. The first report of natural infection of tomato by EuYMV was done in open-field crops in four Brazilian states (Duarte et al. 2021a). This emergence of tomato-adapted EuYMV isolates could be a potencial threat since the original host of this begomovirus (*E. heterophylla*) is a common weed in open fields due to the high level of tolerance to the main herbicide (metribuzin) used in this vegetable crop (Duarte et al. 2021a).

Three new hosts were detected for **SimMV** using species-specific primers. These detections are associated with three samples of Solanaceae species collected in the Distrito Federal, DF–033 (in potato, *S, tuberosum*), DF–401 (in *N. physalodes*), DF–776 (also in *N. physalodes*) and *Physalis angulata* collected in the municipality of Guaraí, state of Tocantins (TO–065) (**Figure 3**). Detection of **SimMV** was also carried out in a Fabaceae sample (DF–763) collected in the Distrito Federal in *P. vulgaris*, a host already known in the characterization of samples collected in the state of Goiás (Fernandes-Acioli et al. 2011). Among the SimMV hosts known so far are: *N. benthamiana* (Jovel et al. 2004), an uncharacterized *Sida* species (Jovel et al. 2004), *Sidastrum micranthum* (Jovel et al. 2004), *Abelmoschus esculentus* (Aranha et al. 2011), *C. chinense* (Kitajima et al. 2020), *G. max* (Fernandes et al. 2009), an uncharacterized *Malva* species (Jeske et al. 2010), *Nicotiana tabacum* (Kitajima et al. 2020), *Oxalis latifolia* (Kitajima et al. 2020) and tomatoes (Aranha et al. 2011). In recent work, plants of commercial cotton cultivars (*Gossypium hirsutum*) and germplasm accessions of *G. hirsutum*, *G. barbadense* and *G. mustelium* displaying mosaic symptoms were recognized as hosts of SimMV (Hoffmann et al. 2022). After invasion and dissemination of the *B. tabaci*

MEAM 1 in Brazil, SimMV showed a rapid increase in host numbers, especially among plants of the Malvaceae and Solanaceae families. This observation emphasizes the need for continuous and extensive studies to assess the current epidemiological importance of SimMV for crops such as beans and tomatoes (Fernandes-Acioli et al. 2011).

Species-specific primers for the monopartite begomovirus **ToMoLCV** led to the detection of eight new hosts across different regions of Brazil (**Figure 3**). The presence of ToMoLCV was detected in a sample of *Senna obtusifolia* (BA–104), from Jequié in Bahia state; in samples of *C. baccatum* var. *praetermissum* (DF–267), *S. rostratum* (DF–300), *C. frutescens* (DF–558) and *N. physalodes* (DF–587) collected in the Federal District; in a sample of *N. physalodes* (GO–041), and the Fabaceae *Vigna unguiculata* (GO–051), collected in Goianápolis and in Goiânia (both in Goiás State), respectively. Samples infected with **ToMoLCV** were obtained in *S. americanum* (SP–130) and scarlet eggplant (*S. aethiopicum* var. gilo; SP–150) in Tupã and Bragança Paulista in São Paulo state. Thus far, ToMoLCV records were restricted to the tomato (Ferro et al. 2017) and to the eggplant (*S. melongena*) crops (Kitajima et al. 2020). The polyphagous habit of *B. tabaci* MEAM1, combined with the specificity of the relationship with the begomovirus and the frequent association of weeds with cultivated areas, prove to be decisive for the geographic distribution of **ToMoLCV**. Phylogenetic analyzes that indicate a local diversification of ToMoLCV over hundreds of years and its recent dissemination throughout the country, confirm the importance of the factors mentioned above for expanding the host range (Souza et al. 2022).

Two members of the genus *Topilevirus* were also detected by species-specific primers: tomato apical leaf curl virus – **ToALCV** in a sample of *N. physalodes*, collected in Goianápolis, in the state of Goiás (GO–041) and tomato associated geminivirus 1 (**TAG1**) in a *C. chinense* sample collected in the Distrito Federal (DF–445). The first characterizations of both tomato associated geminivirus 1 in Brazil (Fontenele et al. 2017) and tomato apical leaf curl virus in Argentina (Vaghi-Medina et al. 2018) were carried out only in tomatoes. In the Midwest region of Brazil, ToALCV was detected in tomatoes in the Federal District (Batista et al. 2019). Therefore, both *N. physalodes* and *C. chinense* are novel hosts of *Topilevirus* species. The role of the planthopper *Micrutalis maleifera* (Membracidae) as a vector was indicated by the analysis of amino acids in the coat protein, however there is still a need for transmission assays to confirm this hypothesis (Vaghi-Medina et al. 2018).

The begomovirus with the highest number of samples detected was ToSRV (**Figure 4**), concentrated mainly in the Midwest region (**Figure 5**). This result corroborates a series of studies that pointed out the high adaptability of the virus and its predominance in vegetable

crops and weeds in this region (Reis et al. 2020; Pereira-Silva et al. 2022; Oliveira et al. 2024). In second place with the highest number of detections is ToMoLCV (**Figure 4**) with seven detections in the Midwest region (**Figure 5**) revealing a wider geographic distribution than expected for this begomovirus, which is strongly associated with the Northeast region, the climate semi-arid and high temperatures found in this region (Souza et al. 2022; Ferro et al. 2017; Mituti et al. 2019; Oliveira et al. 2024). The significant number of begomovirus detections in the Midwest region can be related to the relevance of this region for the extensive production of soybeans and beans (members of the Fabaceae family) and fresh-market and processing tomatoes (Solanaceae family) as well as the weed hosts associated with these crops. (Bergamin Filho et al. 2020).

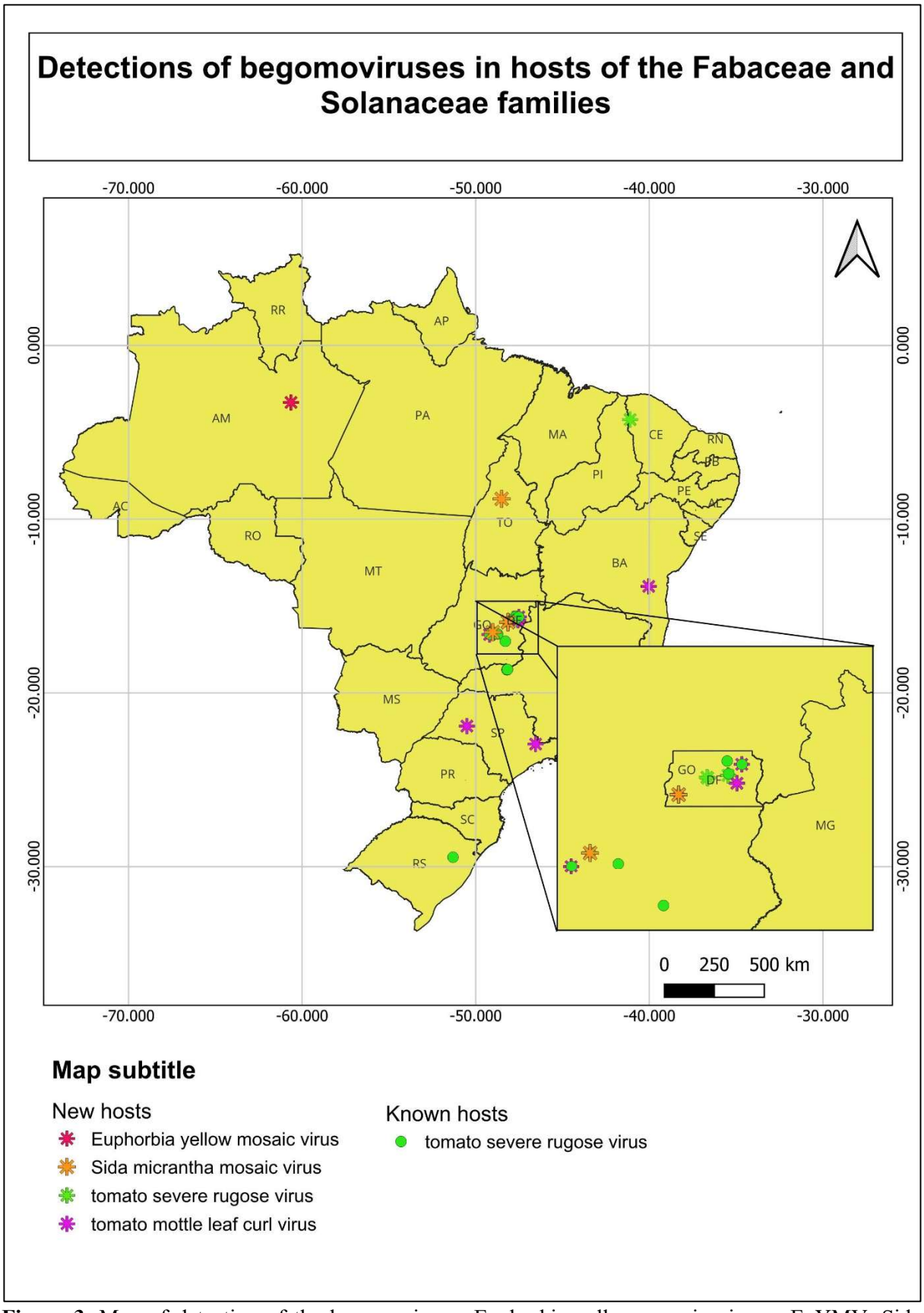

Figure 3. Map of detection of the begomoviruses Euphorbia yellow mosaic virus – EuYMV, Sida micrantha mosaic virus – SimMV, tomato mottle leaf curl virus – ToMoLCV and tomato severe rugose virus – ToSRV in a pool of 87 foliar samples from Fabaceae and Solanaceae species collected across the five Brazilian macroregions (North, Northeast, South, Southeast, and Midwest)

Table 6. Positive samples for viruses of the *Geminiviridae* family and their respective host species, detected by PCR using species-specific primers in a set of 87 leaf samples of Fabaceae and Solanaceae species collected in the five Brazilian macroregions (North, Northeast, South, Southeast and Central-West). Among the total samples tested with the species-specific primers described above, 32 presented positive results and another 55 presented no results for the primers used.

Virus acronyms	**Positive samples by regions**									
	North		**North East**		**South**		**Southeast**		**Midwest**	
	Sample	**Host**	**Sample**	**Host**	**Sample**	**Host**	**Sample**	**Host**	**Sample**	**Host**
EuYMV	AM-046	*Capsicum frutenscens*							DF–020	*Physalis angulata*
									DF–079	*Solanum melongena*
SimMV	TO–065	*Physalis angulata*							DF–776	*Nicandra physalodes*
									DF-033	*Solanum tuberosum*
									DF–401	*Nicandra physalodes*
									DF–763	*Phaseolus vulgaris*
									DF–776	*Nicandra physalodes*
ToMoLCV			BA–104	*Senna obtusifolia*			SP-130	*Solanum americanum*	GO–041	*Nicandra physalodes*
							SP-150	*Solanum aethiopicum*	DF–033	*Solanum tuberosum*
									DF–267	*Capsicum baccatum*
									DF–300	*Solanum rostratum*
									DF–558	*Capsicum frutescens*
									DF–587	*Nicandra physalodes*
									GO–041	*Nicandra physalodes*
									GO–051	*Vigna unguiculata*
ToSRV			CE–051	*Capsicum frutenscens*	RS–085	*Solanum lycopersicum*	MG-336	*Nicandra physalodes*	DF–345	*Nicandra physalodes*
							MG-337	*Nicandra physalodes*	DF–368	*Desmodium incanum*
							MG-355	*Nicandra physalodes*	DF–445	*Capsicum frutenscens*
									DF–486	*Nicandra physalodes*
									DF–509	*Solanum aethiopicum*
									DF–558	*Capsicum frutenscens*
									DF–587	*Nicandra physalodes*
									GO–203	*Nicandra physalodes*

					GO–253	*Nicandra physalodes*
					GO–312	*Nicandra physalodes*
					GO–521	*Nicandra physalodes*
ToALCV					GO–041	*Nicandra physalodes*
TAG-1					DF–445	*Capsicum frutenscens*

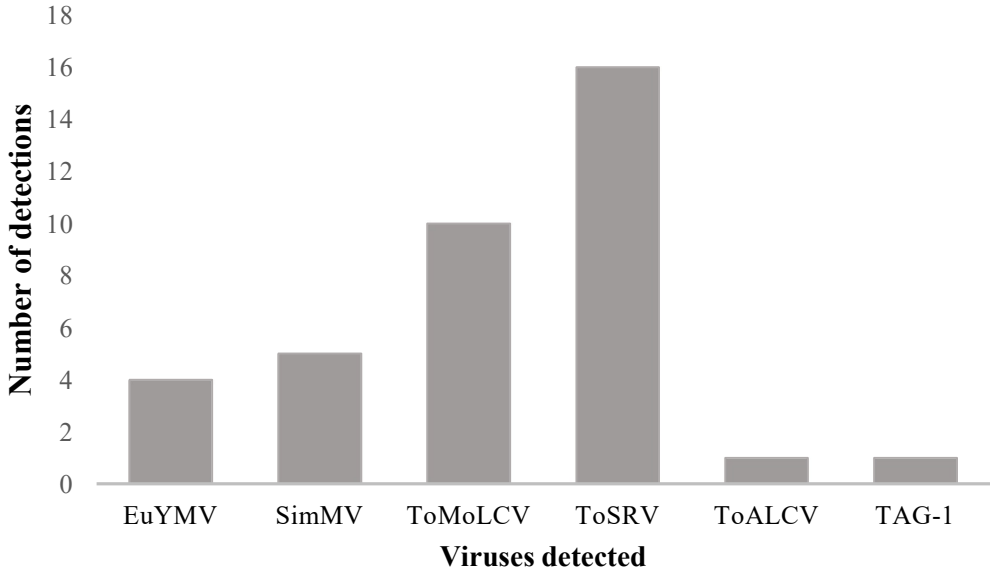

Figure 4. Number of overall detections of viruses from the genus *Begomovirus* in a pool of 87 foliar samples from Fabaceae and Solanaceae species collected across the five Brazilian macroregions. Euphorbia yellow mosaic virus – EuYMV, Sida micrantha mosaic virus – SimMV, tomato mottle leaf curl virus – ToMoLCV and tomato severe rugose virus – ToSRV) and the genus *Topilevirus* (tomato apical leaf curl virus – ToALCV and tomato associated geminivirus 1 – TAG1).

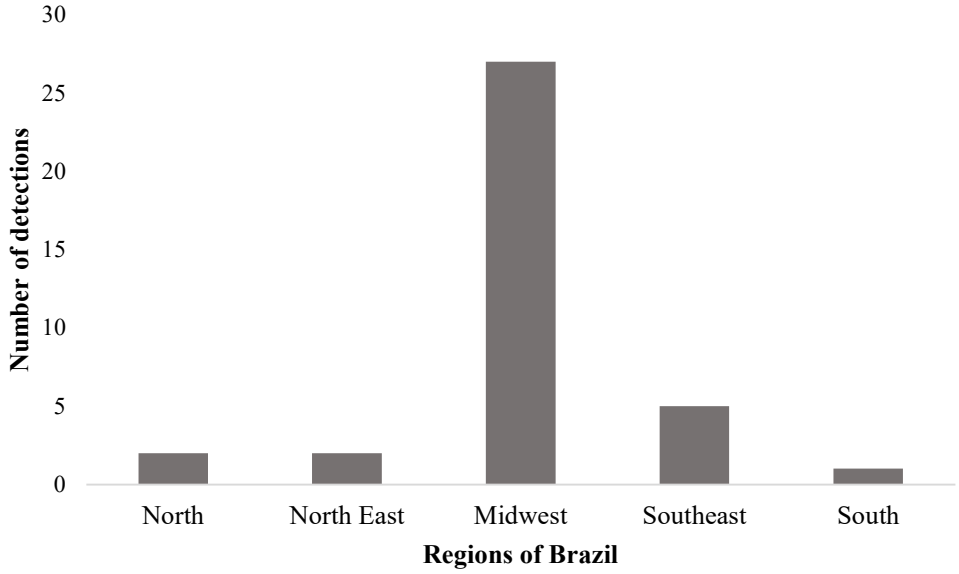

Figure 5. The overall number of begomovirus detections in a pool of 87 foliar samples from Fabaceae and Solanaceae species collected across the five Brazilian macroregions (North, North East, South, Southeast, and Midwest). Euphorbia yellow mosaic virus – EuYMV, Sida micrantha mosaic virus – SimMV, tomato mottle leaf curl virus – ToMoLCV and tomato severe rugose virus – ToSRV) in samples from the pool of the Fabaceae and Solanaceae families distributed across regions of Brazil.

4. Conclusion

Here we demonstrate again the applicability of HTS platforms and their potential to elucidate virome dynamics when combined with molecular detections by PCR with species-specific primers that are among the main applications in large-scale epidemiological studies. The tools used here proved to be effective in clarifying the current scenario of viral diversity present in tomato-associated weeds and the role of these hosts as viral reservoirs, especially in the Fabaceae and Solanaceae families found in areas adjacent or within to tomato fields in Brazil. The geographic distribution of important begomoviruses, such as ToSRV and ToMoLCV, which were also found to be predominant in weeds, can provide guidelines for regional action of breeding programs of tomato as well as major field crops such as beans, soybeans and cotton.

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CHAPTER 3

Natural infection of the tomato-associated weeds *Macroptilium lathyroides*, *Sida acuta*, *Sida ulmifolia*, and *Sida tenuicarpa* by Euphorbia yellow mosaic virus isolates in Brazil.

Natural infection of the tomato-associated weeds *Macroptilium lathyroides*, *Sida acuta*, *Sida ulmifolia*, and *Sida tenuicarpa* by Euphorbia yellow mosaic virus isolates in Brazil.

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Abstract

Euphorbia yellow mosaic virus (EuYMV) is a bipartite begomovirus initially reported in *Euphorbia heterophylla* (family Euphorbiacae) in Central Brazil. In recent years, the viral host range has expanded to members of the Lamiaceae, Malvaceae, Fabaceae, and Solanaceae families, including tomatoes (*Solanum lycopersicum* L.). The emergence of EuYMV isolates adapted to tomato is a major concern, since *E. heterophylla* is a common weed in tropical regions and has a high level of tolerance to the main herbicides used in this vegetable crop. In this context, we carried out High-Throughput Sequencing (HTS) analysis of begomoviruses associated with weeds infesting tomato fields allowed the detection of *Sida tenuicarpa* and *Macroptilium lathyroides* (both in Bahia state; Northeast Brazil) and *S. ulmifolia* and *S. acuta* (both from Tocantins state; North Brazil) as novel hosts of EuYMV. Our results highlight a new the role of novel weed hosts as epidemiological reservoirs of EuYMV isolates, representing a permanent source of field inoculum for plantings employing susceptible tomato cultivars.

The genus *Begomovirus* (*Geminiviridae* family) encompasses monopartite (only DNA-A as genomic component) and bipartite (two genomic components, DNA-A and DNA-B) species (Fiallo-Olivé and Navas-Castillo 2023). Both DNA components have a common region (RC) that guides replication and assists in bidirectional transcription. Six to nine ORFs (open reading frames) are present in the genome of bipartite begomoviruses: Two ORFs are in the viral direction (AV1 and AV2) and four to seven in the complementary strand (AC1, AC2, AC3, AC4, AC5, AC6, and AC7) (Li et al. 2015; Roshan et al. 2018; Ruhel and Chakraborty 2019; Fiallo-Olivé et al. 2021; Hanley-Bowdoin et al. 2000; Wang et al. 2022; Liu et al. 2023). In monopartite begomoviruses, the genomic DNA is homologous to the bipartite DNA-A (Ward and Lazarowitz 1999). In bipartite begomoviruses, two additional non-overlapping ORFs are found in the DNA-B component: a nuclear transport protein in the viral direction and a movement protein in the complementary direction (Fiallo-Olivé and Navas-Castillo 2023). The entire DNA-A sequence is employed for taxonomic demarcation of the genus *Begomovirus*, identity equals to or greater than 91% identity is currently used for classification in the same species (Brown et al. 2015). Differences in the iterons and subdomains of the REP protein have been employed to classify viral species (Arguello-Astorga et al. 2001; Cantú-Iris et al. 2019).

Begomoviruses are efficiently transmitted by members of the *Bemisia tabaci* cryptic species complex (Liu et al. 2012). Field surveys in Brazil indicated efficient invasion of the vector *B. tabaci Middle East-Asia Minor* 1 (MEAM 1 = biotype B), which is associated with more than 25 tomato-infecting begomoviruses (Reis et al. 2020; Oliveira et al. 2024). The polyphagous nature of *B. tabaci* complex vectors allowed the transmission of begomoviruses to new hosts, including weeds and native flora species. Weeds can host more than one begomovirus in mixed infections, which provides a favorable environment for viral recombination and evolution (Roshan et al. 2018; Syller 2012).

Euphorbia heterophylla L. is native to tropical and subtropical Americas, considered a weed of great economic importance (Bridges et al. 1992; Willard and Griffin 1993). Euphorbia mosaic virus was described in the 1950s infecting *E. heterophylla* (= *E. prunifolia*), being the first report of a begomovirus imfecting weed in Brazil (Costa and Bennett, 1950). Euphorbia yellow mosaic virus (EuYMV) was the first fully characterized bipartite begomovirus in *E. heterophylla* from leaf samples collected in the state of Goiás and the Distrito Federal, Central Brazil (Fernandes et al. 2011). The DNA-A component of EuYMV shared 87.3% nucleotide sequence identity with Euphorbia Peru mosaic virus (EuMPV), which indicated the emergence of a novel begomovirus according to the demarcation criteria for new *Begomovirus* species (Brown et al. 2015). The DNA-B sequences of EuYMV displayed 56.8% identity with the euphorbia mosaic virus (EuMV) isolate from Mexico. Phylogenetic analyzes demonstrated that the virus was related to a different lineage called Euphorbia yellow mosaic virus (EuYMV) in Central America.

Many reports are available of EuYMV isolates able to naturally infect *Crotalaria juncea* (Barreto et al. 2013), *Leonurus sibiricus* (Ferro et al. 2017), *Macroptilium atropurpureum* (Silva et al. 2012), *Sida santaremensis* (Tavares et al. 2012), *Glycine max* (Kitajima 2020) and *Phaseolus vulgaris* (Kitajima 2020). In a study of begomovirus diversity in non-cultivated plants in the Mexican North Pacific area, EuYMV was also detected using High-Throughput Sequencing – HTS (Rodríguez-Negrete et al. 2019).

Experimental inoculation assays indicated that EuYMV is capable of experimentally infecting the Solanaceae species *Datura stramonium*, *Nicotiana benthamiana*, *Capsicum annuum* and tomatoes (*Solanum lycopersicum*) (Fernandes et al. 2011; Barreto et al. 2013). *Capsicum chinense* was also reported as a natural host of EuYMV in the Amazon region (Catarino et al. 2020). More recently, the natural host

range of EuYMV was also expanded to tomatoes (Duarte et al. 2021). The emergence of tomato-adapted EuYMV isolates is a major concern since *E. heterophylla* is a common weed across tropical regions due to the high level of tolerance to the major herbicides used in this vegetable crop. In this context, we carried out High-Throughput Sequencing (HTS) analysis of begomoviruses associated with Malvaceae and Fabaceae weeds infesting tomato fields across all macroregions of Brazil searching for potential new natural hosts of EuYMV.

A pool was established with 78 foliar samples of weeds from the Malvaceae family with begomovirus-like symptoms (apical and interveinal chlorosis, yellow spots, golden mosaic, mosaic, mottle, severe rough mosaic, leaf deformation and dwarfism) occurring in areas within or adjacent to tomato fields. These samples were collected during the years 2001–2020 across eight Brazilian states and the Distrito Federal (**Table 1**). Total genomic DNA was extracted with a modified 2X CTAB buffer and organic solvents (Boiteux et al. 1999). Purified total DNA was used as template in Rolling Circle Amplification (RCA) assays (Inoue-Nagata et al. 2004). The set of samples was subjected to HTS on an Illumina NovaSeq 6000 platform. The sequences obtained from this sequencing were analyzed according to the following workflow: **(1)** read elimination low quality; **(2)** reassembly of the sequences using the CLC Genomics Workbench 23.0.1 program; and **(3)** validation of contigs via BLASTn algorithms against the GenBank ssDNA virus database (http://www.ncbi.nlm.nih.gov/). The viral contigs were annotated and the reads obtained were mapped back to the annotated genome using the 'Map to reference' tool available in Geneious R11.1 software (Kearse et al. 2012). After assembly, the contigs were analyzed by comparison with the RefSeq viral database (National Center for Biotechnology Information - NCBI) under very high stringency conditions (minimum percentage match = 98%).

Once the viral genomes were properly assembled, annotated, and analyzed, it was possible to detect the presence of EuYMV-like contigs. In order to detect the EuYMV isolates in individual samples, species-specific primers for EuYMV DNA-A were employed (Reis et al. 2020). The degenerate universal primers (Rojas et al. 1993) were also employed to confirm the presence of both genomic segments of EuYMV in each Malvaceae sample. Both PCR assays for detection were performed with a total volume of 12.5 µL containing 1.25 µL of *Taq* polymerase buffer (100 mM Tris-HCL, pH 8.3 and 500 mM KCl), 0.40 µL of $MgCl_2$ (50 mM), 0.25 µL of dNTPs (10 mM), 0.25 µL of each primer (forward and reverse), 2 µL of RCA, 8.0 µL of Milli-Q water (Millipore, Bedford,

MA, USA) and 0.1 µL of *Taq* DNA polymerase (5 U/µL). The reactions for detection of EuYMV DNA-A were amplified in a thermocycler (Bio-Rad Laboratories, Hercules, CA) programmed for 35 cycles with the following conditions: 94ºC for 3 minutes, initial denaturation; 94º C for 30 seconds, denaturation; 52°C for 45 seconds, annealing; 72º C for 3 minutes, extension; and the final extension, 72ºC for 10 minutes. To detect EuYMV DNA-B in samples, the protocol adopted for the pair of primers PBL1v2040 and PCRc1 (Rojas et al. 1993) consisted of temperatures of 94ºC for 3 minutes, initial denaturation; 94ºC for 30 seconds, denaturation; 55ºC for 45 seconds, annealing; 72ºC for 40 seconds, extension and 72ºC for 7 minutes, final extension. Amplicons with sizes of approximately 690 pb, expected for the detection of DNA-B, and 2610 pb expected for DNA-A were observed by electrophoresis in a 1% agarose gel stained with ethidium bromide and visualized under UV light. Accurate identification of EuYMV host species was performed by PCR assays followed by dideoxy Sanger amplicon sequencing of the maturase (*matK*) genes (Wicke and Quandt 2009; Kar et al. 2015) (**Table 2**). For sequence comparison and confirmation of begomovirus species, MUSCLE alignment was performed using the Sequence Demarcation Tool – SDT 1.2 (Muhine et al. 2014) according to Brown et al. (2015) employing 43 DNA-A sequences representative of geographic regions and the EuYMV host range (Table 3). For DNA-B comparison, 26 sequences related to EuYMV were obtained from GenBank (April 2024), and also subjected to MUSCLE alignment through SDT 1.2. Assessments of iterons and conserved motifs in the common region (CR) between EuYMV DNA-A and DNA-B isolates were performed according to Arguello-Astorga and Ruiz-Medrano (2001). Additional analyzes on all available isolates for divergence of the structural motif of helix 4 (Arguello-Astorga et al. 2004; Cantú-Íris et al. 2019).

From the contig assembly, a complete EuYMV DNA-A sequence was obtained from the analyzed pool. The DNA-A sequence corresponding to contig 67 showed 96.09% identity with the EuYMV isolate KY559430. Contig 67 (PP911363) had 37,325 read coverage and a size of 2610 nucleotides. When the open reading frames (ORFs) were annotated, the following sizes were observed: in the viral sense, AV1 (coat protein – CP), 750 nucleotides, and in the complementary sense, AC1 (replication-associated protein – REP), 1080 nucleotides; AC2 (transcription activator – TrAP), 390 nucleotides; AC3 (replication enhancer – REn), 399 nucleotides; AC4 (symptom determinants), 363 nucleotides (**Fig 1A**) and AC5, 318 nucleotides. The cognate analysis performed according to that proposed by Arguello-Astorga and Ruiz Medrano (2001), indicated in

the common region an identity distance of 99.43% between DNA-A (contig 67) and DNA-B (contig 54) (PP911364). The ORFs annotated for contig 54 presented the following sizes: BC1 (movement protein - MP), 885 nts and BV1 (nuclear transport protein - NSP), 771 nts (**Fig 1B**). The analyses indicated as iteron the sequence TGGTGTCC and the RepIRD domain MPRNPNSFRLS, differing in one amino acid from the reference MPRNPNSFRLT presented in Arguello-Astorga and Ruiz Medrano (2001).

Analysis from MUSCLE alignments performed by SDT allowed comparison with the complete DNA-A (PP911363) (Fig 2) and DNA-B (PP911364) (Fig 3) sequences of EuYMV available in GenBank, which confirmed the identity of the virus as EuYMV. Levels of identity among the 43 EuYMV DNA-A (PP911363) sequences ranged from 95 to 99%. Sequence comparisons indicated greater identity of the EuYMV DNA-A contig 67 LVV isolate with isolates described in the state of Amazonas, the EuYMV isolate (MK990328) characterized in the state of Amazonas from a sample of *Capsicum chinense* (Catarino et al. 2020), and another EuYMV isolate (KY559430), reported in the city of Manaus, Amazonas, from *Euphorbia heterophylla* (Mar et al. 2017). PCR assays using specific primers identified four new hosts of EuYMV that were detected in the sample pool. In the state of Bahia, the DNA-A (PP911363) and DNA-B (PP911364) components of EuYMV were detected in samples BA–098 (collected in the city of Irecê, in 2007) and BA–107 (collected in the city of Milagres, in 2011) corresponding to *Sida tenuicarpa* and *Macroptilium lathyroides*, respectively, representing the first reports of EuYMV in this state in two new hosts. Two other detections of both genomic components of EuYMV were made in samples from the state of Tocantins, samples TO–250 (collected in the city of Natividade, in 2009) and TO–304 (collected in the city of Miranorte, in 2009) corresponding to *Sida ulmifolia* and *Sida acuta*, respectively, two new hosts of EuYMV in a location previously free of reports of the virus. The botanical identification of the hosts was confirmed through morphological analyses and use of sequence information from the maturase K gene, which indicated the following hosts: *Sida tenuicarpa* (100% coverage and 97.87% identity of the matK gene), *Macroptilium lathyroides* (100% coverage and 99.37% identity of the matK gene), *Sida ulmifolia* (100% coverage and 99.85% identity of the matK gene) and *Sida acuta* (100% coverage and 99.86% identity of the matK gene).

Until this study, the following were known hosts of EuYMV: *E. heterophylla*, *C. annuum*, *Datura stramonium* and *Nicotiana benthamiana*, in the Midwest region

(Fernandes et al. 2011), and *Glycine max*, *Phaseolus vulgaris* and *Macroptilium atropurpureum* as infected plants in the field (Kitajima et al. 2020). Tomato samples collected in four Brazilian states exhibited EuYMV infection (Duarte et al. 2021), as well as *Capsicum chinense* samples collected on properties in Amazonas state (cities Presidente Figueiredo and Iranduba) (Catarino et al. 2020). *Macroptilium lathyroides* is a species of the Fabaceae family native to Guyane, Brazil and Paraguay, and as a ruderal plant it has a certain frequency throughout the Brazilian territory, mainly infesting orchards, roadsides, lawns and vacant lots (Silva et al. 2020). The genus *Sida* spp. is associated with around 70 viruses, however *Sida tenuicarpa* and *Sida ulmifolia* are not yet reported as hosts of begomovirus. The species *Sida acuta* is related to several satellites and five begomoviruses, Sida yellow mosaic Yucatan virus (Hernández-Zepeda et al. 2007), Sida yellow mosaic virus (Xiong and Zhou 2006), Sida angular mosaic virus (Silva et al. 2012), Sida golden yellow mosaic virus (Godinho et al. 2014) and Sida yellow spot virus (Godinho et al. 2014), the last three being characterized in Brazil.

In conclusion, HTS analysis of begomoviruses associated with weeds infesting tomato fields allowed the detection of Sida tenuicarpa and Macroptilium lathyroides (both in the state of Bahia; Northeastern Brazil) and S. ulmifolia and S. acuta (both in the state of Tocantins; Northern Brazil) as new hosts of EuYMV and the role of these weeds as epidemiological reservoirs of EuYMV isolates, representing a permanent source of inoculum in tomato plantations.

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Data availability

The data supporting the presented results are in the possession of the author and available upon reasonable request.

Declarations

Ethical approval The authors confirm that there are no ethical issues in publishing the article and there are no human or animal participants involved in this study.

Conflict of interest The authors declare that they have no conflict of interests.

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Table 1. Information about the samples of plants from the Malvaceae family that make up the pool sequenced in this work, organized according to the region of origin of collection (city and year) and isolate code according to the Begomovirus Collection of CNPH (Brasília-DF, Brazil).

Region	City and year	Isolate code
North	Iranduba (2016); Iranduba (2016); Manacapuru (2016); Matinha (2005); Colmeia (2005); Palmas (2008); Palmas (2008); Arraias (2008); Arraias (2008); Formoso (2008); Araguaina (2008); Dueré (2008); Natividade (2009); Natividade (2009); Natividade (2009); Chapada de Natividade (2009); Lajeado (2009); Lajeado (2009); Palmas (2009); Miranorte (2009); IFTO (2010) and Novo Acordo (2013)	AM–028; AM–030; AM–044; TO–016; TO–025; TO–100; TO–105; TO–124; TO–134; TO–175; TO–229; TO–242; TO–250; TO–259; TO–270; TO–273; TO–275; TO–285; TO–304; TO–307, and TO–324
North East	Praia do Espelho (2007); Irecê (2007); Jequié (2011); Maracaz (2011); Jaguaquara (2011); Jequié (2011); Milagres (2011); Jequié (2011); Utinga (2011); Juazeiro (2012); Juazeiro (2012); João Dourado (2016); Jaguaquara (2019); Jaguaquara (2019); Jaguaquara (2019); Jaguaquara (2019); Ibiapina (2012); Ubajara (2012); Ubajara (2017); Chã Grande (2007); Comancin de São Félix (2007) and Santa Maria Boa Vista (2016)	BA–004; BA–018; BA–082; BA–088; BA–098; BA–103; BA–107; BA–109; BA–138; BA–169; BA–170; BA–180; BA–189; BA–190; BA–192; BA–193; CE–060; CE–061; CE–075; PE–006; PE–009; and PE–146
Midwest	Lago Azul (2003); Ponte Alta (2003); CNPH (2009); CNPH (2010); Taquara (2010); Planaltina (2011); Brazlândia (2019); Brazlândia (2019); Abadiânia (2003); Goianápolis (2003); São João da Aliança (2007); Campos Belos (2008); Cavalcante (2009); Cavalcante (2009); Bonfinópolis (2010); Bonfinópolis (2010); Goianápolis (2010); Pontalina (2013); Padre Bernardo (2019) and Caldas Novas (2020)	DF–036; DF–069; DF–275; DF–332; DF–358; DF–394; DF–707; DF–708; GO–235; GO–243; GO–366; GO–413; GO–431; GO–440; GO–460; GO–462; GO–472; GO–548; GO–623, and GO–628
Southeast	Venda Nova (2001); Alto Caxixe (2011); São Roque (2012); Rio Possmouse (2012); Iraí (2002); Monte Carmelo (2008); Paty do Alferes (2006); Paty do Alferes (2006); Paty do Alferes (2006); Paty do Alferes (2006); São José do Ubá (2006); Vassouras (2016) and Vassouras (2016)	ES–002; ES–047; ES–072; ES–077; MG–032; MG–059; RJ–010; RJ–011; RJ–012; RJ–013; RJ–014; RJ–055 and RJ–056
South	Tijucas (2010)	SC–035

Table 2. Information about primers used for EuYMV detection and primers for amplification of the *matK* and *rbcL* genes used for botanical identification of begomovirus hosts.

Target region of the primers	Primer name	Primer sequence 5'-3'	Reference
Euphorbia yellow mosaic virus EuYMV DNA-A	EuYMV-A-R-For	GGGGTTCCAAGTCCAATAAAGATGA	Reis et al. 2020
	EuYMV-A-R-Rev	CAGACACCTTATATTTGCCGGATTC	
DNA-A	PAL1v1978_F	GCATCTGCAGGCCCAACTYGTCTTTYCCNGT	Rojas et al. 1993
	PAR1c2040_R	AATACTGCAGGGCTTYCTRTACATRGG	
DNA-B	PBL1v2040_F	GCCTCTGCAGCARTGRTCKATCTTCATACA	Rojas et al. 1993
	PCRc1_R	CTAGCTGCAGCATATTTACRARWATGCCA	
Barcode primers for *mat*K and *rbc*L genes	*mat*K_F	GCATAAATATAYTCCYGAAARATAAGTGG	Wicke and Quandt 2009 Kar et al. 2015
	*mat*k_R	TGGGTTGCTAACTCAATGG	
	*rbc*L_F	ATGTCACCACAAACAGAGACTAAAGC	Levin et al. 2003
	*rbc*L_R	GTAAAATCAAGTCCACCRCG	

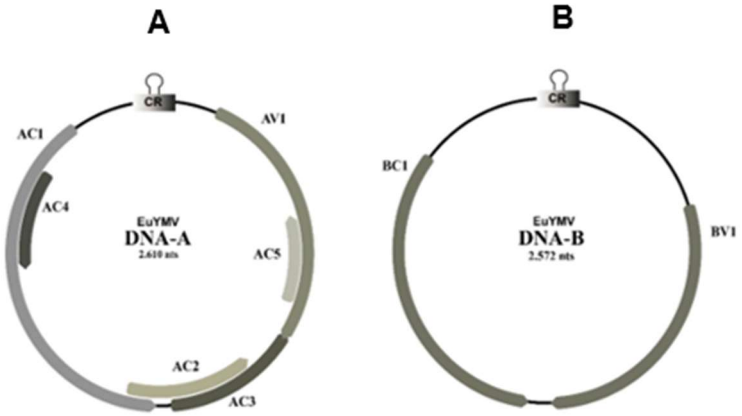

Fig 1A. Genomic organization of DNA-A (PP911363 contig 67). In the DNA-A component, five ORFs (open reading frames) were annotated: AV1 (CP), in the viral sense and AC1 (Rep), AC2 (TrAP), AC3 (Ren), AC4 and AC5. **Fig 1B.** Genomic organization of DNA-B (PP911364 contig 54). In the DNA-B component, two ORFs were annotated: BV1 (NSP), in the viral sense and BC1 (MP), in the complementary sense.

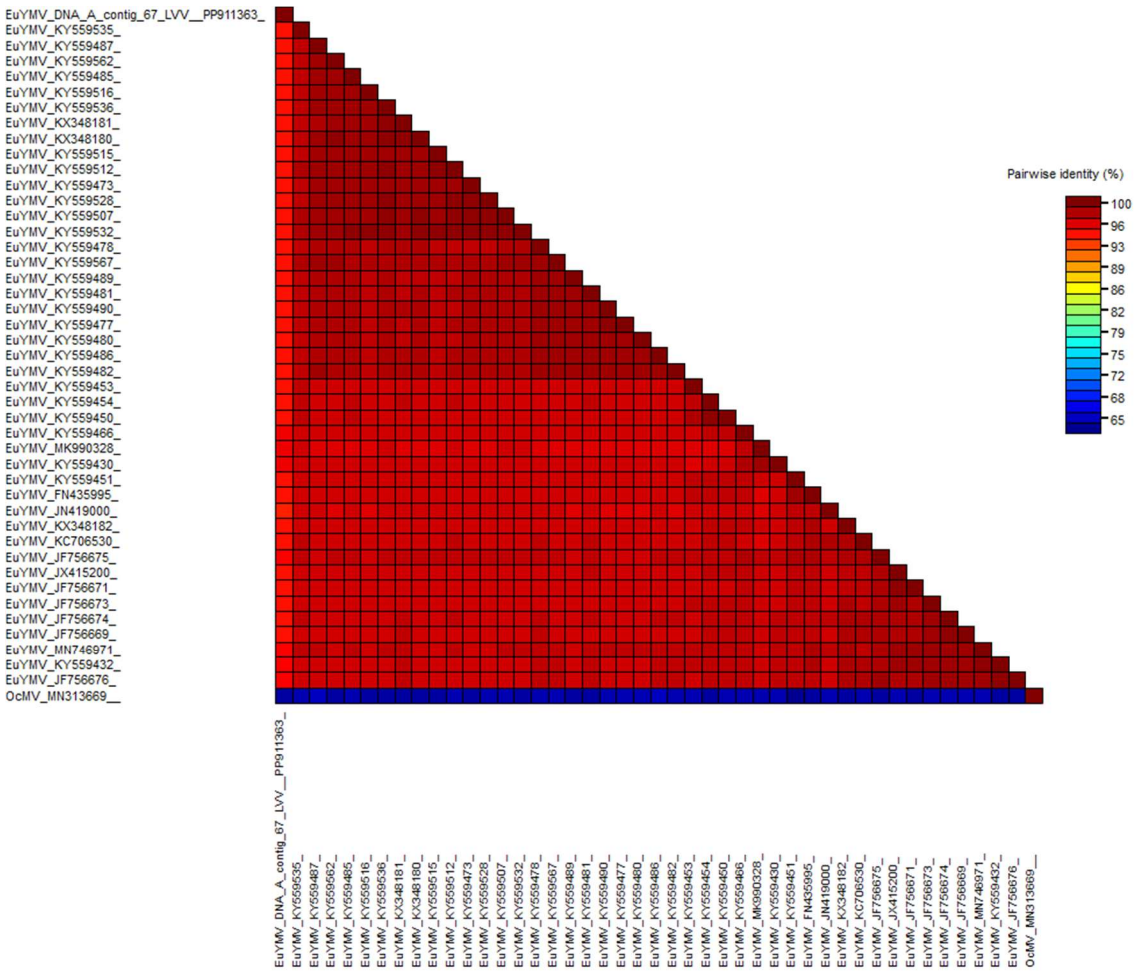

Fig 2. SDT (Sequence Demarcation Tool) of 43 complete DNA-A sequences with identities and distances between isolates described as Euphorbia yellow mosaic virus (EuYMV) and Ocimum mosaic virus (OcMV). The EuYMV sequence analyzed in this study is positioned at the extreme left, corresponding to EuYMV DNA-A contig 67 LVV (PP911363). The GenBank accession numbers corresponding to each viral isolate are associated with the acronym EuYMV. Nucleotide identity between isolates ranges from 63 to 100%.

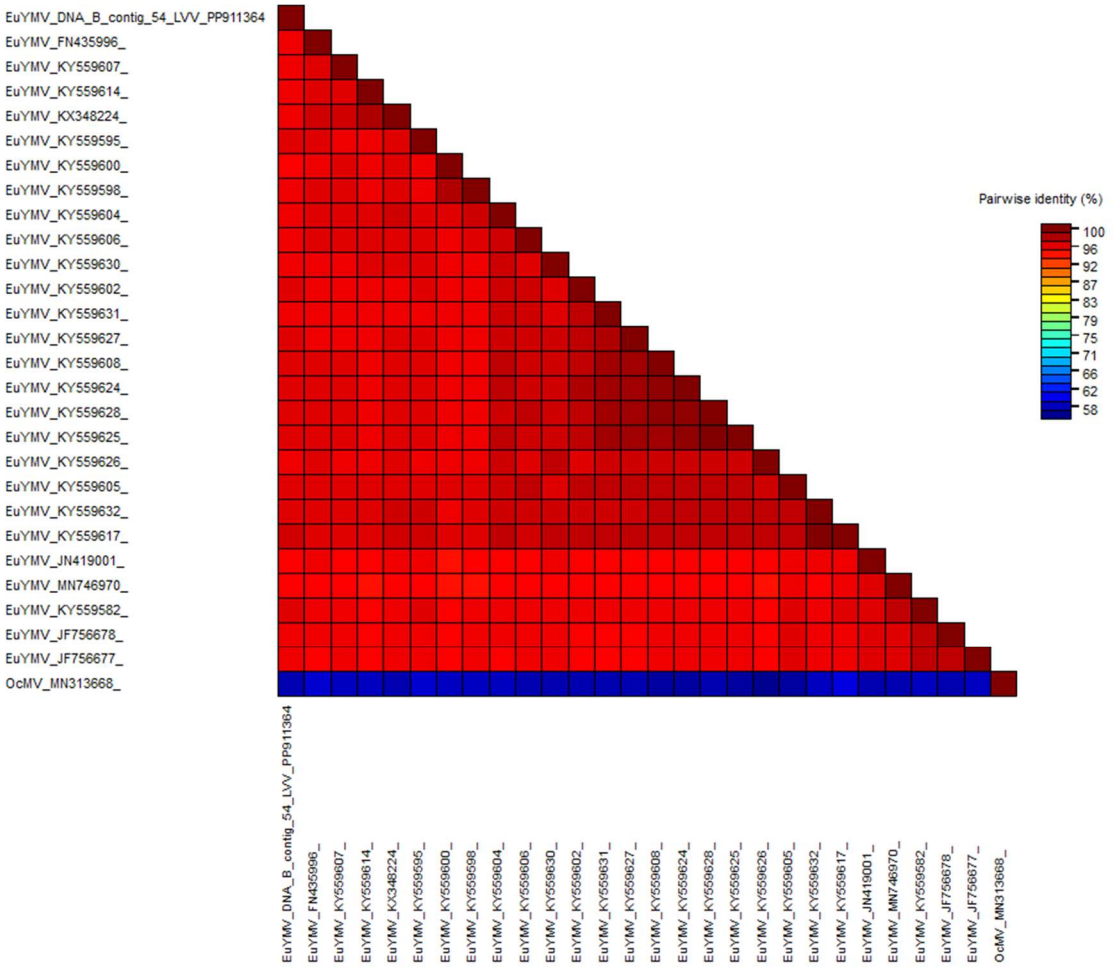

Fig 3. SDT (Sequence Demarcation Tool) of 26 complete DNA-B sequences with identities and distances between isolates described as Euphorbia yellow mosaic virus (EuYMV) and Ocimum mosaic virus (SiMMV). The EuYMV sequence analyzed in this study is positioned at the extreme left, corresponding to EuYMV DNA-B contig 54 LVV(PP911364). The GenBank accession numbers corresponding to each viral isolate are associated with the acronym EuYMV. Nucleotide identity between isolates ranges from 56 to 100%.

Table 3. Information on 43 Euphorbia yellow mosaic virus (EuYMV) isolates used in sequence comparison via MUSCLE alignment of SDT.

Accession	Host	Botanic family	Sequence Length (nts)	Local of collection[a]
JX415200	*Euphorbia heterophylla*	Euphorbiaceae	2611	GO
KY559477	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
KX348182	*Leonurus sibiricus*	Lamiaceae	2618	MS
KY559536	*Euphorbia heterophylla*	Euphorbiaceae	2618	RS
KX348181	*Sida* sp.	Malvaceae	2618	RS
KY559482	*Euphorbia heterophylla*	Euphorbiaceae	2618	PR
KY559486	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
JN419000	*Macroptilium atropurpureum*	Fabaceae	2609	PE
KY559489	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
KY559562	*Euphorbia heterophylla*	Euphorbiaceae	2616	RS
KY559485	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
KC706530	*Euphorbia* sp.	Euphorbiaceae	2606	MG
KY559473	*Euphorbia heterophylla*	Euphorbiaceae	2617	PR
KY559490	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
KY559516	*Euphorbia heterophylla*	Euphorbiaceae	2618	RS
JF756671	*Euphorbia heterophylla*	Euphorbiaceae	2609	GO
KY559454	*Euphorbia heterophylla*	Euphorbiaceae	2618	ES
KY559480	*Euphorbia heterophylla*	Euphorbiaceae	2619	MS
KY559450	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
JF756676	*Euphorbia heterophylla*	Euphorbiaceae	2610	MS
KY559430	*Euphorbia heterophylla*	Euphorbiaceae	2610	GO
KY559567	*Euphorbia heterophylla*	Euphorbiaceae	2619	AM
FN435995	*Euphorbia* sp.	Euphorbiaceae	2610	RS

JF756674	*Euphorbia heterophylla*	Euphorbiaceae	2610	MS
KY559515	*Euphorbia heterophylla*	Euphorbiaceae	2617	GO
KY559478	*Euphorbia heterophylla*	Euphorbiaceae	2619	RS
KY559528	*Euphorbia heterophylla*	Euphorbiaceae	2617	PR
KY559487	*Euphorbia heterophylla*	Euphorbiaceae	2619	RS
KY559481	*Euphorbia heterophylla*	Euphorbiaceae	2619	PR
JF756669	*Euphorbia heterophylla*	Euphorbiaceae	2610	PR
KY559451	*Euphorbia heterophylla*	Euphorbiaceae	2609	DF
KY559512	*Capsicum chinense*	Solanaceae	2617	MS
MK990328	*Euphorbia heterophylla*	Euphorbiaceae	2611	RS
KY559507	*Euphorbia heterophylla*	Euphorbiaceae	2617	AM
KY559535	*Euphorbia heterophylla*	Euphorbiaceae	2610	RS
KY559432	*Euphorbia heterophylla*	Euphorbiaceae	2611	RS
KY559532	*Euphorbia heterophylla*	Euphorbiaceae	2617	GO
JF756675	*Euphorbia heterophylla*	Euphorbiaceae	2610	RS
MN746971	*Solanum lycopersicum* (tomato)	Solanaceae	2609	MG
KY559453	*Euphorbia heterophylla*	Euphorbiaceae	2618	MS
JF756673	*Euphorbia heterophylla*	Euphorbiaceae	2611	DF
KX348180	*Sida* sp.	Malvaceae	2619	RS
KY559466	*Euphorbia heterophylla*	Euphorbiaceae	2610	MG
MN313669	*Ocimum gratissimum*	Lamiaceae	2794	Uganda

[a] *States of Brazil:* AM: Amazonas; DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; MG: Minas Gerais; MS: Mato Grosso do Sul; PE: Pernambuco; PR: Paraná; RS: Rio Grande do Sul.

CHAPTER 4

Final considerations

1. Current scenario of interactions among begomoviruses, weeds and, crops.

Bemisia tabaci (Order Hemiptera and family Aleyrodidae) *Middle East Asia Minor1* – MEAM 1 (previously known as biotype B) and *B. tabaci Mediterranean* – MED (previously known as biotype Q) show a global distribution and they are related to have high competence tranmissiom of a wide range of viruses classified in different genera, including *Begomovirus*, *Crinivirus*, *Torradovirus*, and *Ipomovirus* among others. The genus *Begomovirus* (family *Geminiviridae*) comprises by far the most important group of pathogens infecting tomatoes (*Solanum lycopersicum* L.) in Brazil. Begomoviruses are subdivided according to the number of molecules of DNA encapsidated by twinned particles: monopartite begomovirus (a single genomic DNA-A) and bipartite begomovirus (two DNA components, referred to as DNA-A and DNA-B). Tomato severe rugose virus – ToSRV (a bipartite species) and tomato mottle leaf curl virus – ToMoLCV (a monopartite species) are the prevalent begomoviruses in the country. ToMoLCV is predominant in the Northeast region, but it is widespread in the country, being present across seven biomes across all five macro-geographic regions of Brazil (Oliveira et al. 2024). On the other hand, ToSRV shows a high adaptability, infecting a large number of hosts and being present in different regions of the country. These attributes of ToSRV may also explain its prevalence in the Midwest region.

The ubiquitous presence of the polyphagous whitefly vector *B. tabaci* MEAM 1 throughout the Brazilian territory with the presence of semi-persistent host weeds contributed to an increase in the host range and outbreaks of begomoviruses in many crops, including tomato. In the present work, studies were performed using HTS (High-throughput sequencing) and PCR and, allowed detections and molecular characterization of three bipartite begomoviruses: **1. ToSRV** was the most frequently detected begomovirus with 16 hosts, three samples of *Capsicum frutescens*, one sample of *Desmodium incanum*, ten samples of *Nicandra physalodes*, one sample of *Solanum aethiopicum* and one sample of *Solanum lycopersicum* (**Table 1**). Among these hosts, the virus was detected by the first time in *Desmodium incanum* (family Fabaceae) and, *Solanum aethiopicum* (family Solanaceae). **2. Euphorbia yellow mosaic virus (EuYMV)** was detected by the first time in solanaceous hosts: *Capsicum frutescens*, *Nicandra physalodes*, *Physalis angulata* and *Solanum melongena*. **3. Sida micrantha mosaic virus (SimMV)** was detected in three solanaceous hosts: *Nicandra physalodes*,

Physalis angulata and *Solanum tuberosum*. **4. ToMoLCV** was detected in six new solanaceous hosts: *Capsicum baccatum*, *Capsicum frutescens*, *Nicandra physalodes*, *Solanum aethiopicum*, *Solanum americanum*, and *Solanum rostratum*; and two fabaceous hosts: *Senna obtusifolia* and *Vigna unguiculata*.

As previously mentioned, ToMoLCV and ToSRV are the two best established begomoviruses and, frequently are occurring throughout Brazilian using alternative hosts, corroborating surveys in tomato cultivation that point to this population behavior, especially for ToSRV. From a total of 26 detections carried out in this work for these two begomoviruses, three were fabaceous and 23 solanaceous. These results corroborate the role of weeds and minor crops of these families as reservoirs of begomovirus, and pointing out the potential influence of these plants on the epidemiological cycle of detected begomoviruses, on the genetic structure of these populations and the consequent damage caused to areas cultivated with tomato and areas with these minor crops.

Considering aspects related to the geographic distribution of the detected begomoviruses, the results obtained confirm the predominance of ToSRV in the Midwest region where frequent reports have occurred in recent decades in tomato crops. The presence of EuYMV in the North and Midwest regions was also observed in cultivated plants, reaffirming the hypothesis of virus sharing hosts from the same botanical family. A similar situation occurs with SimMV, whose results consolidate its presence in the Midwest, but indicate an expansion towards the North region with detection carried out in a sample collected in the state of Tocantins. Another significant result in geographic region is the presence of ToMoLCV in the Midwest and Southeast regions, a monopartite begomovirus initially restricted to the Northeast region.

Presenting the results obtained in this work and their concatenation with those present in databases and recent bibliographic references, a greater concentration of the number of hosts from Fabaceae and Solanaceae families in the Midwest region of Brazil is observed, as well as the progression of numbers of hosts in the regions Northeast and Midwest, demonstrating the importance of surveys and characterizations such as those carried out in this thesis, to assist tomato breeding programs and to improve both vector and weed control practices currently employed in production fields.

Table 1. Begomovirus species present in weeds and minor crops classified in Fabaceae and Solanaceae families in Brazil.

Species	Host	State[1]	Reference
Bean golden mosaic virus	*Macroptilium lathyroides*	MG	Xavier et al. (2021)
Blainvillea yellow spot virus	*Physalis*	MG	Rocha et al. (2013)
Cleome leaf crumple virus	*Phaseolus* spp.	AL	Wyant et al. (2012)
*Cowpea bright yellow mosaic virus**	*Vigna unguiculata*	PE	Fonseca et al. (2012)
Euphorbia yellow mosaic virus	*Crotalaria*	GO	Barreto et al. (2013)
	Capsicum chinense	AM	Catarino et al. (2020)
	Capsicum frutescens	**AM**	**Honorato et al. (2024) – Present Thesis**
	Physalis angulata	**DF**	**Honorato et al. (2024) – Present Thesis**
	Solanum melongena	**DF**	**Honorato et al. (2024) – Present Thesis**
	Nicandra physalodes	**DF**	
Macroptilium bright mosaic virus	*Macroptilium lathyroides*	AL	Passos et al. (2016)
Macroptilium bright yellow interveinal virus	*Macroptilium erythroloma*	DF	Batista et al. (2021)
Macroptilium common mosaic virus	*Macroptilium lathyroides*	AL	Passos et al. (2016)
Macroptilium yellow net virus	*Macroptilium lathyroides*	AL	Silva et al. (2012)
Macroptilium yellow spot virus	*Canavalia* sp.	AL	Silva et al. (2012)
Macroptilium yellow vein virus	*Macroptilium lathyroides*	AL	Sobrinho et al. (2014)
Sida micrantha mosaic virus	*Phaseolus vulgaris*	GO	Fonseca et al. (2010)
	Nicandra physalodes	**DF**	**Honorato et al. (2024) – Present Thesis**
	Physalis angulata	**TO**	**Honorato et al. (2024) – Present Thesis**
Sida yellow blotch virus	*Phaseolus lunatus*	PB	Fontenele et al. (2016)
Soybean chlorotic spot virus	*Macroptilium lathyroides*	MG	Sobrinho et al. (2016)
Tomato crinkle leaf yellows virus	*Macroptilium atropurpureum*	PE	Silva et al. (2012)
Tomato interveinal chlorosis virus*	***Rhynchosia minima***	**-**	**Melo et al. (2024)**
Tomato mottle leaf curl virus	***Senna obtusifolia***	**BA**	**Honorato et al. (2024) – Present Thesis**
	Capsicum baccatum	**DF**	**Honorato et al. (2024) – Present Thesis**
	Solanum rostratum	**DF**	**Honorato et al. (2024) – Present Thesis**
	Capsicum frutescens	**DF**	**Honorato et al. (2024) – Present Thesis**
	Nicandra physalodes	**DF**	**Honorato et al. (2024) – Present Thesis**
	Vigna unguiculata	**GO**	**Honorato et al. (2024) – Present Thesis**
	Solanum americanum	**SP**	**Honorato et al. (2024) – Present Thesis**
	Solanum aethiopicum	**SP**	**Honorato et al. (2024) – Present Thesis**
Tomato severe rugose virus	*Capsicum frutescens*	GO	Bezerra-Agasie et al. (2005)
	Pachyrhizus erosus	DF	Pereira-Silva et al. (2022)
	Solanum aethiopicum	**DF**	**Honorato et al. (2024) – Present Thesis**
	Desmodium incanum	**DF**	**Honorato et al. (2024) – Present Thesis**
	Capsicum frutescens	**CE**	**Honorato et al. (2024) – Present Thesis**

* Sequences deposited in GenBank without corresponding publication.

** In bold is information collected for the three-year period corresponding to the years 2022, 2023 and 2024.

[1] AL: Alagoas; AM: Amazonas; BA: Bahia; CE: Ceará; DF: Distrito Federal; GO: Goiás; MG: Minas Gerais; PB: Paraíba; PE: Pernambuco and TO: Tocantins.

Table 2. Interactions between begomoviruses and hosts from the Fabaceae and Solanaceae families

Begomovirus	Host species detected (number of samples)	New hosts	Known hosts (literature)	TOTAL (Thesis and literature)
EuYMV	*Capsicum frutescens* (1) *Physalis angulata* (1) *Solanum melongena* (1) *Nicandra physalodes* (1)	*Capsicum frutescens* *Physalis angulata* *Solanum melongena* *Nicandra physalodes*	*Capsicum chinense* *Capsicum annum* *Datura stramonium* *Nicotiana benthamiana* *Glycine max* *Macroptilium atropurpureum* *Phaseolus vulgaris* *Solanum lycopersicum*	Twelve hosts from the Fabaceae and Solanaceae families
SimMV	*Solanum tuberosum* (1) *Nicandra physalodes* (3) *Physalis angulata* (1)	*Solanum tuberosum* *Nicandra physalodes* *Physalis angulata*	*Phaseolus vulgaris* *Nicotiana benthamiana* *Capsicum chinense* *Glycine max* *Nicotiana tabacum* *Solanum lycopersicum*	Nine hosts from the Fabaceae and Solanaceae families
ToMoLCV	*Senna obtusifolia* (1) *Capsicum baccatum* var. *praetermissum* (1) *Solanum rostratum* (1) *Capsicum frutescens* (1) *Nicandra physalodes* (2) *Vigna unguiculata* (1) *Solanum americanum* (1) *Solanum aethiopicum* (1) *Solanum tuberosum* (1)	*Senna obtusifolia* *Capsicum baccatum* var. *praetermissum* *Solanum rostratum* *Capsicum frutescens* *Nicandra physalodes* *Vigna unguiculata* *Solanum americanum* *Solanum aethiopicum* *Solanum tuberosum*	*Solanum lycopersicum* *Solanum melongena*	Ten hosts from the Fabaceae and Solanaceae families
ToSRV	*Solanum aethiopicum* (1) *Desmodium incanum* (1) *Nicandra physalodes* (10) *Capsicum frutescens* (3) *Solanum lycopersicum* (1)	*Solanum aethiopicum* *Desmodium incanum*	*Nicandra physalodes* *Capsicum frutescens* *Capsicum baccatum* *Solanum lycopersicum* *Pachyrhizus erosus* *Solanum betaceum* *Solanum torvum*	Nine hosts from the Fabaceae and Solanaceae families
TOTAL	**35 samples detected**	**17 new hosts**		

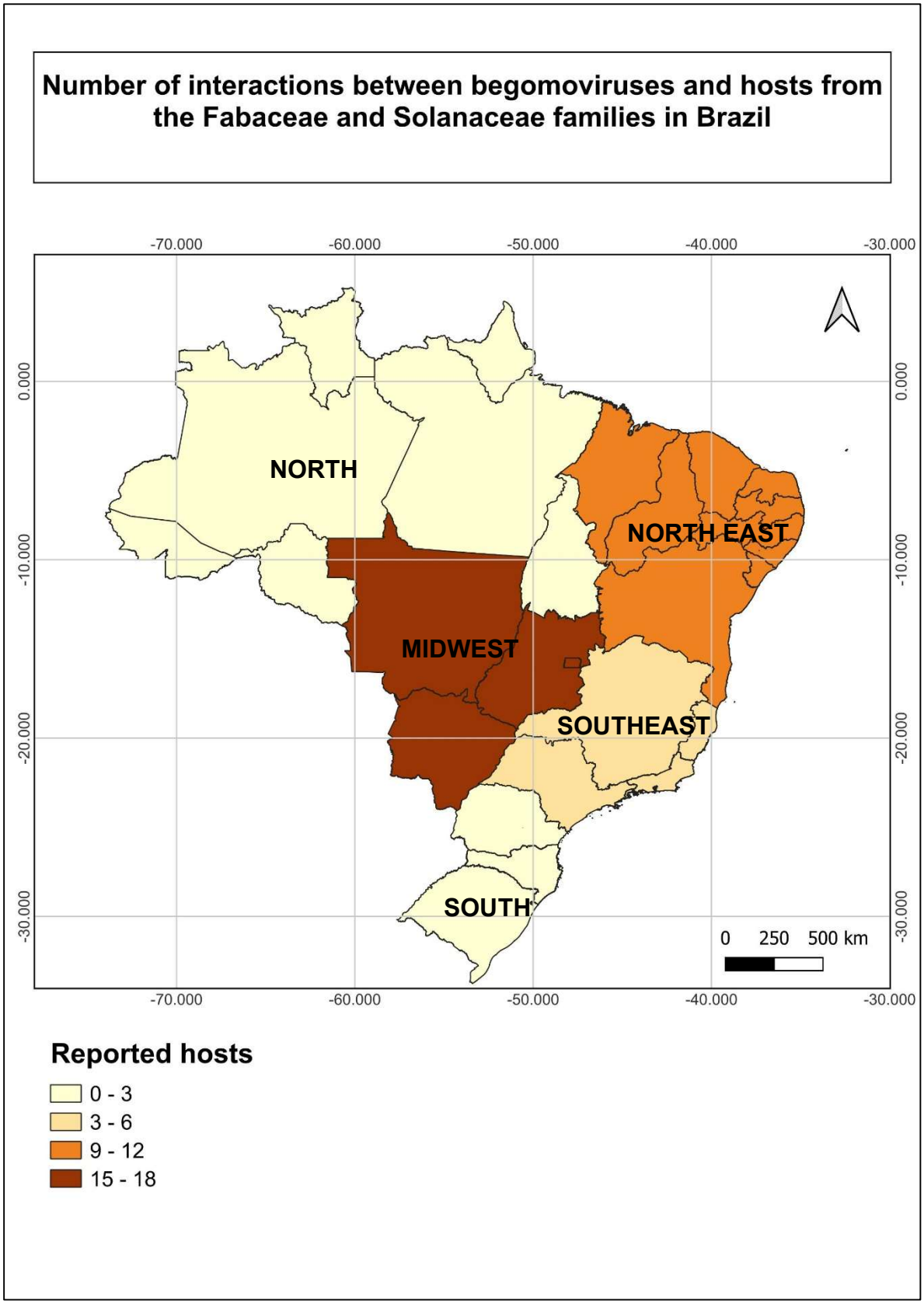

Figure 1. Map of the number of interactions reported between begomovirus and hosts Fabaceae and Solanaceae families by regions of Brazil.

2. Perspectives

Chapter 2

The study of weeds and minor crops virome from the Fabaceae and Solanaceae families resulted in the detection of four *Begomovirus* species (EuYMV, SimMV, ToMoLCV and ToSRV) that find reservoirs in these plants, in which events such as mutation, recombination, and pseudorecombination are favored and can be understood when subjected to more in-depth analysis, allowing for better understanding the epidemiological cycle of these pathogens and possible changes to the genetic structure of these viral populations. The results obtained may also contribute to breeding programs, by providing a broader understanding of the viral species present, and in the management of weeds, knowing the viral species they harbor.

Chapter 3

The EuYMV begomovirus, firstly described in *Euphorbia heterophylla* and tomato, has grown in relevance in the last decade, affecting important crops such as soybeans, beans, and tomatoes, and understanding its geographic expansion and widening of its host range are important results achieved in this chapter. Genomic characterization and phylogenetic analyzis are also relevant for understanding the viral populations detected and different regions.

3. General conclusions

1. ToSRV and ToMoCLV are the most prevalent weed viruses associated with tomato crops in Brazil;

2. The number of hosts for begomoviruses shows a significant increase among plants of the botanical families Fabaceae and Solanaceae;

3. The geographic distribution of important species of *Begomovirus* presents progressive expansion throughout the Brazilian territory, favored, among other aspects, by the presence of weeds adjacent to areas cultivated with tomato;

4. The recently characterized genus *Topilveirus* has increased its host range among plants of the Solanaceae family;

5. This thesis, although developed with a relatively low number of samples, indicates a significantly greater diversity of members of the *Geminiviridae* family present in weeds of the Fabaceae and Solanaceae families, compared to existing reports to date.