Universidade de Brasília
Instituto de Ciências Biológicas
Departamento de Fitopatologia
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Dissertação de Mestrado

Diversidade de vírus de ssDNA e alfasatélites em solanáceas e caracterização molecular de três novas espécies de *Begomovirus* em tomateiro

GIOVANA CURCIO GUIMARÃES

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Trabalho de Conclusão de Curso apresentado à Universidade de Brasília como requisito parcial para obtenção do título de Mestre em Fitopatologia pelo Programa de Pós-Graduação em Fitopatologia

Orientadora

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Agradeço a Deus por guiar meus passos, iluminar meu caminho e ser a fonte inesgotável de amor e graça em minha vida.

Dedico

Dedicatória

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Diversidade de vírus de ssDNA e alfasatélites em solanáceas e caracterização molecular de três novas espécies de *Begomovirus* em tomateiro.

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Figure 2: Pairwise identity analysis using the Sequence Demarcation Tool (SDT) software and root-mean Bayesian phylogenetic tree with GTR+I model with information on DNA–A sequences of *Begomovirus* species obtained from the NCBI database. These viral species are identified with an acronym, abbreviation of the countries where they were described, and their accession numbers.

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Table 6: **DNA–A segments** identified via High-Throughput Sequencing (HTS) in a pool of 129 leaf samples from plants of the Solanaceae family with nucleotide identity values lower than 91%, which are corresponding to putative novel viral species. Information was organized by the code number of the contigs corresponding to the putative novel species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E-value, the correspondent GenBank (GB) accession.

Table 7: Details of recombination breakpoints detected in 14 of the 16 potential novel viral species. Contigs, event, greatest and least relatedness with identities (%), initial and final breakpoint, recombinant region, statistical methods and e-value. Recombination events described in R, G, B, M, C, S, T indicate detection by the RDP, GENCONV, BOOTSCAN, MAXCHI, CHIMAERA, SISCAN and 3SEQ methods, respectively, with the highest p-value presented being determined by the indicated method **bold.**

Resumo geral

A família Solanaceae é uma família botânica diversificada que abrange várias espécies de plantas angiospermas. O tomateiro (*Solanum lycopersicum* L.) é uma das hortaliças mais importantes tanto economicamente quanto socialmente para o Brasil. Os níveis de produtividade dessa cultura sofrem oscilações recorrentes devido a diversos fatores, dentre eles se destacam as doenças tais como as begomoviroses (causadas por um complexo de espécies de begomovírus). Esses patógenos possuem uma ou duas moléculas de DNA circular de fita simples (apresentando os componentes DNA–A e DNA–B) predominam em tomateiro no Novo Mundo, sendo transmitidos por diferentes espécies crípticas do complexo *Bemisia tabaci*. O advento da tecnologia de High Throughput Sequencing (HTS) tem proporcionado a descoberta de novas espécies virais associadas com o cultivo do tomateiro. Neste contexto, o presente trabalho tem como um dos objetivos estudar a diversidade viral no tomateiro e plantas daninhas da família Solanaceae. Para isto, no capítulo dois, cento e vinte e nove (129) amostras foliares de tomateiro e plantas daninhas exibindo sintomas típicos de begomovírus (clorose apical, mosaico dourado e pontuações cloróticas) foram coletadas nas regiões Norte (15), Nordeste (43), Sul (11), Centro Oeste (45), Sudeste (18). As coletas foram realizadas no período de 2003 a 2022, sendo analisadas amostras foliares vários representantes da família Solanaceae. O DNA total extraído foi usado como molde para amplificação por círculo rolante (RCA). Para confirmação da presença de begomovírus por meio de ensaios de PCR usando primers degenerados para o DNA–A (PAR1c496 e PAL1v1978) e para o DNA–B (PBL e CRC). Após esta etapa, RCA das 129 amostras foram empregadas para formar um pool e sequenciadas em uma plataforma Ilumina NovaSeq 6000. Foram obtidos 41.659 contigs sendo que **172** deles exibiram após análises com RefSeqViral identidades com begomovírus (**168**), topilevírus (**1**) gemycircularvírus (**1**) e dois alfasatélites. Dezesseis dos **168** contigs de begomovírus apresentaram identidade nucleotídica abaixo de 91%, sendo consistentes com o critério taxonômico atual de definição de novas espécies dentro do gênero *Begomovirus*. No capítulo três, outros três contigs (C16, C21 e C230) mostraram uma organização genômica típica de begomovírus bipartidos e foram considerados potenciais novas espécies dentro do gênero *Begomovirus*. A partir da utilização de HTS foi possível observar a presença de altos níveis de variabilidade genética e de diversidade de begomovírus em membros da família Solanaceae, indicando um relevante papel como reservatórios virais. Ações de pesquisa estão sendo conduzidas visando a produção de clones infecciosos para desvendar gama de hospedeiras bem como fontes de resistência contra esses novos vírus.

General Abstract

The Solanaceae family is a diverse botanical family that encompasses several species of angiosperm plants. The tomato (*Solanum lycopersicum* L.) is one of the most important vegetables both economically and socially in Brazil. The productivity levels of this crop suffer recurrent fluctuations due to several factors, among which diseases such as begomoviruses (caused by a complex of begomovirus species) stand out. These pathogens have one or two single-stranded circular DNA molecules (presenting the components DNA–A and DNA–B) and predominate in tomato plants in the New World, being transmitted by different cryptic species of the *Bemisia tabaci* complex. The advent of High Throughput Sequencing (HTS) technology has led to the discovery of new viral species associated with tomato cultivation. In this context, the objective of this work is to study viral diversity in tomato and weeds from the Solanaceae family. For this, in chapter two, one hundred and twenty-nine (129) leaf samples from tomato and weed plants exhibiting typical begomovirus symptoms (apical chlorosis, golden mosaic and chlorotic spots) were collected in the North (15), Northeast (43), South (11) Midwest (45), Southeast (18) regions. Collections were carried out from 2003 to 2022, with leaf samples being analyzed from various representatives of the Solanaceae family. The extracted total DNA was used as template for rolling circle amplification (RCA). To confirm the presence of begomoviruses through PCR assays using degenerate primers for DNA–A (PAR1c496 and PAL1v1978) and for DNA–B (PBL and CRC). After this step, RCA of the 129 samples were used to form a pool and sequenced on an Ilumina NovaSeq 6000 platform. 41,659 contigs were obtained, 172 of which showed, after analysis with RefSeqViral, identities with begomovirus (168), topilevirus (1) and gemycircularvirus (1) and two alphasatellites. Sixteen of the 168 begomovirus contigs showed nucleotide identity below 91%, being consistent with the current taxonomic criteria for defining new species within the genus Begomovirus. In chater 3 another three contigs (C16, C21 and C230) showed a genomic organization typical of bipartite begomoviruses and were considered potential new species within the Begomovirus genus. Using HTS, it was possible to observe the presence of high levels of genetic variability and diversity of begomoviruses in members of the Solanaceae family, indicating an important role as viral reservoirs. Research actions are being conducted aimed at producing infectious clones to uncover the range of hosts as well as sources of resistance against these new viruses.

Introdução Geral

Plantas da família Solanaceae, é uma das maiores famílias de plantas dentre as angiospermas que apresentam grande destaque em todo o mundo (Hanÿcinský et al. 2020) por ser composta de espécies como *Solanum lycopersicum* L (tomate)*, Capsicum annuum* L (pimentão) e *Solanum melongena* L (berinjela). Além destas espécies, existem hospedeiras consideradas como reservatórios de patógenos virais, sendo elas plantas daninhas, que crescem lado a lado a culturas de grande importância econômica como o tomate (Singh et al. 2016). Entre estas hosepedeiras estão a jurubeba *(Solanum paniculatum* L), lobeira (*Solanum lycocarpum* L), physalis (*Physalis* L), juá de capote (*Nicandra physalodes* L), maria pretinha (*Solanum americanum* L) e teta de vaca (*Solanum mammosum* L) entre outras.

O tomateiro é uma das culturas mais importantes do Brasil, com um volume de produção de aproximadamente 3,9 milhões de toneladas por ano, sendo o estado de São Paulo o maior produtor (IBGE 2023). No entanto, diversas espécies que formam este grupo de plantas são acometidas por diferentes grupos de patógenos como vírus, bactérias, fungos, nematóides e oomicetos, afetando a qualidade dos frutos (Rasul et al. 2019). Dentre os sintomas causados por vírus estão enrolamento das folhas, mosaico, enrugamento, crescimento reduzido, má formação de brotos e frutos (Kumari et al. 2022).

Entre os fitopatógenos causadores de dano estão os vírus classificados no gênero *Begomovirus*. Este grupo de vírus de plantas pertencem à família *Geminiviridae* que abriga atualmente 445 espécies (ICTV 2023). Os begomovírus apresentam DNA circular de fita simples (ssDNA), sendo relativamente pequenos com cerca de 2,5 a 3,0 kb de tamanho (Brown et al. 2015). O genoma é dividido em monopartido, apenas com um DNA genômico, DNA–A ou bipartido composto por dois componentes distintos denominados DNA–A e DNA–B (Varsani et al. 2017). Nos begomovírus bipartidos encontram-se de cinco a sete genes no componente DNA–A (sentido viral, AV1 e AV2, sentido complementar AC1, AC2, AC3*,* AC4*,* AC5*,* AC6 e AC7) e no componente DNA–B dois genes são encontrados (sentido viral BV1 e sentindo complementar BC1) **(**Zerbini et al. 2017, Zhao et al. 2022, Wang et al. 2022, Liu et al. 2023). Além destes dois componentes*,* existem agentes subvirais de DNA, conhecidos como alfassatélites, betassatélite e deltassatélite (Briddon et al. 2018, Zhou et al. 2013, Lozano et al. 2016). Dentre os begomovírus responsáveis por grandes perdas econômicas estão o bipartido tomato severe rugose virus (ToSRV) (Duarte et al. 2021, Reis et al. 2020 2021) e o monopartido tomato mottle leaf curl virus (ToMoLCV) (Souza et al. 2022) sendo eles amplamente distribuídos na região central e nordeste do Brasil,

respectivamente (Macedo et al. 2019, Gilbertson et al. 2015). A evolução dos begomovírus é impulsionada principalmente por mutação, deriva genética e recombinação (Tourrette et al. 2022).

A transmissão dos begomovírus está associada a um complexo de espécies crípticas de *Bemisia tabaci* (Hemiptera: Aleyrodidae), popularmente conhecida como mosca branca (Ghosh et al. 2021). A partir da década de 1990 as doenças causadas por espécies do gênero *Begomovirus* não eram comuns, entretanto, neste mesmo ano, simultaneamente à entrada no país de *Bemisa tabaci* (grupo genético MEAM–1 antigo biótipo B) (Krause-Sakate et al. 2020, Watanabe et al. 2019) foi observado o aumento nos casos de begomoviroses por possuírem amplo círculo de plantas hospedeiras (hábito polífago) (Ribeiro et al. 2003, Fernandes et al. 2008, Gilbertson et al. 2015).

Atualmente com o advento da tecnologia, o número de vírus encontrados em plantas daninhas e em culturas de grande importancia econômica vem crescendo exponencialmente (Susi et al. 2019). O uso do sequenciamento de alto rendimento (HTS) e técnicas de amplificação para genomas circulares de DNA, como a amplificação por círculo rolante (RCA), possibilitou a geração de dados robustos, qualidade e com bom custo-benefício (Haible et al. 2006, Inoue-Nagata et al. 2004, Mehetre et al. 2019). A partir deste ponto o estudo de metagenômica viral aplicada à virologia de plantas tem possibilitado a descoberta de novas espécies virais em diferentes hospedeiros, como o tomate (Rivarez et al. 2021).

Embora estudos sobre diversidade populacional de begomovírus já tenha sido alvo de investigações, o presente trabalho visa ampliar tais análises em culturas como tomateiro e avaliar o potencial das plantas daninhas. da família Solanaceae, como reservatório viral em diferentes regiões brasileiras.

Neste contexto os principais objetivos do presente trabalho são: (1) efetuar uma análise metagenômica da diversidade de espécies de *Begomovirus* e de agentes subvirais, em cultivo de tomate e plantas daninhas presentes em todas as cinco regiões brasileiras; (2) conduzir estudos de caracterização molecular de três de novas espécies de begomovírus encontrando no Norte e Sul do Brasil.

Espera-se que o presente trabalho amplie os conhecimentos em relação a distribuição geográfica das espécies virais presentes em tomateiro e plantas daninhas, enquanto o estudo do genoma possibilita o conhecimento de possíveis novas ORFs e suas funções quando em interação com vetor ou planta.

Hipótese

Em decorrência do aumento de begomoviroses em tomateiro e em outras hospedeiras (incluindo Solanaceae) no Brasil, acredita-se que ainda exista uma ampla diversidade viral a ser estudada no país em plantas desta importante família botânica. Nesse cenário, diversidade viral em plantas daninhas associadas à cultura de tomateiro pode estar relacionadas ao aumento da variabilidade genética de espécies virais da família *Geminiviridae* e o avanço contínuo de novos begomovírus sugerem que estes fenômenos biológicos estão acontecendo.

Objetivos gerais

O estudo das viroses no tomateiro ultrapassa a esfera científica, assumindo uma relevância crucial nos aspectos sociais e econômicos da cultura do tomate. Socialmente, a produção de tomates representa uma fonte vital de subsistência para comunidades rurais, conectando-se intimamente com laços comunitários e a coesão social. Economicamente, a cultura do tomateiro é um pilar na agricultura comercial, influenciando diretamente a renda dos agricultores, a estabilidade econômica local e até mesmo o comércio internacional de produtos agrícolas. Investir em pesquisa e educação sobre as viroses do tomateiro não apenas aborda desafios fitossanitários, mas também promove estabilidade econômica e a segurança alimentar em escala global, destacando a interconexão entre o estudo científico e o bem-estar socioeconômico das comunidades.

Objetivos específicos

Capítulo 2

1. Avaliar diversidade viral em 129 amostras na família Solanaceae de diferentes regiões brasileiras.
2. Estudar taxas de recombinação em novos virus

Capítulo 3

1. Caracterizar molecularmente três novas espécies de begomovirus bipartidos em tomateiro.
2. Avaliar taxas de recombinção nas três novas espécies a serem decritas

Revisão de literatura

1.1 Família Solanaceae

A família Solanaceae é um grupo monofilético de dicotiledôneas, sendo uma família botânica que engloba uma vasta diversidade de plantas angiospermas, desempenhando um papel crucial na alimentação humana, medicina e horticultura (Gebhardt et al. 2016, Ganaie et al. 2018). Composta por aproximadamente 106 gêneros e mais de 2.700 espécies (Dupin et al. 2017), essa família é distribuída globalmente, ocupando uma variedade de habitats que vão desde regiões tropicais até temperadas (Xu et al. 2017)

Nesta família fazem parte culturas amplamente cultivadas como o tomate (*Solanum lycopersicum* L), sendo uma das culturas mais importantes desta familia (Samuels 2015). O tomate desempenha um papel crucial no cenário econômico global, tanto no setor agrícola quanto industrial, uma vez que é reconhecido como um alimento funcional (Silva et al. 2019). Além de seu papel culinário, o tomate é uma fonte rica de nutrientes, destacando-se o licopeno, um antioxidante associado a benefícios para a saúde (Mazidi et al. 2020)

Outra importante espécie desta familia é a pimenta (*Capsicum* spp.), sendo naturias das zonas tropicais e úmidas da América Central e do Sul (Batiha et al. 2020). As pimentas são amplamente utilizadas na culinária mundial, conferindo sabores picantes aos pratos (Song et al. 2021). Além de seu valor gastronômico, as pimentas contêm compostos, como a capsaicina, que têm propriedades benéficas à saúde, como a promoção da circulação sanguínea (Krisnamurti et al. 2023).

Além destas, a família Solanaceae inclui espécies consideradas daninhas, como *Physalis angulata*, *Solanum nigrum* e *Solanum paniculatum*. *P. angulata*, é reconhecida como planta daninha devido à sua habilidade de colonizar áreas agrícolas, competindo com culturas desejáveis e impactando negativamente a produtividade (Balah et al. 2022). *S. nigrum*, conhecida como erva-moira, é outra planta daninha comum que pode infestar campos cultivados (Deng et al. 2023). Apesar do status indesejado, estudos indicam que extratos dessa espécie possuem propriedades antioxidantes e antimicrobianas, apontando para possíveis benefícios em pesquisas futuras (Wang et al. 2017). A jurubeba (*Solanum paniculatum*), embora considerada uma planta daninha, possui histórico de uso na medicina popular, evidenciando a complexidade das relações entre plantas e sociedade (Medeiros et al. 2018)

Além disso, a família Solanaceae não se restringe apenas à alimentação e medicina. Muitas de suas espécies são cultivadas por suas flores ornamentais, como a petúnia (Petunia spp.), que é apreciada em jardins e paisagismo (Hančinský et al. 2020).

1.1 Tomateiro: origem, produção e importância econômica no mundo

O tomate (*Solanum lycopersicum* L) é nativo das regiões andinas da Colômbia, Chile, Peru, Ilhas Galápagos, Equador e Bolívia (Peralta et al. 2007, Sims 1980). A domesticação da cultura ocorreu no México nas cidades de Puebla e Veracruz (Long 2013), e sua distribuição aconteceu por meio dos europeus no ano de 1544 em que a cultura foi difundida pelas regiões da Ásia, África e Oriente Médio (vam Dam et al. 2006).

No contexto global a produção mundial atual é de cerca de 100 milhões de toneladas (mT) de frutas frescas por hectare (FAOSTAT 2021). O mercado produtor de tomate é comandado pela China, que no ano de 2021 produziu cerca de 67,5 mT/ha, ou seja, 48% da produção mundial total, sendo o país líder no ranking de produção, seguido por Índia (21,1 mT/ha), Turquia (13 mT/ha), Estados Unidos da América (10,4 mT/ha), Itália (6,6 mT/ha), Egito (6,2 mT/ha), Espanha (4,7 mT/ha), México (4,1 mT/ha), Brasil (3,6 mT/ha) e Nigéria (3,6 mT/ha) (Faostat 2021) (**Figura 1**).
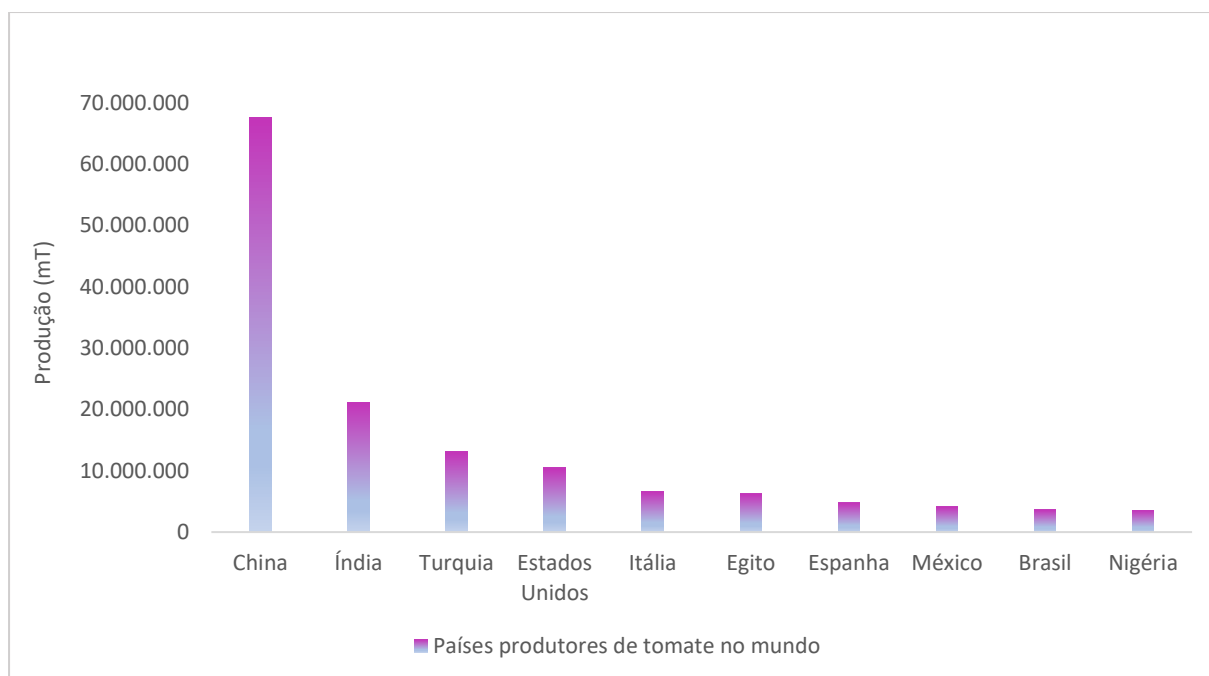
Figura 1: Os dez maiores produtores de tomate do mundo (Faostat 2023).

O tomate é uma das culturas mais importantes do Brasil, com um volume de produção de aproximadamente 3,9 milhões de toneladas por ano (IBGE 2023), sendo o estado de São Paulo o maior produtor com 1.039,7 mil toneladas (t), seguido por Goiás (1. 1021,7 t), Minas Gerais (536,4 t), Paraná (238,8 t), Ceará (183,5 t), Bahia (179,6 t), Rio de Janeiro (136,6 t). Em 2020, o Brasil exportou 104.416 toneladas de tomate fresco, com um valor total de US$ 50,4 milhões (ComexStat 2021). A indústria de tomate processado no Brasil movimenta aproximadamente R$ 4,7 bilhões (US$ 867 milhões), empregando mais de 10.000 pessoas (ABRE 2021).

São Paulo é o estado que se destaca pela produção de tomate industrial (**Figura 2**), cujo produto são molhos e extratos, correspondendo aproximadamente por 27% da produção nacional, seguido do estado de Goiás com produção de 26%, já os outros estados destacam-se pela produção para consumo *in natura* (Treichel 2016, IBGE 2023). Apesar de São Paulo ser hoje o principal produtor, a produção brasileira de tomate para industrialização começou de fato em Pernambuco, no município de Pesqueira, no final do século XVlll (Silva et al. 2006).
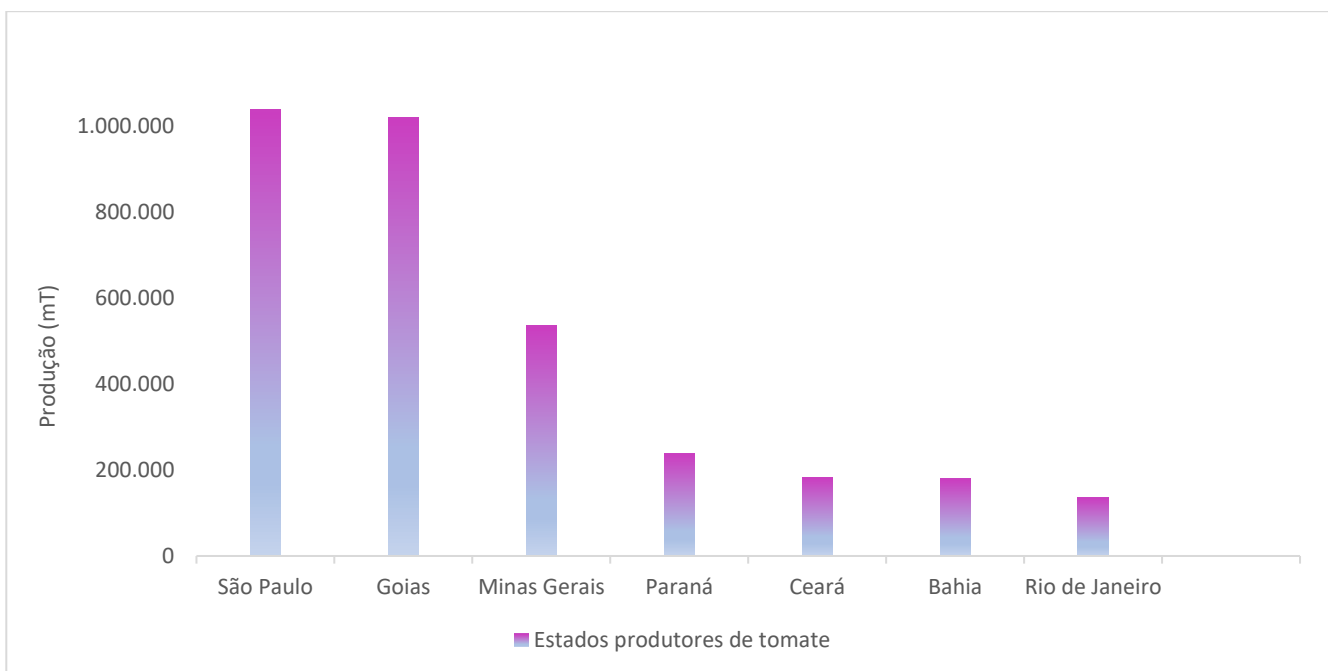
Figura 2: Maiores produtores de tomate do Brasil (IBGE 2023).

Os agricultores frequentemente enfrentam dificuldades com pragas e doenças, condições climáticas desfavoráveis e concorrência de tomates importados (Figueiredo et al. 2008). Estudos vêm sendo realizados por programas de melhoramento com o objetivo aprimorar o desempenho do tomate para otimizar a produção, garantir a qualidade do produto e reduzir custos (Acharya et al. 2018).

1.3 Plantas da família Solanaceae como hospedeiras de patógenos

O tomateiro pode ser afetado por diversas doenças, incluindo aquelas causadas por bactérias, fungos, nematoides e vírus (Lopes et al. 2021). Dentre as doenças bacterianas do tomateiro inclui-se a *Clavibacter michiganensis* subsp. *michiganensis* causadora do cancro bacteriano, a mais importante doença bacteriana desta cultura (Lopes 2017, Panno 2021). Encontra-se também a *Ralstonia solanacearum* causadora da murcha bacteriana, e a *Pseudomonas syringae* pv. tomato, em que apresenta manchas arredondadas de coloração marrom-escura nas folhas (Lopes et al. 2021).

As doenças fúngicas desta cultura incluem a murcha de fusário, causada pelo fungo *Fusarium oxysporum* f. sp. *lycopersici*, e a murcha de verticílio, causada pelo fungo *Verticillium dahliae* (Srinivas et al. 2019, Reis et al. 2006). Outros importantes patógenos são a *Alternaria solani,* responsável por causar a pinta preta (Peixoto et al. 2021), *Botrytis cinerea* causador da podridão cinzenta (Nakajima M, Akutsu K 2014) e *Septoria lycopersici* agente etiológico da septoriose (Panno et al. 2021).

Os nematoides relatados causadores de grandes perdas são frequentemente classificados em *Meloidogyne* spp. (conhecido como nematoides das galhas) e *Pratylenchus* spp. (conhecido como nematoides das lesões radiculares) (Lopes 2021). Há outros gêneros também relatados na cultura do tomate como *Belonolaimus* ssp. e *Rotylenchulus* ssp., porém com menos frequência (Pinheiro et al. 2014).

Os vírus são fitopatógenos que se destacam pelas grandes perdas causadas ao longo dos anos. No Brasil, os principais vírus que afetam as culturas de tomate são classificados nos gêneros *Begomovirus*, *Orthotospovirus*, *Crinivirus*, *Tobamovirus* e *Potyvirus* (Inoue-Nagata et al. 2016b, Mituti et al. 2019). Entretanto, doenças causadas por begomovírus família *Geminiviridae* merecem destaque especial no Brasil pois induzem sintomas graves e ocorrem com maior frequência causando perdas de rendimento (Rivarez et al. 2023).

Tomato severe rugose virus (ToSRV) (Duarte et al. 2021, Reis et al. 2020 2021) e tomato mottle leaf curl virus (ToMoLCV), ambos classificados no gênero *Begomovirus*, são amplamente relatados em tomateiros e causam sintomas como manchas cloróticas, clorose internerval, manchas, mosaico, distorção foliar e nanismo **(Figura 3)** (Souza et al. 2022). Algumas espécies cultivadas de Solanaceae, como o tomate, crescem lado a lado com espécies silvestres da família, algumas das quais são plantas daninhas frequentes (Singh et al. 2016). As plantas daninhas, são componentes importantes dos ecossistemas agrícolas por atuarem muitas vezes na conservação e recuperação do solo, entretanto podem dificultar o crescimento

das culturas ao competir com as plantas por água, nutrientes e luz solar, atuando também como hospedeiras de patógenos, o que resulta em grandes perdas na produção agrícola (Monteiro et al. 2022, Salem et al. 2022).

Atualmente o número de vírus encontrados em plantas daninhas vem crescendo exponencialmente, e acredita-se que existam muitos outros a serem identificados e descritos (Susi et al. 2019). Dentre as plantas daninhas comumente encontradas em campos de cultivo de tomate e culturas de menor expressão pode ser infectadas por diversas espécies de begomovírus, como:

1. Blainvillea yellow spot virus em *Physalis* (L) (Rocha et al. 2013),
2. Physalis yellow spot virus em *Physalis* (L) (Kitajima 2020),
3. Tomato rugose mosaic virus em *Solanum melongena* (L) (Kitajima 2020),
4. Tomato yellow spot virus em *Capsicum annuum* (L) (Kitajima 2020) e
5. Tomato yellow spot virus em *Nicandra physalodes* (L) Gaertn. (Kitajima 2020).

Portanto, a caracterização de begomovírus em hospedeiros não cultivados fornece informações sobre a possível ameaça de novas epidemias (Rivarez et al. 2021).
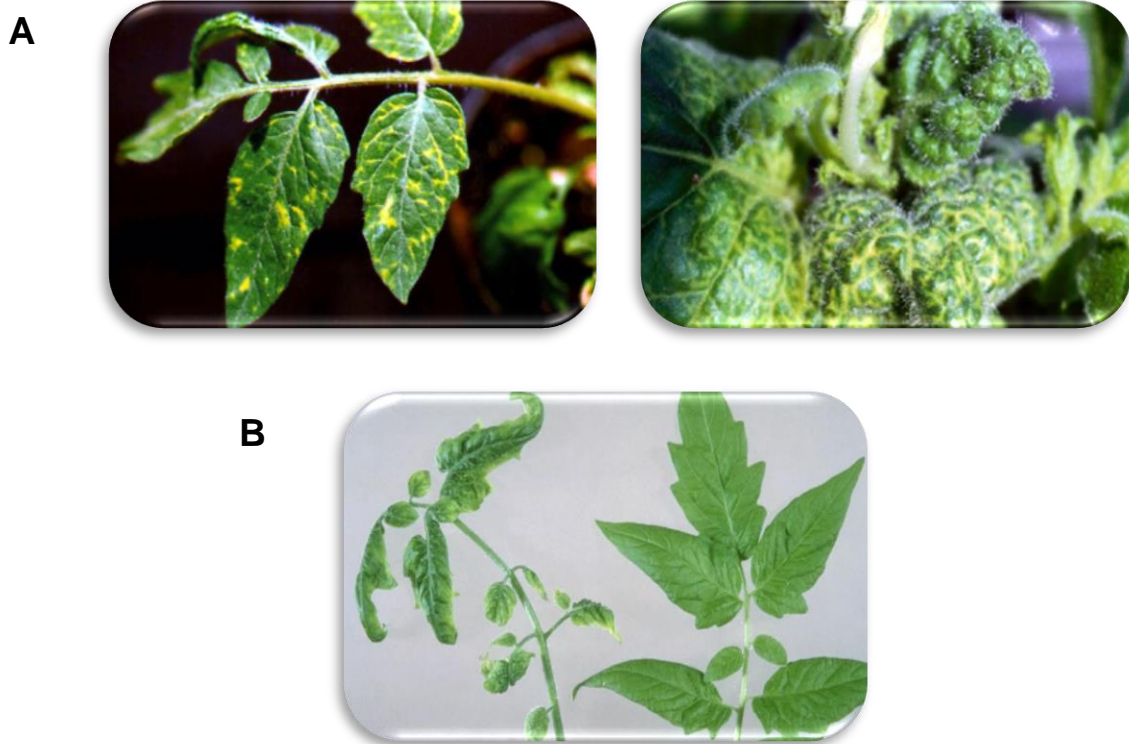
Figura 3: Sintomas causados por begomovirus em tomateiro, como mosaicos, manchas no limbo foliar, amarelecimento, enrolamentos e/ou deformação das folhas, redução no crescimento da planta e deformações nos frutos. (**A)** tomato golden mosaic virus (TGMV) e **(B)** tomato yellow leaf curl (TYLC).

1.4 Família *Geminiviridae*

Geminiviridae é uma família de vírus altamente diversa cujo membros afetam uma ampla gama de hospedeiras, sendo composta por quatorze gêneros: *Becurtovirus, Begomovirus, Capulavirus, Citlodavirus, Curtovirus, Eragrovirus, Grablovirus, Maldovirus, Mastrevirus, Mulcrinelvirus, Opunvirus, Topilevirus, Topocuvirus* e *Turncurtovirus* contendo aproximadamente 520 espécies virais (**Tabela 4**) (ICTV 2023, Zerbini et al. 2017). Os critérios de demarcação para cada um destes gêneros incluem a gama de hospedeiros, insetos vetores, organização genômica e análises filogenéticas (Zerbini et al. 2017, Roumagnac et al. 2022).

Vírus classificados na família *Geminiviridae* possuem genoma de DNA circular de fita simples (ssDNA), sendo relativamente pequenos com cerca de 2,5 a 3,0 kb de tamanho. A estrutura morfológica da partícula é composta por um capsídeo icosaédrico geminado (ICTV 2023). A estrutura genômica da partícula consiste em monopartido, apenas com um DNA genômico - A ou bipartido composto por dois componentes distintos denominados DNA–A e DNA–B (Varsani et al. 2017). Na organização genômica destes vírus encontram-se de cinco a sete genes no componente DNA–A: ORF AV1 (capa proteica - CP), ORF AV2 (proteína de movimento - MP), em sentido viral. Já em sentido complementar, os genes: ORF AC1(Rep – gene que codifica proteína associada a replicação), ORF AC2, (TrAP – gene que codifica a proteína ativadora de transcrição), ORF AC3 (REn – gene que codifica a proteína intensificadora de replicação), ORF AC4 (C4) responsável pelo desenvolvimento de sintomas, movimentação viral e replicação (Rigden et al. 1994, Rojas et al. 2001, Medina – Puche et al. 2021), ORF AC5 (função está associada à patogenicidade e supressão do silenciamento gênico (Li et al. 2015, Zhao et al. 2022). O DNA–B, cujos genes são: ORF BV1 (NSP) e ORF BC1 (MP) no sentido complementar (Li et al. 2021).

Dentre os gêneros desta família, os begomovírus são os mais importantes em termos de números de espécies, sendo associados a sintomas que incluem mosaico, enrolamento de folhas, nanismo, clorose e necrose (Varsani et al. 2014).

Tabela 1: Característica dos gêneros da família *Geminiviridae* contemplando genoma, gênero viral, tipos de vetores e origem de replicação e ORFs.

Genoma	Gênero viral (NE)	Vetor	Origem de replicação (N)[2]	Região intergênica (RI)[2]	ORFs[3]	Referências
Monopartido/bipartido	*Begomovirus* (445)	*Bemisia tabaci*	5'-TAATATTAC-3'	**Uma** região intergênica (RIL) e **uma** região comum (RC) com DNA-B	AV1, AV2, AC1, AC2, AC3, AC4, AC5 BV1 e BC1.	Fiallo-Olivé et al. 2020; ICTV 2023
Monopartido	*Maldovirus* (3)	Não se sabe	5'-TAATATTAC-3'	**Um**a região intergênica (RI)	V1, V2, C1, C2, C3 e C4	Roumagnac et al. 2021; ICTV 20223
	Mastrevirus (45)	*Cicadulina mbila e C. storeyi*	5'-TAATATTAC-3'	**Duas** regiões intergênicas (RIL e RIC)	V1, V2, C1 e C2	Batista et al. 2021; ICTV 2023
	Eragrovirus (1)	Não se sabe	5'-TAA**G**ATT**CC** -3'	**Duas** regiões intergênicas (RI-1 e RI-2)	V1, V2, C1 e C2	Varsani et al. 2014b ICTV 2023
	Becurtovirus (3)	*Circulifer haematoceps*	5'-TAA**G**ATT**CC** - 3'	**Duas** regiões intergênicas (RIL e RIC)	V1, V2, V3, C1 e C2	Varsani et al. 2014a; ICTV 2023
	Topocuvirus (1)	*Micrutalis maleifera*	5'-TAATATTAC 3'	**Uma** região intergênica (RI)	V1, V2, C1, C2, C3 e C4.	Briddon et al. 1996; ICTV, 2022
	Capulavirus (4)	*Aphis craccivora e Dysaphis plantaginea*	5'-TAATATTAC-3'	**Duas** regiões intergênicas (RIL e RIC)	V1, V2, V3, V4, C1, C2 e C3.	Ryckebusch et al. 2020; ICTV 2023
	Citlodavirus (4)	Não se sabe	5'-TAATATTAC-3'	**Duas** regiões interagências (RIL e RIC)	V1, V2, V3, C1 e C2.	Qiu et al. 2020; ICTV 2023
	Curtovirus (3)	*Circulifer tenellus*	5'-TAATATTAC-3'	**Duas** regiões interagências (RIL e RIC)	V1, V2, V3, C1, C2, C3 e C4.	Hanley-Bowdoin et al. 2013; ICTV 2023

Grablovirus (3)	*Spissistilus festinus*	5'-TAATATTAC-3'	**Duas** regiões interagências (RIL e RIC)	V1, V2, V3, C1, C2 e C3.	Bahder et al. 2016; ICTV 2023
Mulcrilevirus (2)	*Tautoneura mori*	5'-TAATATTAC-3'	**Uma** região intergênica (RI)	V1, V2, V3, V4, C1 e C2.	Lu et al. 2021; ICTV 2023
Opunvirus (1)	*Dactylopius* sp.	5'-TAATATTAC-3'	**Uma** região intergênica (RI)	V1, V2, C1, C2, C3, C4.	Fontenele et al. 2020; ICTV 2023
Topilevirus (2)	Não se sabe	5'-TAATATTAC-3'	**Duas** regiões intergênicas (RIL e RIC)	V1, V2, V3, C1, C2 e C3.	Roumagnac et al. 2021; ICTV 2023
Turncurtovirus (3)	*Circulifer haematoceps*	5'-TAATATTAC-3'	**Uma** região intergênica (RI)	V1, V2, C1, C2, C3 e C4.	Hasanvand et al. 2018; ICTV 2023

[1] NE: número de espécies correspondente a cada gênero.

[2] N – nonanucleotídeo (nucleotídeos que diferem entre os gêneros estão destacados em negrito), RI – região intergênica, genoma (região intergênica longa – RIL; região intergênica curta – RIC);

[3] As ORFs (open reading frames) são indicadas como sendo codificadas no sentido viral = V ou sentido complementar = 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;

1.5 Gênero *Begomovirus*: histórico, importância e organização genômica

Begomovirus corresponde ao maior gênero de vírus de plantas pertencentes com 445 espécies (ICTV 2023). Historicamente, o primeiro relato de doença causada por begomovírus no Brasil foi causada pelo tomato golden mosaic virus (TGMV) um begomovírus bipartido do Novo Mundo, no estado de São Paulo em meados da década 1950 (Flores et al. 1960).

Este grupo viral causa uma ampla variedade de sintomas em seus hospedeiros, incluindo mosaicos, manchas no limbo foliar, amarelecimento, enrolamentos e/ou deformação das folhas, redução no crescimento da planta e deformações nos frutos. Desta forma os begomovírus se encontram em alguns casos causando grandes perdas de produção em hortaliças, raízes e fibras em campos abertos em regiões tropicais (Fiallo-Olivé Navas - Castillo 2023). Exemplos de doenças economicamente importantes causadas por begomovírus incluem (i) vírus do mosaico africano da mandioca (african cassava mosaic virus (ACMV)), sendo um dos fatores limitantes da produção de mandioca na África subsaariana (Leg et al. 2021) já no Brasil é enquadrado como Praga Quarentenária Ausente (A1) (EMBRAPA 2019); (ii) vírus do enrolamento da folha amarela do tomate (tomato yellow leaf curl virus (TYLCV)) afeta plantações de tomate em todo o mundo (Yan et al. 2021) e (iii) o vírus do enrolamento da folha do tomateiro de Nova Delhi (tomato leaf curl New Delhi virus (ToLCNDV)) atinge principalmente cucurbitáceas nas regiões Indo-Paquistão e na bacia do Mediterraneo (DVAG 2022).

Os *Begomovirus* encontram-se classificados em Velho Mundo (VM) (África, Ásia, Australásia e Europa), caracterizando-se por possuírem genomas em sua maioria monopartidos, com apenas alguns bipartidos, e Novo Mundo (NM) (Américas) caracterizando-se por apresentarem genoma majoritariamente bipartidos, com alguns monopartidos (Brown et al. 2015, Navas-Castillo & Fiallo-Olivé, 2020). Os begomovírus do Novo Mundo diferenciam-se dos begomovírus do Velho Mundo pela ausência da ORF AV2 (Zerbini et al. 2017, ICTV 2022). De acordo com Torres-Herrera et al (2019) relataram que os ancestrais dos begomovírus do Novo Mundo teriam sido introduzidos nas Américas a partir do Alasca, causando numerosas linhagens secundárias de begomovírus no curso da radiação evolutiva.

Begomovírus bipartidos são constituídos por duas estruturas de DNA circular de fita simples (ssDNA) chamadas DNA–A e DNA–B cada um com 2,5 kb a 2,6 kb (Hanley-Bowdoin et al. 2000, Brown et al. 2015). Nos begomovírus bipartidos encontram-se de cinco a seis genes no componente DNA–A. Estes genes são, no sentido viral, a **AV1/V1** que codifica a proteína capsidial (CP), sendo essencial para o empacotamento, infecção e transporte do vírus no inseto

vetor; **AV2/V2** gene que codifica a proteína de movimento viral (Hanley-Bowdoin et al. 2000, Zhao et al. 2020). V2 também funciona como supressor do silenciamento gênico pós transcricional (SGPT) e silenciamento gênico transcricional (SGT), entretanto este gene não é encontrado em begomovírus pertencentes ao Novo Mundo (Zrachya et al. 2007, Wang et al. 2018, Wang et al. 2022). No sentido complementar tem-se os genes **AC1/C1** que funciona como codificador proteína associada a replicação (Rep) (Hanley-Bowdoin et al. 2000, Fiallo-Olivé et al. 2021); **AC2/C2** gene que codifica a proteína ativadora de transcrição (Trap) sendo essencial para patogenicidade viral (Van et al. 2001, Cao et al. 2023). Além disso, AC2 pode interferir nas respostas de defesa da planta sendo supressora do ácido jasmônico (Rosas-díaz et al. 2016); **AC3/C3** codifica a proteína intensificadora de replicação (REn) aumentando assim o acúmulo de DNA viral (Hanley-Bowdoin et al. 2000, Wu et al. 2021); **AC4/C4** é a menor ORF descrita e possui diversos papeis como desenvolvimento de sintomas, movimentação viral e replicação (Rigden et al. 1994, Rojas et al. 2001, Medina – Puche et al. 2021). Recentemente, estudos mostraram que alguns begomovírus bipartidos também codificam o gene **AC5/C5**, cuja função está associada à patogenicidade e supressão do silenciamento gênico (Li et al. 2015, Zhao et al. 2022). Além destes, o gene **AC6/C6** sobrepondo-se parcialmente às ORFs AV1/A1 e AV2/ V2 codifica um polipéptido de 97 aminoácidos sendo codificada pelo tomate leaf curl China virus (ToLCCNV) foi encontrada especificamente nas mitocôndrias (Wang et al. 2022). Estudos atuais descobriram o novo gene **AC7/C7** sendo codificador de proteínas envolvidas no fator de patogenicidade e supressão do silenciamento de RNA, e que desempenha papel crítico durante a infecção por tomato yellow leaf curl virus (TYLCV) (Liu et al. 2023).

O componente DNA–B possui dois genes que codificam proteínas que estão ligadas ao movimento viral. Os genes, em sentido viral, **BV1** (gene responsável por codificar a proteína de movimento dentro do núcleo - NSP) e a **BC1** (proteína de movimento - MP) (Hanley-Bowdoin et al. 2000, Hanley-Bowdoin et al 2013, Melgarejo et al. 2013). As ORFs dos begomovírus monopartidos incluem os genes **AV1** e **AV2** de sentido viral e os genes de sentido complementar **C1, C2, C3, C4, C5, C6 e C7** (**Figura 3**) *(*Zerbini et al. 2017, Zhao et al. 2022, Wang et al. 2022, Liu et al. 2023).
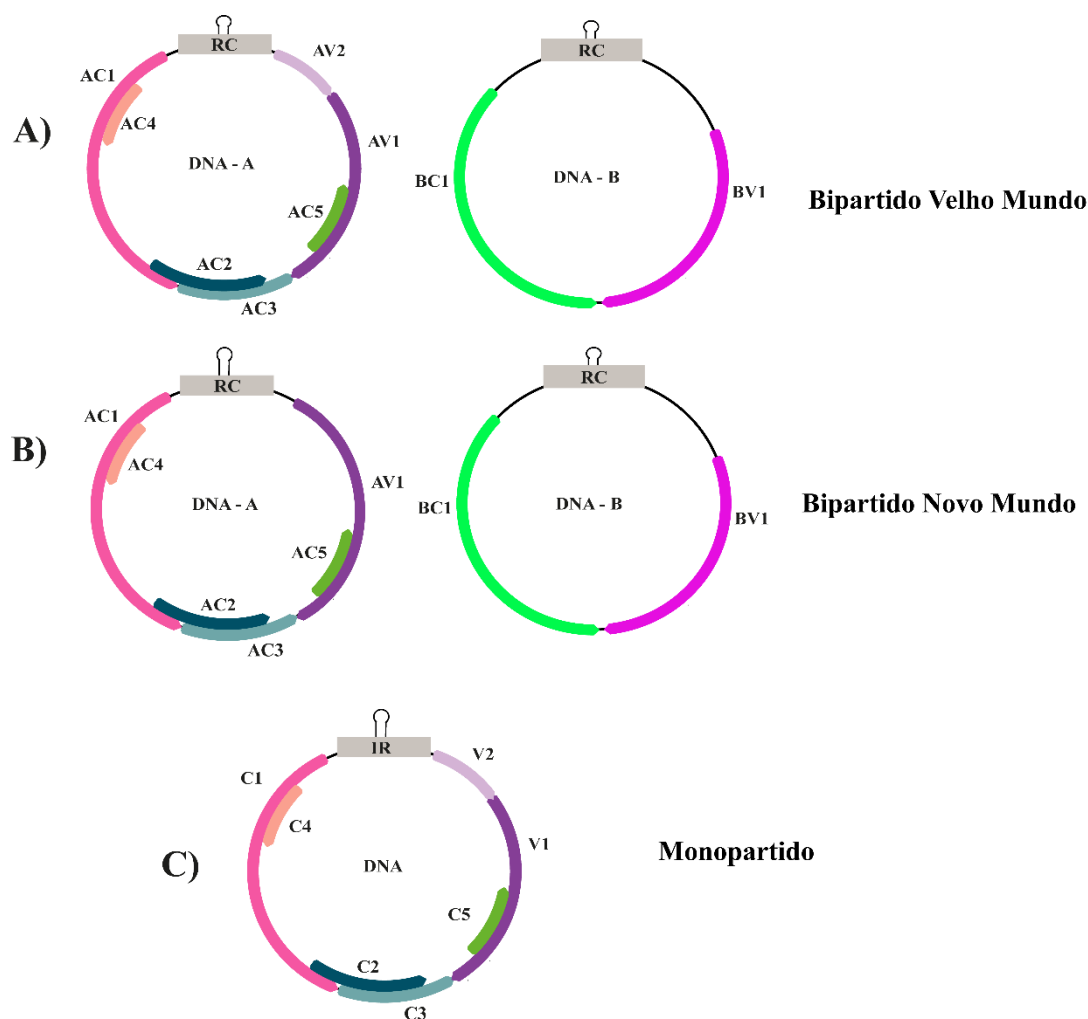
Figura 3: Representação esquemática dos tipos de genomas do gênero *Begomovirus*. **(A)** DNA−A de begomovírus bipartidos do Velho Mundo, cujos genes são: ORF AV1 (capa proteica - CP), ORF AV2 (proteína de movimento - MP), em sentido viral. Já em sentido complementar, os genes: ORF AC1(Rep), ORF AC2, (TrAP), ORF AC3 (REn), ORF AC4 (C4), ORF AC5 (C5). O DNA-B, cujos genes são: ORF BV1 (NSP) e ORF BC1 (MP) no sentido complementar. São mostrados os stem-loops conservados (RC). **(B)** DNA−A de begomovírus bipartidos do Novo Mundo, cujo genoma não tem ORF AV2. **(C)** Genoma de begomovírus monopartido. Figura gerada pela autora.

Abreviação: ORF, quadro de leitura aberta.

Os dois componentes (DNA–A e DNA–B) compartilham entre si uma sequência que varia de 140 a 200 nts de comprimento dentro da região comum (RC), sendo este o ponto de partida para o processo de replicação viral. Na RC há a alça conservada com a sequência 5'-TAATATTAC-3' presente em quase todos os membros da família *Geminiviridae* (Fiallo–Olivé et al. 2021). É também nesta região que se encontram o sítio de clivagem da proteína Rep, o TATABOX e as sequências repetidas específicas dos vírus (iterons) em que a REP se liga a motivos específicos no DNA viral (início da replicação). Os subdomínios conservados (regiões

conservadas em vírus com iterons idênticos) foram denominados "Domínios relacionados ao Iteron (IRD-Rep)" com o objetivo de permitir os agrupamentos de begomovírus com iterons iguais em dois grupos, espécies do Velho Mundo e espécies do Novo Mundo (Arguello Astorga et al. 2001, Arguel Astorga et al. 2004).

Anos depois, Cantu-Iris et al. (2019) realizaram estudos mais aprofundados no tange a caracterização molecular de begomovírus. Neste trabalho foi possível confirmar a existência de uma região conservada entre a origem de replicação e o códon de início do gene da capa proteica (CP). O mesmo trabalho apresentou em um vírus do Novo Mundo (Blechum interveinal chlorosis virus), um segmento de DNA quase palindrômico exibindo um núcleo ACTT– (N7) –AAGT fortemente conservado em comprimento, porém sua sequência é altamente variável entre as espécies virais; uma sequência heptanucleotídica rica em GC e variável quando em sequência; em vírus do Velho Mundo a região CP homologa aos begomovírus exibiu uma organização diferente, com a região TATA–box sobrepondo a metade esquerda do elemento ACTT–N7. Sequências semelhantes ao promotor da capa proteica (CP), foi denominado TACE (TATA–associated composite element) sendo encontrado em diferentes gêneros da família *Geminiviridae*.

Em decorrência da ampla gama de espécies e o surgimento de novos begomovírus, fez se necessário o estudo de novas formas de classificação, sendo assim Brown et al. (2015) realizou um extenso estudo a fim de tornar única a classificação taxonômica deste grupo. De acordo com os critérios estabelecidos por Brown et al. (2015), uma nova espécie, seja ela monopartida ou bipartida, deve conter os níveis de identidade do genoma DNA-A completo inferior a 91% quando comparado a outra espécie qualquer de begomovírus já conhecida. Entretanto, se o vírus compartilha níveis de identidade acima de 91% e abaixo de 94% com genoma completo para todos os isolados descritos para aquela espécie, ele é classificado como nova estirpe.

1.6 *Bemisia tabaci* (Middle East Asian Minor - MEAM 1 (=biotipo B) como vetor de begomovírus em tomateiro.

Os primeiros relatos no Brasil de infecções causadas por begomovírus foram feitos entre as décadas de 1950 e 1970 (Flores et al. 1960, Matyis et al. 1975). A partir da década de 1990 os membros de begomovírus causadores de doenças não eram comuns, entretanto, neste mesmo ano, simultaneamente à entrada no país de *Bemisa tabaci* (grupo genético MEAM-1

antigo biótipo B) foi observado o aumento nos casos de begomoviroses (Ribeiro et al. 2003, Fernandes et al. 2008, Gilbertson et al. 2015).

A transmissão dos begomovírus está associada a um complexo formado por 11 grupos contendo espécies crípticas de *Bemisia tabaci* (Hemiptera: Aleyrodidae), popularmente conhecida como mosca branca (Ghosh et al. 2021). Entres estes grupos, o Oriente Médio-Ásia Menor 1 (MEAM1, antigo biótipo B), Mediterrâneo (MED, antigo biótipo Q) e Novo Mundo (NW, antigo biótipo A) são encontrados na América do Sul e são considerados as mais invasivas dentro deste complexo (Krause-Sakate et al. 2020, Watanabe et al. 2019). Com base na filogenia molecular do gene mitocondrial do citocromo oxidase I (mtCOI), foi proposto que *B. tabaci* consiste em um complexo de mais de 40 espécies crípticas (morfologicamente indistinguíveis) (Xavier et al. 2021) sendo o limite de 3,5% de divergência tem sido usado como critério de demarcação de uma nova espécie dentro deste complexo (Dinsdale et al. 2010, De Barros et al. 2005, Barbosa et al. 2015).

A interação entre vírus e inseto vetor é caracterizada por ser circulativa não propagativa (De Barro et al. 2011) entretanto estudos de transmissão do tomato yellow leaf curl virus (TYLCV) tem mostrado que os begomovírus são aptos a também se replicarem no inseto vetor, em condições específicas (Pakkianathan et al. 2015). O ciclo de infecção dos begomovírus no inseto vetor é dívida em fase de aquisição, em que a mosca branca insere o estile no tecido do floema e assim adquire o vírus, que por sua vez se move para o intestino médio do inseto; e fase de latência, quando os begomovírus acumulam-se no intestino por um longo período, após essa fase o vírus circula por todo corpo do vetor (Czosnek et al. 2017, Roy et al. 2021). Em aproximadamente 7h o vírus se move para glândulas salivares por endocitose a partir daí é inoculado em uma planta hospedeira durante a alimentação (Czosnek et al. 2017).

A *B. tabaci* como vetor de doenças virais principalmente em tomate, é de difícil manejo. Isso se deve às elevadas taxas de crescimento populacional da praga, à rápida evolução da resistência a inseticidas e a localização protegida dos indivíduos na face inferior das folhas (Krause-Sakate et al. 2020). Entretanto, estudos exploratórios com objetivo de desenvolver novas ferramentas de controle são desenvolvidos diariamente. Características físicas de resistência como compostos tóxicos em vacúolos ou tricomas glandulares desempenham papel importante na defesa das plantas contra à mosca branca (Li et al. 2023, Diaz et al. 2021). Em *Solanum* spp, os tricomas glandulares dos tipos IV e VI estão associados a altos níveis de resistência a diversas espécies de *B. tabaci* (Rodrigues – Lopes et al. 2012, Diaz et al. 2021). Outras características físicas como folhas estreitas e mais finas foram associadas ao aumento da resistência à mosca branca em variedades de tomate (Pal et al. 2021). Características

químicas também são grandes aliada no controle de mosca branca. Estudos relataram uma correlação positiva entre componentes fenólicos e a resistência à mosca-branca em berinjela e tomate (Pal et al. 2021). Outros forma de controle é a ativação de proteínas de defesa, o que pode interromper os processos fisiológicos normais dos insetos, incluindo a digestão e absorção de nutrientes (Li et al. 2023)

1.7 Mecanismos de variabilidade e diversidade genética de begomovírus.

É sabido que os begomovírus possuem mecanismos que os permitem ter uma ampla variabilidade genética, devido a diferenças em uma ou mais características do organismo (Bhandari et al. 2017). Estes mecanismos que culminam no surgimento de novas espécies são: mutações, recombinações e pseudo recombinação (Roossinck 1997, Seal et al. 2006).

As mutações ocorrem devido a incorporação incorreta de nucleotídeos durante a replicação viral, gerando variabilidade antes da troca de fragmentos genômicos associados a eventos de recombinação (Roossinck 1997, Seal et al. 2006, Duffy and Holmes 2008). Estudos recentes mostram mutações na proteína capsidial (CP) em isolados de TYLCV do Velho Mundo, em que mostram mutações na serina (aminoácido da CP) causando uma nova conformação de hélice. Consequentemente, esta mutação pode conferir mudanças na interação vetor-planta tendo uma relação direta na infectividade do vírus e possa ser consistente com a rápida propagação em diferentes regiões da Europa e da Ásia (78%) (Nigam et al. 2023).

A recombinação é a troca de fragmentos genômicos entre duas cadeias de DNA durante a replicação (García-Arenal et al. 2003). A recombinação genética em vírus permite que parentais transmitam informações genéticas para os seus recombinantes, desta forma possibilita uma evolução da organização genômica de tal modo a maximizar a capacidade adaptativa e reduzir efeitos deletérios nesses vírus (Lefeuvre & Moriones 2015). Desta forma, em uma análise para identificar possíveis eventos de recombinação, utilizando o software RDP4 (Martin et al. 2020), Fan et al. (2023) inferiu o surgimento de um novo begomovírus, o tomato yellow leaf curl Shuangbai virus (TYLCSbV), sendo provavelmente originado da recombinação do vírus ageratum yellow vein China virus (AYVCNV) e tobacco curl shoot virus (TbCSV). Este evento pode estar relacionado com a eficiência de transmissão pelo vetor *B. tabaci* (Fan et al. 2023).

A pseudorecombinação pode ser definida como uma troca de segmentos homólogos entre dois isolados ou espécies de vírus relacionados durante a infecção mista na célula hospedeira (Sicard et al. 2016, Chen et al. 2021). O efeito da pseudorecombinação foi

demonstrado por Chen et al. (2021), com o squash leaf curl China virus (SLCCNV) e tomato leaf curl New Delhi virus (ToLCNDV), sendo que a pseudorecombinação natural foi confirmada por agroinoculação em curcubitáceas. Ainda no trabalho de Chen et al. (2021), este fenômeno demonstrou que apenas um pseudorecombinate natural de ToLCNDV pode infectar tomate com sucesso, sendo ele o ToLCNDV–A com SLCCNV-B. No entanto, ambos os isolados naturais de ToLCNDV e SLCCNV não apresentaram boa resposta infectiva ao tomate, sendo assim, o componente DNA–B de outro vírus é o determinante responsável pela infecção do tomateiro.

Os begomovírus exibem altas taxas de mutações, recombinação e pseudorecombinação, tanto dentro quanto entre espécies virais, resultando na rápida evolução adaptativa de novas variantes e novas espécies (Rocha et a. 2013, Silva et al. 2014, Souza et al. 2020). Estes fenômenos ocorrem majoritariamente em decorrência da distribuição cosmopolita do inseto vetor, *Bemisia tabaci* (mosca branca) que ao se deslocarem podem encontrar diversas plantas hospedeiras e adquirir vírus durante o processo, o que resulta em infecção única ou mista em plantas suscetíveis encontradas posteriormente (Adkins et al. 2011, Gautam et al. 2023)

O primeiro levantamento nacional de espécies de begomovírus foi realizado por Ribeiro et al. (2003), em que foram coletados 23 isolados entre 1994 e 1999 em campos localizados nas regiões Centro-Oeste, Sudeste e Nordeste. Neste estudo, pelo menos sete novas espécies foram reportadas no país entre elas ToCMoV e ToRMV. Em levantamentos dos últimos cinco anos, Macedo et al. (2018) detectaram um novo vírus, ToLCPVV, infectando tomateiro no Nordeste do Brasil. Com a utilização de HTS, Rego-Machado et al. (2019) detectaram um novo begomovírus bipartido ToICV2 em amostras de tomateiro no estado de Goiás. Mituti et al. (2019) relataram a presença de quatro begomovírus já previamente reportados: ToSRV, ToCmMV, ToMoLCV e ToYVSV em um levantamento nos estados do Nordeste, Centro-Oeste e Sudeste. Reis et al. 2020 realizaram um levantamento com a utilização de HTS, e identificaram a presença de três novos begomovírus em tomates no Brasil. Martins et al. (2021), detectaram uma nova espécie infectando tomateiro no Centro-Oeste brasileiro e propuseram o nome tomato mottle leaf distortion virus (ToMoLDiV). Reis et al. 2023 realizaram um levantamento em que dois novos begomovírus infectando tomate foram descobertos, sendo eles tomato golden net virus (ToGNV) e tomato yellow net virus (ToYNV).

Em levantamentos mais recentes, feitos com base em dados do GenBank (2023), Host Data Base (2023) e Kitajima et al. (2020), mais de 300 vírus foram relatados infectando a

cultura do tomate mundialmente, sendo o gênero *Begomovirus*, com 361 representantes virais (**Figura 3**), sendo 30 deles encontrados da cultura do tomateiro (**Figura 4**).
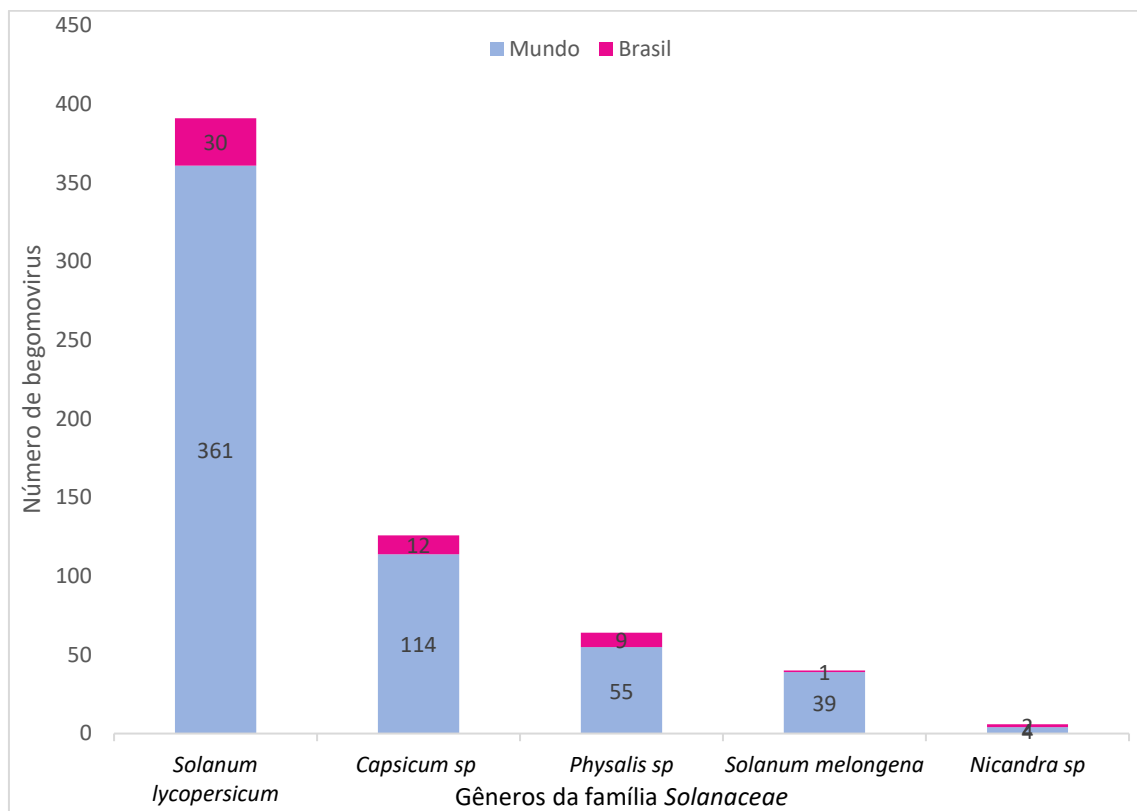
Figura 3: Número de *Begomovirus* relatados em plantas silvestres ou culturas de menor expressão da Família Solanaceae em todo o mundo e Brasil, de acordo com a base de dados do GenBank (2023), Host DataBase (2023) e Kitajima (2020). A maior concentração de begomovirus relatados se encontram em tomateiro (*Solanum lycopersicum*), com 362 espécies no mundo e com 28 encontrada no Brasil. Dentre as plantas daninhas, silvestres e culturas de menor expressão o maior número de begomovirus está relacionado aos gêneros *Capsicum* spp, com 144 espécies no mundo e 12 no Brasil, seguida de *Physalis* spp, *Solanum melongena* e *Nicandra* sp.

Tabela 2: Distribuição regional de 30 espécies de begomovírus infectando o tomateiro (*Solanum lycopersicum*) no Brasil

	Espécie viral	Estado da Federação	Referências
1	*Chino del tomate Amazonas virus*	AM	Fonseca et al. (2011)
2	*Cleome leaf crumple virus*	GO	Fontenele et al. (2017)
3	*Euphorbia yellow mosaic virus*	DF, GO, MG, RS, RJ e SP	Barreto et al. (2013); Macedo et al. (2018); Duarte et al. (2020)
4	*Sida micrantha mosaic virus*	MG, DF e GO	Calegario et al. (2004); Reis et al. (2020)
5	*Sida mottle virus*	SP	Cotrim et al. (2007)
6	*Sida yellow net virus*	AM & RJ	Fernandes (2015)
7	*Tomato bright yellow mosaic virus*	BA	Fonseca et al. (2013)
8	*Tomato bright yellow mottle virus*	TO	Fonseca et al. (2013)
9	*Tomato chlorotic leaf curl virus*	PA	Quadros et al. (2019)
10	*Tomato chlorotic mottle Guyane virus²*	DF	Pereira – Carvalho et al. (2019)
11	*Tomato chlorotic mottle virus*	BA, MG, DF, ES, PE e RJ	Ribeiro et al. (2003); Ribeiro et al. (2007)
12	*Tomato common mosaic virus*	MG, RJ e ES	Castillo-Urquiza et al. (2008); Barbosa et al. (2016); Mituti et al. (2019)
13	*Tomato golden leaf distortion virus*	TO	Fonseca et al. (2013)
14	*Tomato golden mosaic virus*	BA, DF, MG, PR, RN, RJ e SP	Matyis et al. (1975); Hamilton et al. (1984)
15	*Tomato golden net virus¹*	DF	Reis et al. (2023)
16	*Tomato golden vein virus*	GO, DF e MG	Albuquerque et al. (2012); Reis et al. (2020); Reis et al. (2021)
17	*Tomato interveinal chlorosis virus*	PE	Albuquerque et al. (2012)
18	*Tomato interveinal chlorosis virus-2*	GO	Rêgo-Machado et al. (2019)
19	*Tomato leaf curl purple vein virus*	PI	Macedo et al. (2018)
20	*Tomato leaf distortion virus*	MG e RJ	Fonseca et al. (2013)
21	*Tomato mild mosaic virus*	MG e RJ	Castillo-Urquiza et al. (2008)
22	*Tomato mosaic severe dwarf virus²*	DF	Reis et al. (2020)
23	*Tomato mottle leaf curl virus*	MG, GO, DF, ES, RJ, SP, BA, PB e PE	Ribeiro et al. (2003); Chaves et al. (2017); Ferro et al. (2017)
24	*Tomato mottle leaf distortion virus*	GO	Martins et al. (2021)

25	*Tomato rugose yellow leaf curl virus*	RS	Fonseca et al. (2016)
26	*Tomato severe rugose virus*	DF, GO, MG, RJ, SP, PE, SC e RS	Rezende et al. (1997); Cotrim et al. (2007); Duarte et al. (2021)
27	*Tomato yellow leaf deformation dwarf virus²*	DF	Pereira – Carvalho et al. (2020)
28	*Tomato yellow net virus¹*	DF	Reis et al. (2023)
29	*Tomato yellow spot virus*	MG	Calegario et al. (2007)
30	*Tomato yellow vein streak virus*	DF, GO, MG, RS, RJ e SP	Faria et al. (1997); Albuquerque et al. (2010); Reis et al. (2021)

Amazonas (AM); Bahia (BA); Tocantins (TO); Minas Gerais (MG); Distrito Federal (DF); Espírito (ES); Pernambuco (PE); Paraná (PR); Rio Grande do Norte (RN); Rio Grande do Sul (RS); Rio de Janeiro (RJ); São Paulo (SP) e Pará (PA).

¹ não aceitos pelo ICTV

² apenas depositados no GenBank

1.8 DNA satélite associados ao gênero *Begomovirus*

Atualmente estudos tem demonstrado que além dos componentes genômicos DNA–A e DNA–B existem agentes subvirais de DNA, conhecidos como DNA satélites (Rosario et al. 2016) e que existem em associação a begomovírus monopartidos e bipartidos (Rojas et al. 2018). A maior parte dos begomovírus do Velho Mundo e alguns do Novo Mundo estão associados a três tipos de satélites de DNA conhecidos como alfassatélite (anteriormente conhecidos como DNA–1) (Briddon et al. 2018), betassatélite anteriormente conhecidos como DNA-β (Zhou et al. 2013) e deltasatélite (Lozano et al. 2016).

Os **alfassátelites** estão classificados na família *Alphasatellitidae*, subfamília *Geminialphasatellitinae* (ICTV 2023) o tamanho do genoma varia entre 1,5 e 1,7 pb, com a presença de uma região rica em adenina e uma estrutura em gancho, o nonanucleotídeo TAGTATT/AC (Iqbal et al. 2021). Tanto os alfassatélites quanto os betasaatélites são capazes de interagir funcionalmente com diferentes begomovírus auxiliares, sendo assim são frequentemente associados a complexos begomovirus/betassatélites do Velho mundo (Kumar et al. 2017, Kumar et al. 2021). Os alfassatélites podem se replicar autonomamente seus próprios genomas, mas dependem de seu begomovírus auxiliar para infecção sistêmica, encapsidação e transmissão vetorial (Stainton et al. 2017, Zhao et al. 2022). Entretendo, as funções biológicas exatas para alguns ainda permanecem insertas, porém alguns alfassatélites podem modular os sintomas virais e afetar o acúmulo do vírus auxiliar e/ou do DNA betasaatélite durante a infecção (Mar et al. 2017, Luo et al, 2019). No Brasil, os alfassatélites foram encontrados em associação com begomovírus Novo Mundo infectando plantas não cultivadas como *Euphorbia heterophylla* (Euphorbiaceae) e *Cleome affinis* (Cleomaceae) infectadas por begomovírus bipartidos (Paprotka et al. 2010). Mais recentemente no Brasil, Reis et al (2020) detectaram um novo alfassatélite em tomateiro que ainda não foi totalmente caracterizado. O valor de identidade nucleotidica é inferior a 88% sendo este o critério atual usado para classificação de uma nova espécie (ICTV 2023, Briddon et al. 2018).

Os **betassatélites** estão classificados estão classificados na família Tolecusatellitidae, que possui dois gêneros (*Betasatellite* e *Deltasatellite*) (ICTV 2023). Possuem genoma variando entre 1,5 e 1,7 pb, possuem uma região rica em adenosina (rica em A), organização genômica altamente conservada entre todos os betasatélites conhecida como região conservada por satélite (RCS) e um único gene, βC1 (Briddon et al. 2008, Mar et al. 2017). Os betassatélites dependem do begomovírus auxiliar para sua replicação, movimento e transmissão (Kumar et al. 2021). Recentemente, um novo gene codificador da proteína βV1 foi identificado em

betassatélites associado a begomovírus provocando a morte celular programada do tipo resposta de hipersensibilidade (HI), o que contribui para virulência e infecção viral (Hu et al. 2020). O gene βC1 é conhecido por ser um determinante de sintomas e, portanto, aumenta os sintomas em alguns patossistemas e atua também como um supressor transcricional e pós–transcricional do silenciamento gênico (Kumar et al. 2021).

Os **deltassatélites** possuem genoma de aproximadamente 700pd e podem tanto estar associados a begomovirus monopartidos do Velho Mundo (Dry et al. 1997) quanto a bipartidos do Novo Mundo (Fiallo–Olivé et al. 2012). Diferente dos alfassatélites e betassatelites, os deltassatelites não codificam nenhuma proteína, sendo totalmente dependentes do vírus auxiliar para replicação, movimentação nas plantas e transmissão pelo vetor *Bemisa tabaci* (Fiallo–Olivé et al. 2016, Hassan et al. 2016, Ferro et al. 2020). O critério atual para classificação como betassatélite ou deltassatélite é realizada com base na identidade de nucleotídeos do genoma completo devendo ser inferior a 91%.

1.9 Uso do High-Throughput Sequencing (HTS) em virologia vegetal

A detecção e identificação de novos vírus dependem atualmente de uma grande variedade de técnicas, tradicionais e modernas (Adams et al. 2009). O sequenciamento de alto rendimento (*High Throughput Sequencing*, HTS) refere-se às novas tecnologias de sequenciamento de ácidos nucleicos, desenvolvida décadas depois que o método de sequenciamento de DNA Sanger que surgiu pela primeira vez em 1977, dominando por três décadas (Sanger et al. 1977, Mardis 2008).

As tecnologias HTS são diferentes do método Sanger na medida em que fornecem análise massivamente paralelas, rendimento extremamente alto de várias amostras a um custo reduzido (Mardis 2011). Adams et al. 2009 fizeram os primeiros estudos utilizando o sequenciamento de alto de rendimento em virologia de plantas, em que fez análises metagenômicas sem prévio conhecimento do patógeno suspeito.

Entre as plataformas utilizadas estão: 454 GS FLX+/Roche, em fase de descontinuação, foi a primeira plataforma de sequenciamento de alto rendimento disponível comercialmente em 2004, sendo seguida pela Illumina em 2006, pelo sequenciador SOLiD em 2007, pelo Ion Torrent em 2010 e mais recentemente pela Nanopore (**Tabela 6**) (Scholz et al. 2012, Ambardar et al. 2016).

As etapas para detecção viral por meio de análises metagenômicas começam pela extração dos ácidos nucleicos, seguido por enriquecimento do DNA/RNA viral, previamente extraído. Nos casos de vírus de DNA circular, emprega-se a técnica de amplificação por círculo

rolante (RCA), permitindo o enriquecimento de amostras compostas de genoma ssDNA (Inoue-Nagata et al. 2004). De forma geral, a linha de pesquisa seguida para descoberta de genomas virais pela abordagem de metagenômica é: preparação de amostras (bibliotecas) e enriquecimento de DNA viral; análises da qualidade das sequências obtidas bibliotecas por *Illumina Hiseq*; retirar os adaptadores das sequências; montagem de *contigs*; submeter ao *tBLASTx*; aumento dos *contigs* e nova montagem; montagens finais (Blawid et al. 2017).

A partir deste ponto o estudo de metagenômica viral aplicada à virologia de plantas tem possibilitado a descoberta de novos vírus em diferentes hospedeiros, como o tomate (Rivarez et al 2021). Somente em 2020, utilizando a tecnologia de HTS, foram descobertas seis novas espécies de vírus infectando tomateiro, sendo elas quatros begomovirus, New species #1, New species #2, New Species #3 (Reis et al. 2020), tomato fruit blotch virus (ToFBV) (Ciuffo et al. 2020) e um novo *Ilarvirus* cujo nome proposto é Solanum nigrum ilarvirus 1 (Ma et al. 2020). Ainda com uso de HTS, Reis et al. 2023 recobriram dois novos begomovirus monopartidos no Novo Mundo, cujos nomes propostos são tomato golden net virus (ToGNV) e tomato yellow net virus (ToYNV). Nos estudos de Rivarez et al. (2023), foram detectamos 37 vírus conhecidos e 55 novos, que foram classificados em táxons de vírus estabelecidos, e 33 vírus não classificados membros de *Riboviria* em ervas daninhas próximas a plantações de tomate. Dentre os vírus encontrados em tomate e ervas daninhas estão, o tomato alphanucleorhabdovirus 1 (TARV1, Rhabdovirus), tomato betanucleorhabdovirus 1 (TBRV1, *Rhabdovirus*), tomato spotted wilt orthotospovirus (TSWV, *Orthotospovirus*), tomato mosaic virus (ToMV, *Tobamovirus*), potato virus Y (PVY, *Potyvirus*) entre outros.

Tabela 3: Diferentes gerações de plataformas HTS, composição química, comprimento de leitura (bp), tipo de amplificação, princípio e limitações de cada plataforma.

Plataformas HTS	Química	Comprimento de leitura (bp)	Tipo de amplificação	Princípio	Limitações
454 GS FLX+/Roche[1]	Pirosequenciamento	10 - 1000	PCR de emulsão	Detecção de pirofosfato liberado durante a incorporação de nucleotídeos.	Erros de sequenciamento durante a incorporação dos nucleotídeos.
Illumina[2]	Terminadores reversíveis	36 - 300	Ponte de PCR	Terminador reversível para sequenciamento rápido usando dNTPs de marcação única.	Superlotação ou sobreposição de sinais, devido à sobrecarga da amostra.
SOLiD[3]	Sequenciamento de leitura curta	85	PCR de emulsão	Método enzimático de sequenciamento usando DNA ligase.	Leitura curtos levam à imprecisão na montagem da leitura.
Ion Torrent[4]	Torre de íons	200–400	PCR de emulsão	Utiliza semicondutores iônicos que detecta íons H+ gerados durante a incorporação de nucleotídeos.	Perda na intensidade do sinal quando sequências de homopolímero são sequenciadas.
Nanopore[5]	Sequenciamento de leitura longa	média 10.000–30.000	Sem PCR	O método baseia-se na linearização de moléculas ácidos nucleicos e na sua capacidade de se mover através de um poro (nanoporos).	Erro com sequências de baixa complexidade. Possui baixa precisão com menores leituras.

[1] Ronaghi et al. 1996, Slatko et al. 2018, Henson et al. 2012, [2] Slatko et al. 2018, Henson et al. 2012, Buermans et al. 2014, [3] Henson et al. 2012, Shendure et al. 2005, Liu et al. 2012
[4] Slatko et al. 2018, Henson et al. 2012, Rothberg et al. 2011, [5] Amarasinghe et al. 2020, Slatko et al. 2018, Jain et al. 2016.

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CHAPTER 2

Metagenomics and diversity analyses of single-stranded DNA viruses and their associated satellites infecting members of the Solanaceae family in Brazil

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Metagenomics and diversity analyses of single–stranded DNA viruses and their associated satellites infecting members of the Solanaceae family in Brazil

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Abstract

The family *Geminiviridae* encompass a wide array of single-stranded (ss) DNA viruses, including *Begomovirus* species that can cause serious economic losses in tomato and other Solanaceae crops. A subgroup of tomato-infecting begomoviruses is also associated with Solanaceae weeds, which may function as viral reservoirs. Herein, high-throughput sequencing (HTS) was used to carry out a large-scale analysis of the diversity of ssDNA viruses in foliar tissues of tomatoes as well as other Solanaceae crops and weeds. Foliar samples exhibiting begomovirus-like symptoms were field-collected, enriched by rolling circle amplification, subdivided into pools, and sequenced in an Ilumina NovaSeq 6000 platform. A total of 168 begomoviruses were recovered by HTS. Sixteen (16) of them were found to be putative new viral species. Other genera of ssDNA viruses and subviral agents were also detected in our survey, including one topilevirus, one gemycircularvirus, and two alphasatellites. These pathogens displayed nucleotide identities of 99.1%, 99.67%, 99.92%, and 92.96%, with tomato apical leaf curl virus (MH539677), plant associated genomovirus 12 (MT214094), *Alphasatellitidae* species (MT214093), and Euphorbia yellow mosaic alphasatellite (KY559642), respectively. Hence, HTS allowed revealing high levels of 'hidden' genetic diversity of ssDNA viruses and their satellites in Solanaceae, demonstrating once more the potential epidemiological role of cultivated plants and weeds from this botanical family as persistent viral reservoirs.

Key words: *Begomovirus*, Tomato, Weeds,· High-throughput sequencing, HTS.

2.1 Introduction

The *Geminiviridae* is the major family of insect-transmitted, circular, and single-stranded DNA (ssDNA) plant viruses (Ishfaqe et al. 2022). This family comprises 14 genera (*Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrinelvirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus*, and *Turncurtovirus*) with ≈ 520 viral species (ICTV 2023). Classification at the genus level is based upon host range, their associated insect vectors, genomic organization, and phylogenetic relationships (ICTV 2023, Brown et al. 2015, Varsani et al. 2017). Two new viral families (*Pleolipoviridae* and *Genomoviridae*) with circular, non-enveloped ssDNA with genome sizes ranging from 1.800 to 2.400 nucleotides were more recently created (Varsani et al. 2021). The *Genomoviridae* family comprises nine genera: *Gemycircularvirus*, *Gemyduguivirus*, *Gemygorvirus*, *Gemykibivirus*, *Gemykolovirus*, *Gemykrogvirus*, *Gemykroznavirus*, *Gemytondvirus*, and *Gemyvongvirus* (ICTV 2023)

The genus *Begomovirus* comprises ≈ 445 viral species (ICTV 2023), which are characterized by having either monopartite (with only the DNA–A component) or bipartite (with two distinct components: DNA–A and DNA–B) genomes (Brown et al. 2015, Varsani et al. 2017). Three classes of ssDNA subviral agents (alphasatellites, betasatellites, and deltasatellites) associated with both monopartite and bipartite begomoviruses were also characterized (ICTV 2023). The transmission of begomoviruses is carried out by cryptic species of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) complex, which are known as whiteflies (Ghosh and Ghanim 2021). Begomoviruses display three major genetic variability mechanisms (mutation, recombination, and pseudo-recombination), which allow viral adaptation to new hosts as well as the emergence of new diseases (Elena et al. 2014, Naito et al. 2019, Guevara-Rivera et al. 2022).

The tomato (*Solanum lycopersicum* L.) is a member of the Solanaceae family, being one of the most economically important vegetable crops around the world (Kumar et al. 2022). *Begomovirus* infections are responsible for half of emerging plant diseases, which leads to losses equivalent to about a quarter of the expected crop yield (Rivarez et al. 2023). In Brazil, outbreaks of *Begomovirus* species in tomatoes were more often reported after the invasion and widespread dissemination of *B. tabaci* Middle East Asia Minor 1 (MEAM 1 = biotype B) in the early 1990s (Reis et al. 2020, Reis et al. 2021). A very rapid geographical dispersal of the begomoviruses was observed in Neotropical areas due to their wide host range, high

transmissibility of viral species, and the broad adaptation of *B. tabaci* MEAM 1 to different environmental conditions and host plants (Watanabe et al. 2019, Krause-Sakate et al. 2020).

Extensive studies carried out subsequently the invasion of *B. tabaci* MEAM1 revealed overwhelming high rates of diversity of begomovirus species occurring in Brazilian regions (Reis et al. 2020). Currently, 30 infecting tomato begomoviruses have been reported in Brazil, the majority of which are already accepted by the International Committee on Taxonomy of Viruses (ICTV). To date, tomato severe rugose virus (ToSRV; a bipartite species) and tomato mottle leaf curl virus (ToMoLCV; a monopartite species) are the most economically important begomoviruses infecting tomatoes as well as many cultivated and weed hosts across all geographical Brazilian regions (Duarte et al. 2020, Duarte et al., 2021b, Reis et al. 2020, Reis et al. 2021, Pereira-Silva et al. 2022). Furthermore, begomoviruses initially described in weeds have been also occasionally reported infecting tomato plants (Macedo et al. 2018, Duarte et al. 2021a, Cotrim et al. al. 2007, Pereira-Silva et al. 2022).

In this scenario, research interest has increased towards exploring the diversity of viruses occurring in weeds across different agro-ecosystems using High-throughput sequencing (HTS) as the main analytical tool (Sommers et al. 2021, Krause-Sakate et al. 2020). Viral metagenomics studies based on HTS in tomato and weed species frequently associated with this crop can generate important information for understanding the epidemiology of begomoviruses (Rivarez et al. 2021). HTS enables the generation of broad and comprehensive genomic data, allowing the characterization of complete viral genomes, functional genes, regulatory regions, and genetic variations that may be related to viral pathogenicity (Bejerman et al. 2020).

New members of the family *Geminiviridae* (including monopartite and bipartite begomoviruses) have been discovered in Brazil via HTS-based metagenomic studies (Reis et al. 2020, Reis et al. 2022, Reis et al. 2023). In fact, HTS studies revealed a large viral diversity in tomato and weeds encompassing a total of 125 viruses, including 79 new species of which 65 were found exclusively in weeds (Rivarez et al. 2023). Current findings corroborate the notion that weeds are major reservoirs of viruses, which might pose persistent threats to agricultural production and food security around the world (Rong et al. 2023). The knowledge about the viral diversity present across agro-ecosystems provides outstanding tool to predict the emergence of more virulent variants (McLeish et al. 2021). In this context, the objective of the present work was to carry out metagenomic analyses of ssDNA viruses in Solanaceae crops and weeds across different Brazilian regions with the aim of cataloging their viral diversity and verifying the role of hosts as potential viral reservoirs.

2.2 Material and Methods

2.2.1 Sample collection – A collection encompassing 129 foliar samples exhibiting begomovirus-like symptoms were field-collected from a wide array of plant species from the Solanaceae family. Samples were collected between 2003 and 2022 in all macro-regions of Brazil (**Table 1**). Distinct years and places of collection were the major selection criteria for inclusion of a single sample in the subsequent analyses.

2.2.2 DNA extraction – DNA from the samples was extracted with a modified protocol 2X CTAB buffer and organic solvents (Boiteux et al. 1999) and stored in a freezer (–20º C). The DNA quality was evaluated by electrophoresis on 1% agarose gels stained with ethidium bromide and visualized under UV light. DNA quantification was performed using a NanoDrop One apparatus (Thermo Scientific).

2.2.3 PCR tests with degenerate primers for begomovirus detection and enrichment of ssDNAs by Rolling Circle Amplification (RCA) – Total DNA obtained from each sample was used as a template for RCA essentially as previously described (Reis et al. 2020). Confirmation of begomovirus infection in the samples after RCA was performed using the degenerated primer pairs: PAL1v1978 (5'–GCA TCT GCA GGC CCA CAT **Y**GT CTT **Y**CC NGT–3') and PAR1c496 (5'–CAT GCT GCA GTA CAT **Y**GG CCT **Y**TT **D**AC CC–3') for the DNA–A component and PBL1v2040 (5'–GCC TCT GCA GCA **R**TG **R**TC **K**AT CTT CAT ACA–3') and PRCc1 (5'–CTA GCT GCA GCA TAT TTA C**R**A **RW**A TGC CA–3') for the DNA–B component (Rojas et al. 1993).

2.2.4 Preparation of pools and High-Throughput Sequencing (HTS) – The RCA samples were grouped in a pool comprising samples from the North (15), Northeast (42), South (11), Southeast (18), Midwest (43) Brazilian regions (**Table 1**). Samples were then sent to HTS on an Illumina NovaSeq-6000 platform.

2.2.5 Viral sequence analyses – The resulting data were analysed using the CLC Genomics Workbench 7.5 software (Qiagen) and subsequently analysed with the Geneious® R11.1 software (Kearse et al. 2012). The methodology used was essentially as previously described (Nery et al. 2020, Nery et al. 2023). The reads obtained were mapped to the contig of potential

viruses aiming to obtain the final contig sequences. The individual contigs were extended with the help of the Geneious® R11.1 software and the 'Map to reference' tool (90 to 99% minimum overlap identity parameter) with mapping in the reads file provided by HTS. All contigs were subjected to comparisons with viral sequences present in the GenBank using BLASTn algorithm. To annotate ORFs (Open Reading Frames), the MUSCLE alignment was used in the Geneious® R11.1 software based on the reference genome. After *de novo* assembly, contigs from the pools was submitted to a taxonomic prediction analysis with the standard classification parameters using the Kaiju web server (http://Kaiju.binf.ku.dk/server) (Menzel et al. 2016). Based upon this analysis, contig sequences predicted to be of viral origin were identified. Sequences with larger sizes were selected and assembled, and alignments were also carried out with reference genomes that displayed higher identity levels (Menzel et al. 2016). The Geneious® R11.1 software was also used to assemble the viral genomes, annotate, and align the assembled sequences. In the case of putative new species, we also carried out analyses of ORFs, the intergenic regions (in monopartite viruses) and the common region (in bipartite viruses). Figures were elaborated as described by Reis et al. (2021). For comparison between isolates and viral species, the sequences were subjected to pairwise MUSCLE multiple alignment using the SDT program (Muhire et al. 2014).

2.2.6 Phylogenetic and recombination analyses – The phylogenetic relationships obtained in these studies were compared with the virus sequences available in the GenBank database. Analyses were performed using the complete DNA–A genome as criteria for begomoviruses (Brown et al. 2015). Sequences were aligned using MUSCLE 3.8.425 software (Edgar 2004) The tree was generated with IQtree 2.2.0 software with Bayesian information criterion model TIM3+F+R5. The figures were created using Adobe Illustrator CC and EvolView software. The sequences were then analysed for the presence of possible recombination events using the RDP5 software (Martin et al. 2021).

3. Results

High-throughput sequencing (HTS) provided the following data: 41,659 contigs were obtained and 172 of them displayed identity to begomoviruses (168), topilevirus (1), gemycircularvirus (1) and alphasatellites (2 contigs). Of the 168 contigs related to begomoviruses, 73 correspond to DNA–A (**Table 2**) and 50 to DNA–B (**Table 3**) components, with 45 corresponding to putative monopartite begomoviruses (**Table 4**). Sixteen (16) DNA–A-related contigs showed nucleotide identity below 91%, consistent with the current taxonomic criteria for defining new

species within the genus *Begomovirus* (**Figure 2 and Table 5**). The MUSCLE sequence alignments of these 16 contigs displayed identity levels ranging from 66% to 89%. The contigs C195, C18367, C2046, and C5175 showed 99.10%, 99.67 99.92%, and 92.96% identity with tomato apical leaf curl virus (MH539677), plant associated genomovirus 12 (MT214094), Alphasatellitidae species (MT214093) and Euphorbia yellow mosaic alphasatellite (KY559642), respectively **(Table 6)**.

Recombination analyses using MUSCLE alignments of all 16 complete DNA–A sequences of the putatively new begomoviruses were carried out. The criterium adopted for confirmation of a bona fide recombination event was the significant detection by at least four out of seven following statistical methods: RDP (Martin et al. 2005), GENECONV (Padidam et al. 1999), BootScan (Martin et al. 2005), MaxChi (Smith et al. 1992), Chimaera (Posada et al. 2002), SiScan (Gibbs et al. 2000), and 3Seq (Boni et al. 2007). These analyses provided strong evidence that at least 26 recombination events are present across 14 of the 16 DNA–A genomes analyzed **(Table 7)**.

4. Discussion

Our HTS analyses allowed the assembly of 168 DNA–A sequences related to begomoviruses. Seventy-three (73) contigs were related to DNA–A and 50 contigs to DNA–B. The detection of more DNA–A in relation to DNA–B genomes may indicate component-sharing events with different begomoviruses using the same DNA–B component for intra- and intercellular movement (Briddon et al. 2015). Thes events were also detected by Reis et al. (2020) using HTS and PCR assays. In their work, isolate GO–023 was found to be in mixed infection with tomato common mosaic virus (ToCMoV), and isolate MG–388 was in mixed infection with ToSRV. And the absence of the DNA–B component of ToCmMV also suggests that these isolates may be using components from these co-infecting species. However, this hypothesis still needs to be further investigated.

After pairwise phylogenetic and identity analyses (**Figures 1 and 2**), it was possible to identify in field-collected samples high levels of begomovirus diversity in association with distinct Solanaceae species and genera. This can be explained by the polyphagous habit of *B. tabaci* MEAM 1, which is potentially able to transmit begomoviruses across distinct members of this botanical family. The promiscuous Solanaceae host exploitation by this vector and its efficient viral transmission under field conditions may explain the profuse emergence of novel tomato-infecting begomoviruses (Rivarez et al. 2023, Czoneck et al. 2017). The observed viral diversity might be also explained by the mechanisms that generate genetic variability (mutation,

recombination and pseudo-recombination). Events of recombination and pseudo-recombination are intensified by the occurrence of mixed infections, which leads to high intra-host diversity within this viral group (Reis et al. 2021, Dye et al. 2023).

Patterns of interspecies recombination and several hot and cold genomic spots for recombination have been described among members of the genus *Begomovirus* (Prasanna et a. 2007). Herein, we analysed the recombination breakpoints present in a dataset of tomato-infecting begomoviruses and a large number of recombination events were detected **(Table 7).** Most recombination events occurred in the genomic regions encompassing AV1 ORF (CP), the AC1 ORF (Rep), and the AC3 (Ren). These results are consistent with those obtained in analyses of members of the *Geminiviridae* family, which show that the Rep and the intergenic region between the AV1 and AC3 ORFs are recombination hot spots (Lefeuvre et al. 2007, Lefeuvre et al. 2015). On the other hand, the presence of recombination cold spots were also identified within ORF V2 and in part of ORF AV1 (Lefeuvre et al. 2009), which is also in agreement with our findings. It is worth noting that recombination breakpoints are not randomly assigned to hot and cold spots. These patterns are strongly influenced by biochemical, biophysical factors and recombination tolerance (Albuquerque et al. 2012).

Two neotropical tomato-infecting begomoviruses such as tomato chlorotic mottle virus (ToCMoV) and ToSRV display kinship in relation the recombination events (Rocha et al. 2013). One possible explanation is that the intraspecies variability reflects the genetic variability of their hosts (Rocha et al. 2013). However, there is little information that quantifies the frequency of mixed infections in Solanaceae species by members of the neotropical complex of *Begomovirus* species (Reis et al. 2020). Further studies analyzing mutation frequencies of viral populations (ideally from the same begomovirus) infecting cultivated and non-cultivated hosts should be carried out to verify this hypothesis. Herein, we also obtained a partial sequence (331 nts) of a *Gemycircularvirus* (family *Genomoviridae*) isolate with 100% identity to plant associated genomovirus 12 (MT214094; Reis et al. 2022) (**Table 5**). Three gemycircularviruses were previously identified and described in samples of *Momorcadia charantia*, *Euphorbia heterophylla*, and *Larrea tridentata* in Brazil (de Rezende et al. 2018, Reis et al. 2022).

In our field survey, two contigs (C2046 and C5175) corresponding to alphasatellites were detected with 1322 nucleotides and 745 nucleotides, respectively (**Table 5**). Alphasatellite DNA molecules are subviral agents found in association with *Begomovirus* (Kumar et al. 2021), which are currently classified in the family *Alphasatellitidae*, subfamily *Geminialphasatellitinae* (ICTV 2023). The first alphasatellite isolate identified in the present

study shows the highest identity level (100%) with another New World species found in four different samples from the Federal District in previous metagenomics analysis of tomato samples (Reis et al. 2020). The alphasatellite found here is most likely from the genus *Clecrusatellite*, which was previously reported in constant association with New World bipartite *Begomovirus* in Neotropical regions (Paprotka et al. 2010, Romay et al. 2010). This alphasatellite was previously found in constant association with tomato golden vein virus (TGVV) and ToMLCV in tomato samples (Reis et al. 2020). The second alphasatellite detected here displayed 92% identity with Euphorbia yellow mosaic alphasatellite (EuYMA – KY559642). However, the sequence of this subviral agent is yet partial. EuYMA was first reported in association with Euphorbia yellow mosaic virus (EuYMV) in *E. heterophylla* in Brazil (Mar et al. 2016). The criterion currently used for classifying a new species within the family Geminialphasatellitinae is nucleotide identity of less than 88% in comparison with complete sequences of all available alpha satellite genomes (ICTV 2023, Briddon et al. 2018).

A single *Topilevirus* (2874 nucleotides in size) with high identity (99%) to tomato apical leaf curl virus (ToALCV) was also detected in Solanaceae samples (**Table 4**). Thus far, ToALCV has been found infecting only tomatoes in Argentina (Medina et al. 2018) and Brazil (Batista et al. 2019). Therefore, we will carry out more extensive analyzes in order identify the original host of the sample(s) that were positive to ToALCV since our survey includes distinct Solanaceae species.

In conclusion, HTS allowed revealing high levels of 'hidden' genetic diversity of ssDNA viruses and their satellites in Solanaceae, demonstrating once more the potential epidemiological role of cultivated plants and weeds from this botanical family as persistent viral reservoirs.

Table 1: Information about the 129 samples of symptomatic leaves classified in the Solanaceae family between 2003 and 2022 in all macro-regions of Brazil, with respective codes, hosts, scientific name of the hosts, location, and year of collection.

Macro geographical region	Isolate code	Original host	Host (scientific name)	Collection site (year)
North	TO-033	*Capsicum*	*Capsicum* species	Matinha (2005)
	AM-003	Sweet-pepper	*Capsicum annuum*	Iranduba (2007)
	TO-089			Aragominas (2008)
	TO-065	*Physalis*	*Physalis* species	Guaraí (2007)
	TO-173			Araguaina (2008)
	AC-003	Scarlet eggplant	*Solanum aethiopicum*	Porto (Acre) (2016)
	TO-053			Gurupi (2007)
	AC-005	Tomato	*Solanum lycopersicum*	Porto (2016)
	AM-036			Iranduba (2016)
	AM-038			
	AM-042			
	PA-001			Altamira (2016)
	TO-046			Gurupi (2008)
	TO-090			Aragominas (2008)
	TO-093			
Northeast	PE-002	*Capsicum*	*Capsicum* species	Chã Grande (2007)
	PE-070	*Solanum americanum*	*Solanum americanum*	Senharó (2010)
	BA-027	Tomato	*Solanum lycopersicum*	América Dourada (2007)
	BA-028			
	BA-029			
	BA-030			
	BA-031			
	BA-033			
	BA-034			
	BA-035			
	BA-036			
	BA-037			

BA-045			Igarachi (2009)
BA-060			Irecê (2010)
BA-073			Jaguaquara (2010)
BA-124			Utinga (2011)
BA-128			Município Wagner (2011)
BA-137			Irecê (2011)
BA-155			
BA-156			
BA-157			
BA-158			
BA-159			
BA-160			
BA-161			
BA-162			
BA-163			
BA-164			
BA-166			Juazeiro (2012)
BA-173			Poções (2014)
BA-182			Aracatu (2017)
BA-186			
CE-027			Ibiapina (2010)
CE-031			
PI-002			Guadalupe (2016)
PI-003			
PI-004			
PI-005			
RN-001			Jandaíra (2012)
BA-013	Jurubeba	*Solanum paniculatum*	Irecê (2007)
BA-017			
RAL-756			Alagoas (2022)

Midwest	**DF-559**	*Capsicum*	*Capsicum annuum*	Gama (2013)
	DF-285		*Capsicum* species	
	GO-363			Goiânia (2007)
	RDF-794			Gama (2022)
	RDF-819			Brasília / EEB (2022)
	DF-284	Nicandra	*Nicandra physalodes*	Gama (2009)
	DF-369			
	DF-799			
	GO-624			Padre Bernardo (2019)
	GO-639			Teresópolis de Goiás (2020)
	DF-178	*Solanum viarum*	*Solanum viarum*	Núcleo Rural São José (2005)
	DF-393			Planaltina (2011)
	DF-307	*Physalis*	*Physalis* species	Gama (2009)
	DF-272	Scarlet eggplant	*Solanum aethiopicum*	
	GO-012	*Solanum americanum*	*Solanum americanum*	Itapaci (2002)
	RDF-780	Tree tomato	*Solanum betaceum*	Gama (2022)
	RDF-781			
	GO-057	Fruit of the Wolf	*Solanum lycocarpum*	Goianápolis (2003)
	GO-242	Fruit of the Wolf		Alexânia (2003)
	DF-048	Tomato	*Solanum lycopersicum*	Gama (2003)
	DF-169			Núcleo Rural São José (2005)
	DF-208			Taquara (2005)
	DF-221			
	DF-391			Planaltina (2011)
	DF-480			Rajadinha (2012)
	GO-045			Goianápolis (2003)
	GO-123			
	GO-188			
	GO-321			Leopoldo de Bulhões (2004)
	GO-324			

	GO-325			
	GO-393			Itaberaí (2006)
	GO-482			Goianápolis (2010)
	GO-488			Goianápolis (2010)
	RDF-797			Gama (2022)
	RDF-752			Brasília / EEB (2021)
	RDF-816			Brasília / EEB (2022)
	RDF-818			Brasília / EEB (2022)
	DF-301	*Solanum mammosum*	*Solanum mammosum*	Gama (2022)
	DF-259	Eggplant	*Solanum melongena*	Gama (2022)
	RDF-815	Eggplant	*Solanum melongena*	Brasília / EEB (2022)
	GO-592	*Solanum viarum*	*Solanum viarum*	Luziânia (2014)
	DF-021	Cubiu	*Solanum sessiliflorum*	Gama (2003)
Southeast	SP-100	Nicandra	*Nicandra physalodes*	Capão Bonito (2011)
	ES-309	Scarlet eggplant	*Solanum aethiopicum*	Venda Nova do Imigrante (2021)
	SP-031	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-037	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-054	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-053	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-055	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-056	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-057	Tomato	*Solanum lycopersicum*	Piracicaba (2008)
	SP-166	Tomato	*Solanum lycopersicum*	Santo Antônio da Posse (2015)
	SP-285	Tomato	*Solanum lycopersicum*	São Paulo IAC (2020)
	ES-310	Tomato	*Solanum lycopersicum*	Venda Nova do Imigrante (2021)
	MG-042	Tomato	*Solanum lycopersicum*	Araguari (2001)
	MG-048	Tomato	*Solanum lycopersicum*	Araguari (2008)
	MG-433	Tomato	*Solanum lycopersicum*	Cascalho Rico (2019)
	MG-435	Tomato	*Solanum lycopersicum*	Araguari (2020)
	MG-438	Tomato	*Solanum lycopersicum*	Piracaiba (2020)

	SP-216	Eggplant	*Solanum melongena*	Bragança Paulista (2016)
South	**PR-115**	Tomato	*Solanum lycopersicum*	Ortigueiras (2010)
	RS-019			Torres (2009)
	RS-071			Morro Redondo (2013)
	SC-001			Florianópolis (2006)
	SC-012			Santo Amaro da Imperatriz (2006)
	SC-036			Caçador (2011)
	SC-044			Santo Amaro da Imperatriz (2011)
	SC-060			
	SC-062			
	PR-144			Mauá da Serra (2015)
	RS-070	Eggplant	*Solanum melongena*	Canguçu (2013)

Table 2: Discovery of *Begomovirus* species via High-Throughput Sequencing (HTS) in a pool of 129 leaf samples of botanical species from the Solanaceae family. Information was organized by the code number of the contigs corresponding to **DNA–A segments** from putative *Begomovirus* species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E–value, the correspondent GenBank (GB) accession, and description of the virus associated with each contig. Contigs highlighted in gray represent potential new viral species, viruses with "*" correspond to isolates of the same species.

Contig	Read coverage	Size (nucleotides)	Coverage (%)	Identity (%)	E-Value	GB Accession	Description
381	127,99	2642	100%	95.37%	0	MW600767.1	Cnidoscolus mosaic leaf deformation virus DNA-A
65	6,369	2520	98%	82.89%	0	NC_040184.1	Cnidoscolus mosaic leaf deformation virus DNA-A
104	244,683	2670	100%	83.77%	0	MZ465583.1	
103	10,403	2669	100%	88.95%	0	MW600767.1	
68	186,988	2698	89%	84.20%	0	MZ019476.1	Euphorbia severe leaf golden mosaic virus DNA-A
82	477,141	2686	100%	93.56%	0	KC706535.1	Sida micrantha mosaic virus DNA-A *
78	134,112	2677	100%	84.66%	0	MT214092.1	Sida micrantha mosaic virus DNA-A
3318	61,725	2616	86%	83.14%	0	EU908734.1	
215	109,025	2677	100%	84.43%	0	MT214092.1	
373	180,882	2677	100%	85.03%	0	MT214092.1	
2	628,706	2622	100%	96.80%	0	MT214086.1	Tomato chlorotic mottle virus DNA-A*
21	954,522	2623	100%	92.99%	0	NC_003664.1	
74	705,14	2622	100%	95.85%	0	NC_003664.1	
909	596,438	2624	100%	95.69%	0	NC_003664.1	
910	696,273	2622	100%	95.27%	0	NC_003664.1	
105	788,723	2624	100%	97.87%	0	NC_003664.1	
134	971,269	2623	100%	92.23%	0	NC_003664.1	
478	945,285	2623	100%	97.20%	0	NC_003664.1	
568	939,395	2624	100%	96.38%	0	NC_003664.1	
882	768,302	2624	100%	92.31%	0	MT215003.1	
151	876,477	2623	100%	93.75%	0	NC_003664.1	

844	689,312	2622	100%	96.12%	0	NC_003664.1	
5515	1,013,517	2622	100%	92.80%	0	NC_003664.1	
918	981,553	2612	100%	92.46%	0	NC_003664.1	
11644	458,413	2618	93%	86.66%	0	NC_003664.1	
98	785,086	2677	90%	89.86%	0	MT214086.1	
8425	1,239,912	2623	100%	91.20%	0	NC_003664.1	Tomato chlorotic mottle virus DNA-A
17	707,063	2629	92%	91.47%	0	NC_003664.1	
92	947,135	2622	100%	90.58%	0	MT214086.1	
18237	386,598	2666	92%	90.23%	0	NC_003664.1	
73	403,706	2623	99%	92.43%	0	NC_043122.1	Tomato golden leaf distortion virus DNA-A
19	206,402	2561	100%	98.91%	0	MN928612.1	Tomato golden vein virus DNA-A *
55	206,402	2561	100%	98.91%	0	MN928612.1	Tomato golden vein virus DNA-A *
30	203,856	2561	100%	98.98%	0	MN928612.1	Tomato golden vein virus DNA-A *
465	206,402	2561	100%	98.91%	0	MN928612.1	Tomato golden vein virus DNA-A *
91	354,598	2561	100%	96.56%	0	MN928612.1	Tomato golden vein virus DNA-A *
20	206,306	2561	100%	98.87%	0	MN928612.1	Tomato golden vein virus DNA-A *
8277	206,306	2561	100%	98.87%	0	MN928612.1	Tomato golden vein virus DNA-A *
69	206,306	2561	100%	98.87%	0	MN928612.1	Tomato golden vein virus DNA-A *
90	206,306	2561	100%	98.87%	0	MN928612.1	Tomato golden vein virus DNA-A *
93	206,306	2561	100%	98.87%	0	MN928612.1	Tomato golden vein virus DNA-A *
7877	519,879	2590	97%	90.57%	0	MN928612.1	Tomato golden vein virus DNA-A *
1781	530,047	2665	98%	84.00%	0	MN928610.1	Tomato golden vein virus DNA-A
1441	808,237	2628	100%	96.33%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *
903	992,11	2655	100%	92.73%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *
484	784,735	2632	92%	93.05%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *
72	835,337	2611	100%	97.56%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *
4902	532,784	2649	100%	96.79%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *
748	603,521	2633	92%	87.55%	0	MT215006.1	Tomato rugose mosaic virus DNA-A *

47	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
9	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
32	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
166	1,060,393	2607	93%	97.16%	0	MW653951.1	Tomato severe rugose virus DNA-A *
21349	3,308,994	2593	100%	98.96%	0	MW560614.1	Tomato severe rugose virus DNA-A *
652	1,006,938	2593	100%	97.26%	0	MW560614.1	Tomato severe rugose virus DNA-A *
27	3,175,648	2593	100%	97.92%	0	MW560614.1	Tomato severe rugose virus DNA-A *
1320	869,168	2593	100%	93.80%	0	MT215001.1	Tomato severe rugose virus DNA-A *
113	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
14	1,067,782	2607	93%	97.24%	0	MW653951.1	Tomato severe rugose virus DNA-A *
196	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
205	988,144	2595	100%	95.02%	0	MW560614.1	Tomato severe rugose virus DNA-A *
399	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
387	1,067,500	2593	100%	97.03%	0	MW560614.1	Tomato severe rugose virus DNA-A *
15212	1,069,654	2592	100%	97.30%	0	MW560614.1	Tomato severe rugose virus DNA-A *
7369	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
1697	1,093,868	2593	100%	98.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
5908	1,145,219	2601	100%	96.32%	0	MW560614.1	Tomato severe rugose virus DNA-A *
1404	2,128,316	2593	100%	97.88%	0	MT215001.1	Tomato severe rugose virus DNA-A *
1729	1,142,009	2587	100%	97.15%	0	MW596524.1	Tomato severe rugose virus DNA-A *
374	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
4884	1,250,881	2593	100%	99.65%	0	MW560614.1	Tomato severe rugose virus DNA-A *
5203	839,029	2593	100%	94.87%	0	MW560614.1	Tomato severe rugose virus DNA-A *
102	669,82	2593	100%	91.41%	0	MT215001.1	Tomato severe rugose virus DNA-A

Table 3: Discovery of *Begomovirus* species via High-Throughput Sequencing (HTS) in a pool of 129 leaf samples of botanical species from the Solanaceae family. Information was organized by the code number of the contigs corresponding to **DNA–B segments** from putative *Begomovirus* species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E–value, the correspondent GenBank (GB) accession, and description of the virus associated with each contig.

Contig code number	Read the coverage	Size (nucleotides)	Coverage(%)	Identity(%)	E-Value	GB Accession	Description
62	10,121	2545	91%	83.16%	0	NC_040184.1	
237	7556	2630	98%	81.29%	0	NC_040184.1	
63	6,306	2519	97%	82.98%	0	NC_040184.1	Cnidoscolus mosaic leaf deformation virus DNA-B
866	7556	2630	98%	81.29%	0	NC_040184.1	
116	153,604	2578	87%	81.34%	0	NC_040184.1	
7	788,307	2626	85%	91.01%	0	AJ557452.1	
99	31,716	2656	100%	92.55%	0	KX348193.1	
2679	23,054	2677	85%	83.97%	0	EU908734.1	Sida micrantha mosaic virus DNA-B
694	31,716	2656	100%	92.55%	0	KX348193.1	
3574	82,536	2615	86%	83.17%	0	EU908734.1	
389	79,696	2590	95%	98.94%	0	MT214087.1	Tomato chlorotic mottle virus DNA-B
12	109,825	2533	100%	97.48%	0	MN928611.1	
25	77,395	2593	100%	87.91%	0	MN928611.1	
16	326,078	2533	100%	97.29%	0	MN928611.1	
8103	119,366	2569	88%	97.73%	0	MN928611.1	
15	116,583	2610	95%	97.51%	0	MT733807.1	
13	118,251	2533	100%	97.44%	0	MT733807.1	Tomato golden vein virus DNA-B
26	118,251	2533	100%	97.44%	0	MT733807.1	
266	118,251	2533	100%	97.44%	0	MT733807.1	
8423	126,282	2568	85%	97.92%	0	MT733807.1	
14460	1,844,894	2608	73%	88.13%	0	MN928611.1	
1287	104,336	2538	93%	96.24%	0	MN928611.1	

3114	111,369	2550	76%	93.86%	0	MN928611.1	
3412	17,939	2628	79%	80.84%	0	MK087039.1	Tomato interveinal chlorosis virus-2 DNA-B
167	19,045	2566	85%	81.62%	0	MK087039.1	
83	763	2626	86%	78.93%	0	JN381822.1	Tomato rugose yellow leaf curl virus DNA-B
1	3,771,655	2570	100%	98.29%	0	MW653952.1	
203	2,704,903	2615	86%	96.51%	0	MW653952.1	
994	2,090,888	2567	82%	91.13%	0	MW653952.1	
40	3,771,655	2570	100%	98.29%	0	MW653952.1	
6	3,530,928	2545	100%	97.40%	0	MW653952.1	
39	3,398,765	2570	100%	98.53%	0	MW653952.1	
2166	2,443,137	2617	81%	90.39%	0	MW653952.1	
250	2,092,813	2541	87%	91.69%	0	MW653952.1	
18	3,771,655	2570	100%	98.29%	0	MW653952.1	
3	3,771,655	2570	100%	98.29%	0	MW653952.1	
5	3,771,656	2570	100%	98.29%	0	MW653952.1	
11	3,771,655	2570	100%	98.29%	0	MW653952.1	Tomato severe rugose virus DNA-B
38	3,771,655	2570	100%	98.29%	0	MW653952.1	
4	3,771,655	2570	100%	98.29%	0	MW653952.1	
5436	3,771,655	2570	100%	98.29%	0	MW653952.1	
4114	3,771,655	2570	100%	98.29%	0	MW653952.1	
2975	3,771,655	2570	100%	98.29%	0	MW653952.1	
523	3,771,655	2570	100%	98.29%	0	MW653952.1	
114	3,771,655	2570	100%	98.29%	0	MW653952.1	
468	3,771,655	2570	100%	98.29%	0	MW653952.1	
17574	2,937,750	2567	100%	92.27%	0	MW653952.1	
2258	3,771,655	2570	100%	98.29%	0	MW653952.1	
2717	3,771,655	2570	100%	98.29%	0	MW653952.1	
77	3,771,655	2570	100%	98.29%	0	MW653952.1	

Table 4: Discovery of *Begomovirus* monopartite, single-stranded DNA (ssDNA) viruses via High-Throughput Sequencing (HTS) in a pool of 129 leaf samples of botanical species from the Solanaceae family. Information was organized by the code number of the contigs corresponding to putative monopartite ss DNA viral species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E−value, the correspondent GenBank (GB) accession, and description of the virus associated with each contig. Contigs highlighted in gray represent potential new viral species, viruses with "*" correspond to isolates of the same species.

Contig	Read the coverage	Size (nucleotides)	Coverage(%)	Identity(%)	E-Value	GB Accession	Description	Genus
61	169,754	2629	100%	90.73%	0	KY196216.1	Tomato leaf curl purple vein virus*	
8	52,032	2655	92%	82.77%	0	KY196221.1	Tomato leaf curl purple vein virus	
10	752,97	2631	100%	98.52%	0	JF803247.1	Tomato mottle leaf curl virus*	
22	516,847	2632	100%	96.21%	0	JF803247.1	Tomato mottle leaf curl virus*	
23	727,601	2633	100%	96.32%	0	JF803247.1	Tomato mottle leaf curl virus*	
24	399,646	2652	96%	91.97%	0	JF803247.1	Tomato mottle leaf curl virus	
28	891,545	2617	96%	89.57%	0	JF803247.1	Tomato mottle leaf curl virus	
29	588,871	2632	100%	89.97%	0	ON419922.1	Tomato mottle leaf curl virus	
41	471,13	2646	95%	93.31%	0	JF803247.1	Tomato mottle leaf curl virus*	
42	506,401	2662	98%	96.32%	0	JF803247.1	Tomato mottle leaf curl virus*	
43	562,264	2661	96%	91.11%	0	JF803247.1	Tomato mottle leaf curl virus	
44	822,966	2632	100%	88.79%	0	ON419894.1	Tomato mottle leaf curl virus	*Begomovirus*
46	468,234	2662	96%	88.1%	0	JF803247.1	Tomato mottle leaf curl virus	
50	441,794	2627	100%	95.19%	0	JF803247.1	Tomato mottle leaf curl virus*	
51	240,636	2617	98%	95.16%	0	JF803247.1	Tomato mottle leaf curl virus*	
57	468,234	2662	96%	88.1%	0	JF803247.1	Tomato mottle leaf curl virus	
59	558,285	2626	92%	91.63%	0	JF803247.1	Tomato mottle leaf curl virus	
60	280,967	2632	100%	95.9%	0	ON419894.1	Tomato mottle leaf curl virus*	
67	424,252	2632	100%	93.7%	0	ON419894.1	Tomato mottle leaf curl virus*	
75	758,596	2636	96%	99.45%	0	JF803247.1	Tomato mottle leaf curl virus*	
76	1,001,010	2630	100%	95.44%	0	JF803247.1	Tomato mottle leaf curl virus*	
79	562,595	2656	96%	90.97%	0	JF803247.1	Tomato mottle leaf curl virus	
96	462,488	2643	94%	93.65%	0	JF803247.1	Tomato mottle leaf curl virus*	

100	468,234	2662	96%	88.41%	0	JF803247.1	Tomato mottle leaf curl virus
106	816,521	2632	100%	88.07%	0	ON419894.1	Tomato mottle leaf curl virus
149	1,827,904	2606	90%	90.11%	0	JF803251.1	Tomato mottle leaf curl virus
321	566,05	2632	100%	96.28%	0	JF803247.1	Tomato mottle leaf curl virus*
326	468,234	2662	96%	88.1%	0	JF803247.1	Tomato mottle leaf curl virus
335	783,943	2631	100%	99.51%	0	JF803247.1	Tomato mottle leaf curl virus*
341	621,39	2612	93%	93.08%	0	MT214088.1	Tomato mottle leaf curl virus*
396	753,589	2650	94%	93.74%	0	JF803247.1	Tomato mottle leaf curl virus*
424	468,234	2662	96%	88.1%	0	JF803247.1	Tomato mottle leaf curl virus
642	454,501	2632	100%	98.29%	0	JF803247.1	Tomato mottle leaf curl virus*
747	980,752	2669	100%	88.8%	0	ON419894.1	Tomato mottle leaf curl virus
1093	468,234	2653	96%	88.41%	0	JF803247.1	Tomato mottle leaf curl virus
1514	882,862	2632	100%	89.65%	0	JF803247.1	Tomato mottle leaf curl virus
1971	491,508	2632	100%	91.31%	0	ON419922.1	Tomato mottle leaf curl virus
2637	779,153	2631	96%	99.45%	0	JF803247.1	Tomato mottle leaf curl virus*
2721	533,294	2632	100%	95.3%	0	JF803247.1	Tomato mottle leaf curl virus*
2854	546,559	2647	94%	96.66%	0	JF803247.1	Tomato mottle leaf curl virus*
4921	496,154	2632	100%	93.71%	0	ON419894.1	Tomato mottle leaf curl virus*
4928	619,979	2638	100%	90.78%	0	JF803247.1	Tomato mottle leaf curl virus*
5158	502,357	2604	93%	88.17%	0	JF803247.1	Tomato mottle leaf curl virus*
5318	530,753	2632	100%	95.22%	0	JF803247.1	Tomato mottle leaf curl virus*
9807	516,677	2632	97%	95.56%	0	JF803247.1	Tomato mottle leaf curl virus*

Table 5: Discovery of single-stranded DNA satellites, gemonovirus e *Topilevirus* via High-Throughput Sequencing (HTS) in a pool of 129 leaf samples of botanical species from the Solanaceae family. Information was organized by the code number of the contigs corresponding to the subviral species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E-value, the correspondent GenBank (GB) accession, and description of the virus associated with each contig.

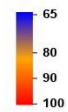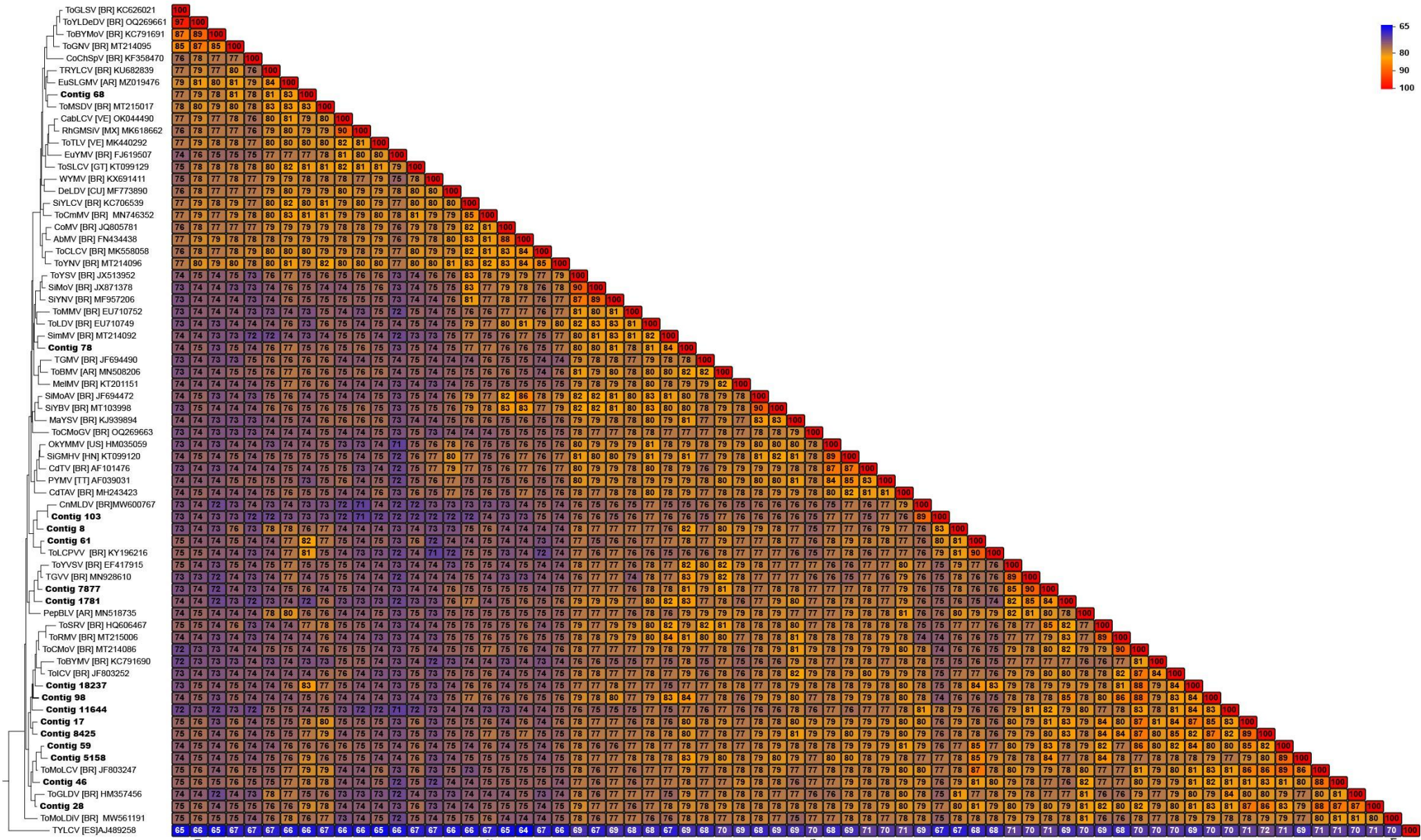| Contig	Reads	Size	Coverage	Identity	E-Value	GB accession	Virus description	Genus/family
5175	59	745	94%	92.96%	0	KY559642.1	Euphorbia yellow mosaic alphasatellite	*Begomovirus*
2046	355	1322	100%	99.92%	0	MT214093.1	Alphasatellitidae sp.	*Alphasatellitidae* sp.
18367	4	331	100%	99.67%	0	MT214094.1	Plant associated genomovirus 12	*Genomoviridae*
195	9,489	2874	100%	99.10%	0	MH539677.1	Tomato apical leaf curl virus	*Topilevirus*

Table 6: **DNA-A segments** identified via high-throughput sequencing (HTS) in a pool of 129 leaf samples from plants of the Solanaceae family with nucleotide identity values lower than 91%, which are corresponding to putative novel viral species. Information was organized by the code number of the contigs corresponding to the putative novel species, read coverage, genome size of the assembled virus, BLASTn coverage, nucleotide sequence identity of the assembled virus, E-value, the correspondent GenBank (GB) accession, and description of the virus closely related with each contig.

Contig	Read	Size	Coverage	Identity	E-Value	GB accession	Virus closely related
103	10.403	2669	100%	88%	0	MW600767	Cnidoscolus mosaic leaf deformation virus (CnMLDV)
68	186.988	2698	89%	80%	0	MZ019476	Euphorbia severe leaf golden mosaic virus (EuSLGMV)
78	134.112	2677	100%	85%	0	MT214092	Sida micrantha mosaic virus (SimMV)
17	707.063	2629	92%	89%	0	MT214086	Tomato chlorotic mottle virus (ToCMoV)
98	785.086	2677	90%	85%	0	MT214086	
8425	1.239.912	2623	100%	88%	0	MT214086	
11644	458.413	2618	93%	82%	0	MT214086	
18237	386.598	2666	92%	84%	0	MT214086	
1781	530.047	2665	98%	82%	0	MN928610	Tomato golden vein virus (TGVV)
7877	519.879	2590	97%	89%	0	MN928610	
8	52.032	2655	92%	79%	0	KY196221	Tomato leaf curl purple vein virus (ToLCPVV)
61	169.754	2629	100%	90%	0	KY196216	
28	891.545	2617	96%	88%	0	JF803247	Tomato mottle leaf curl virus (ToMoLCV)
46	468.234	2662	96%	87%	0	JF803247	
59	558.285	2626	92%	89%	0	JF803247	
5158	502.357	2604	93%	85%	0	JF803247	

Table 7: Details of recombination breakpoints detected in 14 of the 16 potential novel viral species. Contigs, event, greatest and least relatedness with identities (%), initial and final breakpoint, recombinant region, statistical methods and e-value. Recombination events described in R, G, B, M, C, S, T indicate detection by the RDP, GENCONV, BOOTSCAN, MAXCHI, CHIMAERA, SISCAN and 3SEQ methods, respectively, with the highest p-value presented being determined by the indicated method **bold.**

Contig	Event	Major parent (% identity)	Minor parent (% identity)	Breakpoint Begin (ORF)	Breakpoint End (ORF)	Recombinant region	Methods	p-value
08	01	Tomato yellow leaf deformation dwarf virus (77%)	Contig 103 (100%)	2002	2639	AC1(Rep) e C4	R **G** B M C S T	$3,547 \times 10^{-49}$
17	02	Tomato chlorotic mottle virus (ToCMoV) (95%)	Contig 28 (97%)	113	333	IR e AV1 (CP)	R **G** B M C S T	$1,801 \times 10^{-23}$
	03	Contig 59 (87%)	Tomato severe rugose virus (ToSRV) (99%)	623	1696	AV1 (CP), AC2 (Trap), AC3 (Ren), AC1 (Rep)	**R** G B M C S T	$5,238 \times 10^{-77}$
46	04	Contig 5158 (79%)	Contig 28 (95%)	311	816	AV1 (CP)	R **G** B M C S T	$3,717 \times 10^{-17}$
	05	Tomato mosaic severe dwarf virus (ToMSDV) (82%)	Contig 8425 (99%)	1820	2500	AC1 (Rep)	R **G** B M C S T	$1,515 \times 10^{-29}$
59	06	Contig 17 (94%)	Contig 5158 (99%)	57	539	RI e AV1 (CP)	R G **B** M C S T	$1,834 \times 10^{-46}$
	07	Contig 18237 (87%)	Contig 61 (99%)	540	1680	AV1 (CP), AC2 (Trap), AC3 (Ren), AC1 (Rep)	R G **B** M C S T	$6,144 \times 10^{-73}$
61	08	Unknown	Tomato mosaic severe dwarf virus (ToMSDV) (100%)	450	681	AV1 (CP)	R **G** B M C S T	$1,893 \times 10^{-10}$
	09	Tomato leaf curl purple vein virus (ToLCPVV) (94%)	Contig 5158 (100%)	799	1701	AV1 (CP), AC2 (Trap), AC3 (Ren), AC1 (Rep)	R **G** B M C S T	$1,308 \times 10^{-59}$
68	10	Unknown	Contig 28 (100%)	867	1305	AV1 (CP), AC2 (Trap), AC3 (Ren)	R G **B** M C S T	$2,088 \times 10^{-12}$
	11	Unknown	Contig 18237 (100%)	155	853	RI, AV1 (CP)	R G **B** M C S T	$4,737 \times 10^{-60}$
78	12	Sida micrantha mosaic virus (SMMV) (85%)	Contig 8 (99%)	900	2039	AV1 (CP), AC2 (Trap), AC3 (Ren), AC1 (Rep)	R G **B** M C S T	$1,972 \times 10^{-33}$
	13	Sida golden mosaic Honduras virus (SiGMHV) (81%)	Tomato golden vein virua (TGVV) (98%)	914	1592	AV1 (CP), AC2 (Trap), AC3 (Ren), AC1 (Rep)	R **G** B M C S T	$3,184 \times 10^{-40}$

98	14	Sida mosaic Alagoas virus (SiMoAV) (85%)	Contig 18237 (98%)	1293	1762	AC2 (Trap), AC3 (Ren), AC1 (Rep)	R G **B** M C S T	$9,682 \times 10^{-10}$
103	15	Unknown	Sida golden mosaic Honduras virus (SiGMHV) (81%)	1967	2157	AC1 (Rep)	R G B M **C** S T	$8,871 \times 10^{-8}$
1781	16	Unknown	7877 (99%)	1978	2630	AC1 (Rep), AC4	R G **B** M C S T	$6,943 \times 10^{-87}$
	17	Unknown (ToCMoV)	Contig 8425 (100%)	468	877	AV1 (CP)	R **G** B M C S T	$8,824 \times 10^{-29}$
5158	18	Unknown (SiMoV)	Contig 78 (99%)	1947	2416	AC1 (Rep) e AC4	**R** G B M C S T	$2,875 \times 10^{-25}$
7877	19	Tomato golden vein virus (TGVV) (97%)	Contig 18237 (99%)	1055	1314	AC2 (Trap) e AC3 (Ren)	R G **B** M C S T	$4,728 \times 10^{-28}$
11644	20	Tomato chlorotic mottle virus (ToCMoV) (87%)	Contig 103 (100%)	670	1083	AV1 (CP) e AC3 (Ren)	R G B M **C** S T	$2,063 \times 10^{-40}$
	21	Unknown (ToMoLCV)	Tomato chlorotic mottle virus (ToCMoV) (99%)	1084	1601	AC1 (Rep), AC2 (Trap), AC3 (Ren)	R G **B** M C S T	$4,262 \times 10^{-31}$
	22	Tomato chlorotic mottle virus (ToCMoV) (86%)	contig 7877 (100%)	1602	2044	AC1 (Rep) e AC4	**R** G B M C S T	$1,016 \times 10^{-44}$
	23	Cotton chlorotic spot virus (CoChSpV) (70%)	Tomato chlorotic mottle virus (ToCMoV) (94%)	2047	2612	RI, AC1 (Rep) e AC4	R G B M C S T	$2,413 \times 10^{-33}$
18237	24	Tomato chlorotic mottle virus (ToCMoV) (94%)	Tomato leaf curl purple vein virus (ToLCPVV) (96%)	88	458	RI e AV1 (CP)	R G B M C S T	$8,319 \times 10^{-79}$
	25	Unknown (Contig 59)	Contig 61 (100%)	2504	7	RI	R **G** B M C S T	$4,282 \times 10^{-51}$
	26	Tomato chlorotic mottle virus (ToCMoV) (97%)	Unknown	489	975	AV1	**R** G B M C **S** T	$5,449 \times 10^{-13}$

Figure 1: Pairwise identity analysis using the Sequence Demarcation Tool (SDT) software and root-mean Bayesian phylogenetic tree with GTR+I model with information on the DNA-A sequences of Begomovirus species obtained from the database of NCBI. These species are identified with an acronym. abbreviation of the countries where they were described and accession number. Viral names in full: tomato golden leaf spot virus isolate (ToGLSV (BR) - KC626021), tomato yellow leaf deformation dwarf virus (ToYLDeDV (BR) - OQ269661), tomato bright yellow mottle virus (ToBYMoV (BR) - KC791691), cotton chlorotic spot virus (CoChSpV (BR) - KF358470), Euphorbia severe leaf golden mosaic virus (EuSLGMV (AR) - MZ019476), contig 68, tomato mosaic severe dwarf virus (ToMSDV (BR) - MT215017), cabbage leaf curl virus (CabLCV (VE) - OK044490), Rhynchosia golden mosaic Sinaloa virus (RhGMSiV (MX) - MK618662), tomato twisted leaf virus (ToTLV (VE) - MK440292), Euphorbia yellow mosaic virus (EuYMV (BR) - FJ619507), tomato severe leaf curl virus (ToSLCV (GT) - KT099129), wissadula yellow mosaic virus (WYMV (BR) KX691411), Desmodium leaf distortion virus (DeLDV (CU) - MF773890), Sida yellow blotch virus (SiYBV (BR) - MT103998), tomato common mosaic virus (ToCmMV (BR) - MN746352), tomato chlorotic leaf curl virus (ToCLCV (BR) - MK558058), tomato yellow net virus (ToGNV (BR) - MT214095), Corchorus mottle virus (CoMV (BR) - JQ805781), Abutilon Brazil virus (AbMV (BR) - FN434438), Sida mosaic Alagoas virus (SiMoAV (BR) - JF694472), Sida yellow blotch virus (SiYBV (BR) -MT103998), tomato yellow spot virus (ToYSV (BR) - JX513952), Sida mottle virus (SiMoV (BR) - JX871378), Sida yellow net virus (SiYNV (BR) - MF957206), Sida micrantha mosaic virus (SiMMV (BR) - MF957204), contig 78, tomato mild mosaic virus ToMMV (BR) - EU710752, tomato leaf distortion virus (ToLDV (BR) EU710749), tomato yellow net virus (ToGNV (BR) - MT214096), potato yellow mosaic virus (PYMV (TT) - AF039031), Cnidoscolus mosaic leaf deformation virus (CnMLDV (BR) - MZ465583), contig 103, contig 8, contig 61, tomato leaf curl purple vein virus (ToLCPVV (BR) - KY196216), tomato yellow vein streak virus (ToYVSV (BR) - EF417915), tomato golden vein virus (TGVV (BR) - MT733806), contig 7877, contig 1781, pepper blistering leaf virus (PepBLV (AR) MN518735), tomato severe rugose virus (ToSRV (BR) - HQ606467), tomato rugose mosaic virus (ToRMV (BR) - MT215006), tomato chlorotic mottle virus (ToCMoV (BR) AF490004), tomato bright yellow mosaic virus (ToBYMV (BR) - KC791690), tomato interveinal chlorosis virus (ToICV (BR) - JF803252), contig 18237, contig 98, contig 11644, contig 8425, contig 59, contig 5158, tomato mottle leaf curl virus (ToMoLCV (BR) - ON419947), contig 46, tomato golden leaf distortion virus (ToGLDV (BR) -HM357456), contig 28, tomato mottle leaf distortion virus (ToMoLDiV (BR) - MW561191), tomato golden mosaic virus (TGMV (BR) - JF694490), tomato yellow leaf curl virus (TYLCV (ES) - AJ489258). Country abbreviations AR (Argentina), BR (Brazil), CU (Cuba), Spain (ES), United States (USA), Guatemala (GT), Uruguay (UY), Honduras (HN), Mexico (MX), Venezuela (VE), Trinidad and Tobago (TT).
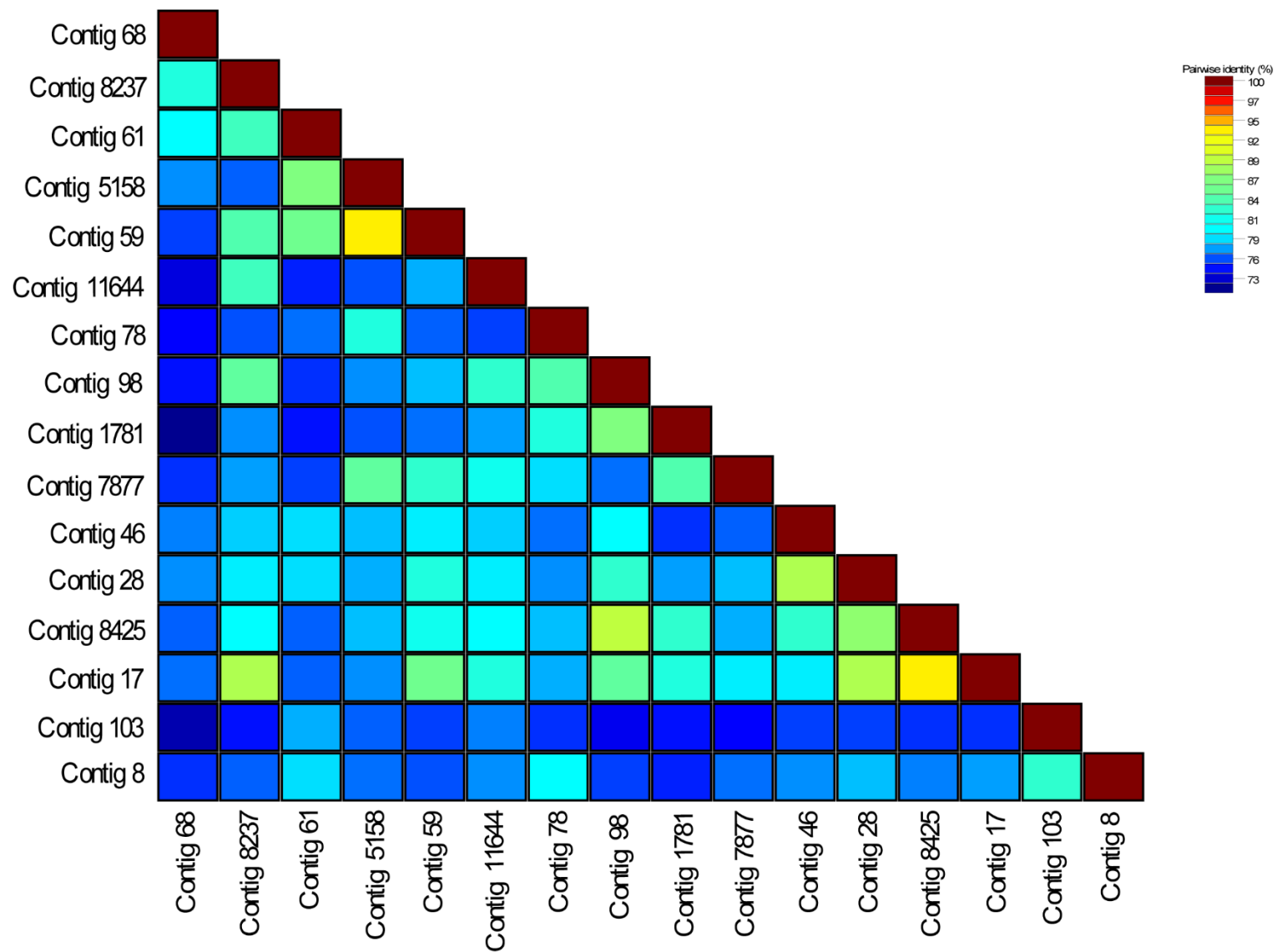
Figure 2: Pairwise identity analysis of *Begomovirus* DNA-A sequence information performed using Sequence Demarcation Tool (SDT) and the MUSCLE alignment. The nucleotide identity values ranged from 66 to 89%.

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CHAPTER 3

Characterization of tomato apical golden net virus and the recombinants tomato chlorotic net virus and tomato bright yellow mottle virus: Three novel Neotropical bipartite begomoviruses detected via High-Throughput Sequencing

To be submitted to Archives of Virology as Annotated Sequence

Characterization of tomato apical golden net virus and the recombinants tomato chlorotic net virus and tomato bright yellow mottle virus: Three novel Neotropical bipartite begomoviruses detected via High-Throughput Sequencing.

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Abstract

Begomoviruses (*Begomovirus*; Geminiviridae) can infect a wide range of Solanaceae hosts, particularly the tomato (*Solanum lycopersicum* L.) crop. Herein, three novel tomato-infecting begomoviruses were discovered in Mauá da Serra–PR (South Brazil; isolate PR–144) in Araguaina–TO (North Brazilian region; isolate TO–167) via High-Throughput Sequencing. Phylogenetic analyzes showed that PR–144 is closer related to melochia mosaic virus (KT201151), whereas TO–167 is a recombinant species closely related to tomato bright yellow mottle virus (KC791691). The genomes of these novel begomoviruses were also characterized via Sanger dideoxy sequencing and exhibited DNA–A and DNA–B components with structural features similar to New World bipartite begomoviruses. The names tomato apical golden net virus (PR–144), tomato chlorotic net virus (TO–167) and tomato bright yellow mottle virus are suggested (TO–167). Additional characterization studies with infectious clones of both species are necessary to clarify their host ranges and identify sources of resistance in tomato germplasm.

Geminiviridae (*Geplafuvirales*) is a family of highly diverse viruses that affect a wide range of plants, comprising fourteen genera: *Becurtovirus, Begomovirus, Capulavirus, Citlodavirus, Curtovirus, Eragrovirus, Grablovirus, Maldovirus, Mastrevirus, Mulcrinelvirus, Opunvirus, Topilevirus, Topocuvirus*, and *Turncurtovirus* comprising $\approx$ 520 viral species [1, 2]. The demarcation criteria for each of these genera include host range, insect vectors, genomic organization, and phylogenetic relationships [2, 3].

Begomovirus is the largest group of plant viruses belonging to the *Geminiviridae* family, with 445 species [1]. These viruses are mainly found in tropical and subtropical regions of the world [4]. The genomic organization can be composed of either monopartites, with a single DNA component generally composed of five open reading frames – ORFs (viral sense, V1 and V2 and complementary sense by C1, C2, C3, C4 and C5), or bipartites. containing two DNA components (DNA–A and DNA–B) composed of ORF AV1 (coat protein – CP), ORF AV2 (movement protein – MP), in viral sense. In a complementary sense, the genes: ORF AC1 (Rep), ORF AC2, (TrAP), ORF AC3 (REn), ORF AC4 (C4), ORF AC5, ORF AC6. DNA–B, whose genes are: ORF BV1 (NSP) and ORF BC1 (MP) in the complementary sense. Each component can measure from 2.5–2.6 kb [1, 2, 5, 6]. To be classified as a new monopartite or bipartite species, the new begomovirus must contain identity levels of the complete DNA–A genome lower than 91% when compared to any other species already validated [22]. *Begomovirus* transmission is carried out in a persistent, circulative and non-propagative manner [7, 8] by the cryptic species *Bemisia tabaci* Middle East Asia Minor 1 – MEAM1 (Hemiptera: Aleyrodidae) as well as other insect species of this complex [9]. Symptoms caused by these viruses in agricultural and vegetable crops are typically stunted growth, distorted leaves, mosaic/yellow spots, interveinal chlorosis, as well as smaller fruit size and poor quality [4]. Begomoviruses cause diseases of great economic importance to many hosts of the Solanaceae family [10] with the tomato crop being one of the most important in Brazil, with a production volume of approximately 3.8 million tons per year [11] In this way, a metagenomic analysis was carried out with the aim of describing three new viral species in tomato.

For these analyses, 154 leaf samples with typical symptoms of begomovirus were collected between 2005 and 2017 in the North, Northeast and South regions of the country. Total DNA was extracted using a modified protocol including a modified 2X CTAB buffer and organic solvents [12]. Subsequently, an aliquot of DNA obtained from each sample was used as a template for RCA (Rolling Circle Amplification) [13] and then, to confirm infection by begomovirus, the primers PAL1v1978/PAR1c496 were used for the DNA–A component and PBL1v2040-PRCc1 for the DNA–B component [14]. The samples were grouped into a single pool called BP1, in which the North (13), Northeast (36) and South (24) regions were covered, then the samples were submitted to HTS on the Illumina NovaSeq-6000 platform. of the sequencing were analyzed using the CLC Genomics Workbench 7.5 software (Qiagen) and subsequently analyzed using the Geneious® R11.1 software [15, 16, 17]. Reads were mapped to contigs. The genomes of each contig were extended with the help of the Geneious® R11.1 software together with the Map to Reference tool (90 to 99% minimum overlap identity

parameter) with the reads file from HTS. All contigs were subjected to comparisons with viral sequences present in local banks or GenBank using BLASTn algorithms. To evaluate identities with the aim of analyzing the closest begomoviruses, they were determined with the Species Demarcation Tool v.1.2 (SDT) [18]. The complete genomes were aligned using the MUSCLE tool [19] and used to create the DNA-based phylogenetic tree. The tree was generated with IQtree 2.2.0 software with Bayesian information criterion model TIM3+F+R5. The figures were created using Adobe Illustrator CC and EvolView software. To detect potential recombination events, the RDP 5 software [20] was used.

As results from HTS (Illumina NovaSeq-6000), 7,230,366 reads were obtained, 38,575 contigs of which 137 correspond to segments of viral genomes by analysis via BLASTn. Analysis of three of these contigs indicated that these sequences represent three potential new species. The putative new species #1 was detected in a sample identified as PR–144 collected in Mauá da Serra–PR in May 2015, while the putative new species #2 and putative new species #3 was identified in the sample TO–167 in Araguaina–TO (northern region of Brazil) collected in August 2008. The DNA–A components of the three begomoviruses exhibited 2612 nucleotides, 2657 nucleotides and 2621 nucleotides, respectively, with organization typical of New World bipartite species. The highest identity (80%) for putative new species #1 was with melochia mosaic virus (MelMV – KT201151), highest identity (80%) for putative new species #2 was with tomato bright yellow mottle virus (ToBYMoV – KC791691), highest identity (95%) for putative new species #3 was also with tomato bright yellow mottle virus (ToBYMoV – KC791691 (Figures 1 and 2). The putative new species #3 has already been worked on by our team, but it will be molecularly characterized in this work.

PCR assays with specific primers targeting the DNA–A component of each putative new species were designed using the HTS-derived genomic information. To detect the DNA–B component, the primer pair 'PBL1v2040' / 'PCRc1' [14] was initially used. The DNA–A and DNA–B components from TO–167 and PR–144 were detected after exhaustive PCR tests using total DNA templates extracted from the original field-collected samples. The complete DNA–A sequences of PR–144 isolate was confirmed via Sanger dideoxy sequencing employing primer pair specific for the virus. Sequencing of samples was performed by ACTGene Análises Moleculares Ltd. (Center for Biotechnology, UFRGS, Porto Alegre, RS, Brazil) using the automatic sequencer AB 3500 Genetic Analyzer with Genetic Analyzer with BigDye® Terminator Cycle Sequencing Ready Reaction Kit version 3.1 protocol.

For PR–144, a DNA–B component of 2565 nucleotides was obtained and showed 82.05% with tomato interveinal chlorosis virus and TO–167 displayed a DNA–B component

of 2619 nucleotides with a maximum identity of 87.62% identity with tomato yellow leaf deformation dwarf virus.

The conserved sequence found in most viruses of the *Geminiviridae* family, 5'TAATATTAC-3' located at the origin of replication in the intergenic region (IR), was found in the DNA–A components of both putative new species. Five ORFs were found in DNA–A, of the putative new species detected in the sample PR–144: one in the viral sense AV1 with 765 nucleotides encoding the coat protein gene–CP. In the complementary sense, four ORFs were found: AC1 gene encoding the replication-associated protein with 1047 nucleotides, AC2 gene encoding the transcription activating protein (Trap) with 390 nucleotides, AC3 replication enhancer gene (Ren) with 399 nucleotides and AC4 being the gene that assists in symptom development with 258 nucleotides, for PR–144.

The putative new begomovirus #2 detected in the sample TO–167 displayed the same ORFs with sizes of AV1 (756 nucleotides), AC1 (1086 nucleotides), AC2 (390 nucleotides), AC3 (399 nucleotides), AC4 (255 nucleotides) and AC5 (252 nucleotides) in which the function is associated with pathogenicity and suppression of gene silencing. For the putative new begomovirus #3, five ORFs were found in DNA–A component present in the sample TO–167, one in the viral sense AV1 with 756 nucleotides encoding the coat protein (CP) gene. In the complementary sense, four ORFs were annotated: the AC1 gene (encoding the replication-associated protein with 1080 nucleotides; the AC2 gene encoding the transcription activating protein (Trap) with 390 nucleotides, the AC3 (replication enhancer Ren gene) with 399 nucleotides, and the AC4 (involved in symptom development) with 252 nucleotides. Furthermore, our analyses allowed the identification in the genomes of PR–144 and TO–167 the iterons GGTAC (Rep IRD = MPSHPKRFQIN) and GGAGT (Rep IRD = MPLPPKSFRLQ) [21], respectively. The putative new begomovirus #2 and putative new begomovirus #3 show that they are pseudorecombinants, since they have the same iteron.

The pairwise nucleotide identities of the DNA–A genomes of these new species with those of other begomoviruses were estimated using the Species Demarcation Tool (SDT). Analyzes showed putative new begomovirus #1 shares a range of 73–83%, whereas, putative new begomovirus #2 shares a range of 78–80% and putative new begomovirus #3 share a range of 95% – 73% (Figure 1). Consistent with the initial HTS results, our phylogenetic analysis showed that TO–167 is closer related to ToBYMoV (KC791691) and tomato yellow leaf deformation dwarf virus (ToYLDeDV – OQ269661). In turn, the new PR–144 displayed closer relationship with the legume-infecting MelMV (KT201151) and tomato leaf curl purple vein virus (KY196216) (Figure 1).

Recombination analyzes with RDP5 were also performed. No evidence of recombination events was detected for PR–144. On the other hand, the genome of putative new species #2 displayed evidences of recombination events according to four statistical methods: RDP ($pvalue = 3,779 \ x \ 10^{-03}$), MaxChi ($p\text{-}value = 1,759 \ x \ 10^{-15}$), Chimaera ($p\text{-}value \ 1,126 = x \ 10^{-8}$) e 3Seq ($p\text{-}value = 4,714 \ x \ 10^{-8}$) showed greater kinship with ToMoLCV – ON419947 and lower with tomato severe rugose virus (ToSRV – HQ606467). The initial break point was located at nucleotide 1122 and the end point at nucleotide 1842, involving the end to the middle segment of the Rep gene, the entire Trap and a portion of the Ren gene.

For the putative new species #3 recombination analyzes with RDP 5 were also performed and presented recombination events according to seven statistical methods: RDP (p-value = 2.375 x 10 − 36), GENECONV (p-value = 1.618 x 10^{-23}), BootScan (p-value = 7.186 x 10^{-36}), MaxChi (p-value = 7.026 x 10^{-15}), Chimaera (p-value = 3.631 x 10^{-14}), SiScan (p-value = 6.265 x 10^{-14}), and 3Seq (p-value = 4.352 x 10^{-12}). Our recombination analyzes showed that this putative new begomovirus is closely related to ToBYMoV (KC791691) with 98.4% of similarity and least related to tomato mottle leaf curl virus (ToMoLCV – ON419947) with 96% of similarity. The initial recombination break point was located at nucleotide #1481 and the final break point at nucleotide #1959, involving segments of the Rep and final Trap sequences.

Thus, these three putative new species of bipartite begomoviruses were found by PCR assays with specific primers in field-collected foliar samples of tomato. PR–144 and TO–167 displayed a genomic organization typical of the New World. It is possible to observe that the majority of begomoviruses that infect tomatoes in Neotropical areas have bipartite genomes, with only a small fraction of monopartite species [26]. To date, tomato mottle leaf curl virus (ToMoLCV), tomato leaf curl purple vein virus (ToLCPVV), tomato golden net virus (ToGNV) and tomato yellow net virus (ToYNV) have been reported as monopartite species infecting tomatoes in Brazil with all of them displaying genomic features of other New World begomoviruses [23, 24, 25, 26]

In the present work, the names tomato apical golden net virus (for isolate PR – 144), tomato chlorotic net virus and tomato bright yellow mottle virus (for the recombinant isolates detected in sample TO – 167) are suggested. Most begomoviruses that infect tomato in Neotropical areas have bipartite genomes, with some monopartite [24]. In our genomic analyses, isolates PR-144 and TO-167 lack the V2 (suppressor of silencing/movement) protein, which is present in monopartite and bipartite Old World begomoviruses [25]. Therefore, it is possible to infer that New World viral species may have a different mechanism to perform V2-

related functions, such as the use of a DNA-B component for viral movement. Pseudorecombination is a mechanism deployed by some bipatite viral species to carry out V2-related functions [26, 27]. In this mechanism, a single genomic segment of DNA-B is shared by distinct viruses [28]. Studies carried out indicate that ToRMV and ToSRV isolates are capable of forming viable pseudorecombinants in their natural host, the tomato (Solanum lycopersicum) [28]. In our molecular analyses, tomato chlorotic net virus - ToCNV was detected in sample TO-167 in a mixed infection with tomato bright yellow mottle virus - ToBYMoV, suggesting that they may be sharing the DNA-B segment.

However, additional studies with specific DNA-B primers are needed to prove that tomato chlorotic net virus and tomato bright yellow mottle virus share the same DNA-B sequence. Furthermore, biological characterization with infectious clones is also necessary to clarify their host range and identify sources of resistance in tomato germplasm.

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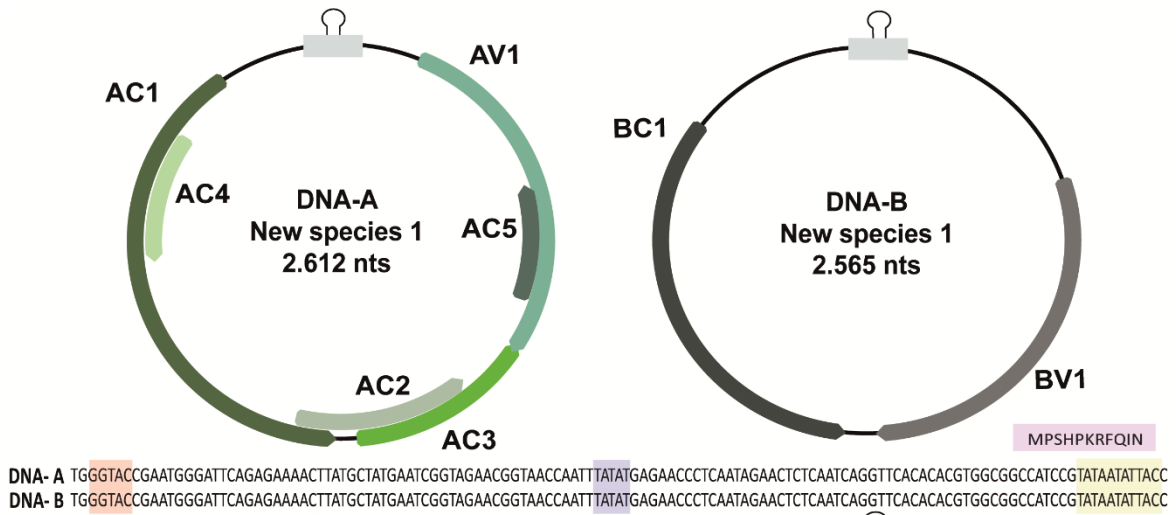
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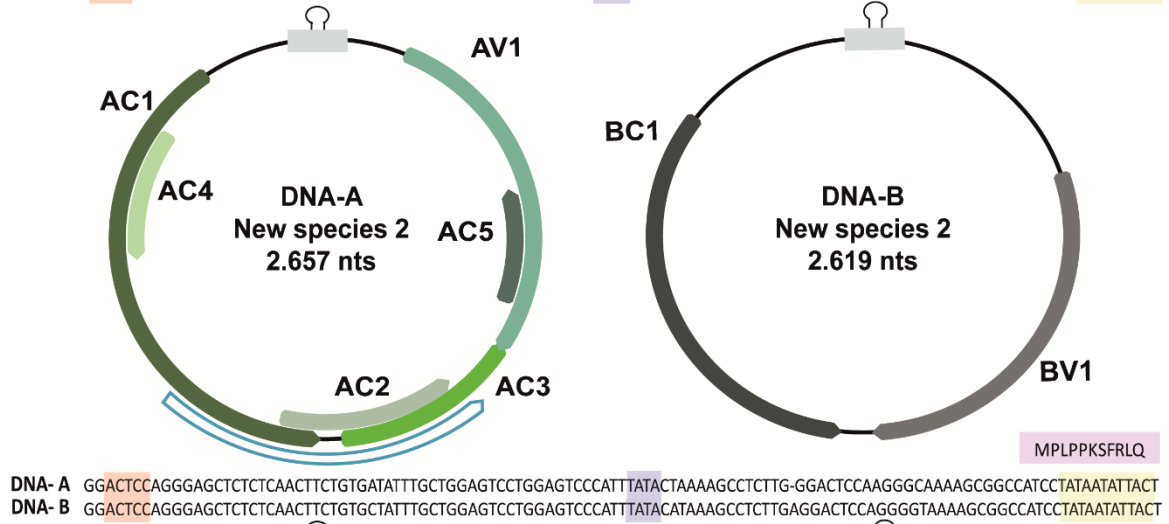
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A

DNA-A New species 1 2.612 nts
AV1, AC5, AC3, AC2, AC4, AC1

DNA-B New species 1 2.565 nts
BC1, BV1

MPSHPKRFQIN

DNA- A TGGGTACCGAATGGGATTCAGAGAAAACTTATGCTATGAATCGGTAGAACGGTAACCAATTTATATGAGAACCCTCAATAGAACTCTCAATCAGGTTCACACACGTGGCGGCCATCCGTATAATATTACC
DNA- B TGGGTACCGAATGGGATTCAGAGAAAACTTATGCTATGAATCGGTAGAACGGTAACCAATTTATATGAGAACCCTCAATAGAACTCTCAATCAGGTTCACACACGTGGCGGCCATCCGTATAATATTACC

B

DNA-A New species 2 2.657 nts
AV1, AC5, AC3, AC2, AC4, AC1

DNA-B New species 2 2.619 nts
BC1, BV1

MPLPPKSFRLQ

DNA- A GGACTCCAGGGAGCTCTCTCAACTTCTGTGATATTTGCTGGAGTCCTGGAGTCCCATTTATACTAAAAGCCTCTTG-GGACTCCAGGGGCAAAAGCGGCCATCCTATAATATTACT
DNA- B GGACTCCAGGGAGCTCTCTCAACTTCTGTGTGCTATTTGCTGGAGTCCTGGAGTCCCATTTATACATAAAGCCTCTTGAGGACTCCAGGGGGTAAAAGCGGCCATCCTATAATATTACT

C

DNA-A New species 3 2.621 nts
AV1, AC3, AC2, AC4, AC1

DNA-B New species 3 2.619 nts
BC1, BV1

MPLPPKSFRLQ

DNA - A CTGGAGTCCTGGAGTCCCATTTATACTAAAAGCCTCTTG-GGACTCCAAGGGCAAAAGCGGCCATCCTATAATATTACT
DNA - B CTGGAGTCCTGGAGTCCCATTTATACATAAAGCCTCTTGAGGACTCCAGGGGGTAAAAGCGGCCATCCTATAATATTACT

CP, Rep, TrAp, REn, RC, AC4, AC5, NSP, MP, Recombination event, Iteron, Região TATA, Nononucleotídeo, Rep IRD
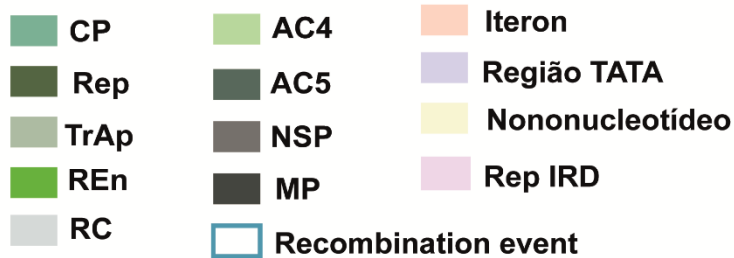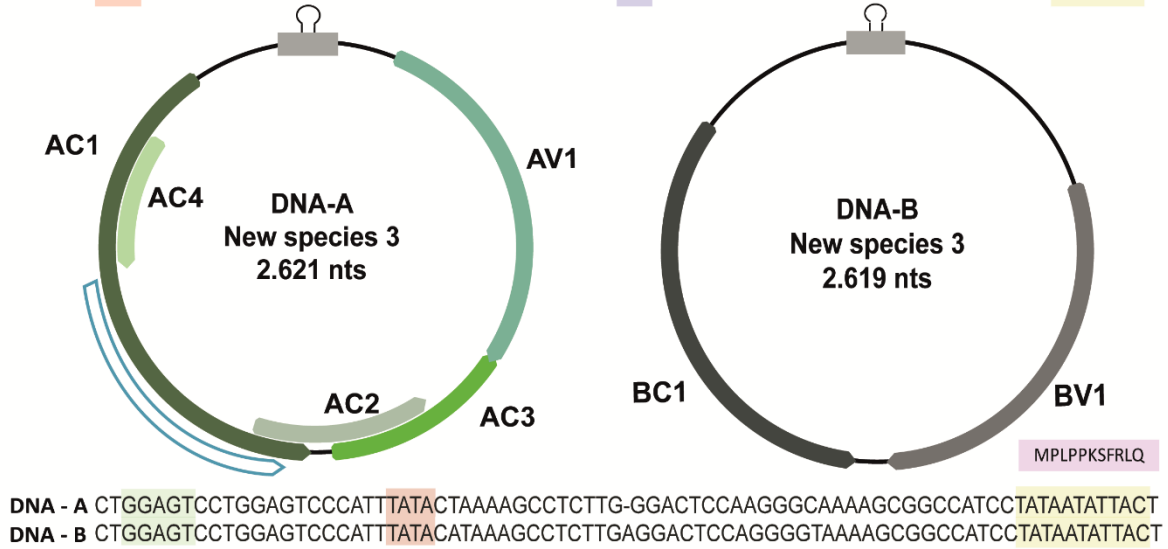
Figure 1: Genomic organization of the three new *Begomovirus* species reported infecting tomato crops in Brazil. Diagrammatic representation of the circular genomes of new PR–144 **(A)**, TO–167 (tomato chlorotic net virus) **(B),** and TO–167 (tomato bright yellow mottle virus) **(C)** and their respective open reading frames and (ORFs) and details of the genomic region that encompasses the Iteron, TATA region and the terminal Rep = IRD (Rep Iteron-Related Domain. The new species apical golden net virus – ToAGNV (PR–144) and tomato chlorotic net virus – ToCNV (TO–167) have ORFs AV1 gene encoding the viral coat protein (CP); AV2, responsible for the movement protein (MP); AC1 viral replication-associated protein (Rep); AC2 encodes transcription activator protein (TrAp); AC3 encodes replication enhancer protein (Ren); AC4 related to symptoms and AC5 related to suppression of gene silencing and pathogenicity. CR = common region, encompassing the clamp. The third species is a monopartite with the ORFs AV1, AC1, AC2, AC3, and AC4, these genes have the same functions as previously described.
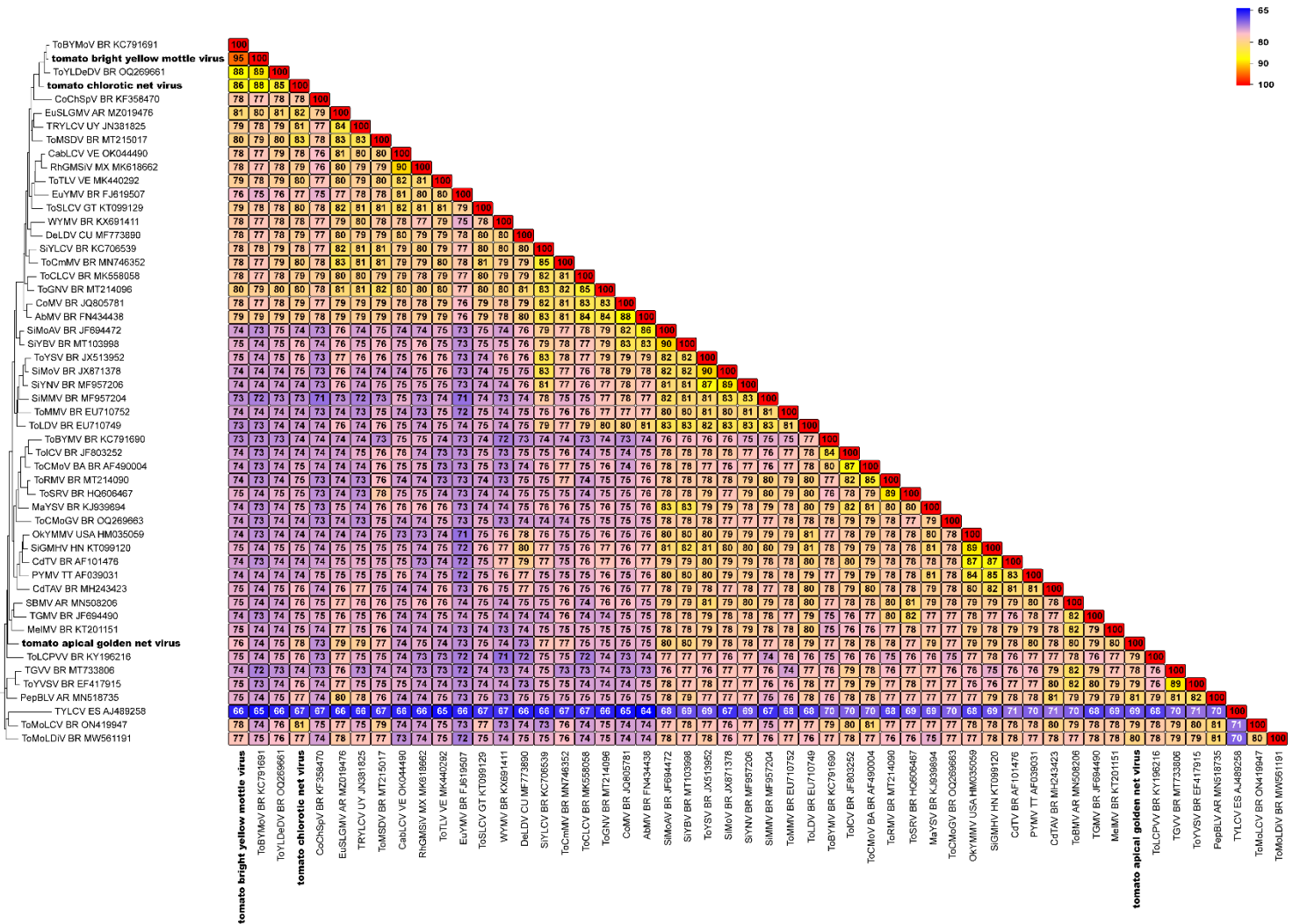
Figure 2: Pairwise identity analysis using the Sequence Demarcation Tool (SDT) software and root-mean Bayesian phylogenetic tree with GTR+I model with information on DNA–A sequences of *Begomovirus* species obtained from the NCBI database. These viral species are identified with an acronym, abbreviation of the countries where they were described, and their accession numbers. Viral names in full:tomato yellow leaf deformation dwarf virus (ToYLDeDV BR OQ269661), tomato bright yellow mottle virus (ToBYMoV BR KC791691), tomato chlorotic net virus, cotton chlorotic spot virus (CoChSpV (BR) KF358470), Euphorbia severe leaf golden mosaic virus (EuSLGMV AR MZ019476), tomato mosaic severe dwarf virus (ToMSDV BR MT215017), cabbage leaf curl virus (CabLCV VE OK044490), Rhynchosia golden mosaic Sinaloa virus (RhGMSiV MX MK618662), tomato twisted leaf virus (ToTLV VE MK440292), Euphorbia yellow mosaic virus (EuYMV BR FJ619507), tomato severe leaf curl virus (ToSLCV GT KT099129), wissadula yellow mosaic virus (WYMV BR KX691411), Desmodium leaf distortion virus (DeLDV CU MF773890), Sida yellow blotch virus (SiYBV BR MT103998), tomato common mosaic virus (ToCmMV BR MN746352), tomato chlorotic leaf curl virus (ToCLCV BR MK558058), tomato yellow net virus (ToGNV BR MT214095), Corchorus mottle virus (CoMV BR JQ805781), Abutilon Brazil virus (AbMV BR FN434438), Sida mosaic Alagoas virus (SiMoAV BR JF694472), Sida yellow blotch virus (SiYBV BR MT103998), Sida mottle virus (SiMoV BR JX871378), Sida yellow net virus (SiYNV BR MF957206), Sida micrantha mosaic virus (SiMMV BR MF957204), tomato mild mosaic virus (ToMMV BR EU710752), tomato leaf distortion virus (ToLDV BR EU710749), tomato yellow net virus (ToGNV BR MT214096), tomato chlorotic mottle Guyane virus (ToCMoGV BR OQ269663), okra yellow mosaic Mexico virus (OkYMMV USA HM035059), Sida golden mosaic Honduras virus (SiGMHV HN KT099120), chino del tomate virus (CdTV BR AF101476), Cnidoscolus mosaic leaf deformation virus (CnMLDV BR MZ465583), tomato yellow vein streak virus (ToYVSV BR EF417915), tomato severe rugose virus

(ToSRV BR HQ606467), tomato rugose mosaic virus (ToRMV BR MT215006), tomato chlorotic mottle virus (ToCMoV BR AF490004), tomato bright yellow mosaic virus (ToBYMV BR KC791690), tomato interveinal chlorosis virus (ToICV BR JF803252), tomato golden leaf distortion virus (ToGLDV BR HM357456), potato yellow mosaic virus (PYMV TT AF039031), chino del tomate Amazonas virus (CdTAV BR MH243423), tomato blistering mosaic virus (ToBMV BR KJ940970), tomato golden mosaic virus (TGMV BR JF694490), tomato leaf curl purple vein virus (ToLCPVV BR KY196216), tomato apical golden net, tomato yellow spot virus (ToYSV BR JX513952), tomato golden vein virus (TGVV BR MT733806), pepper blistering leaf virus (PepBLV AR MN518735), tomato yellow leaf curl virus (TYLCV (ES) AJ489258), tomato mottle leaf curl virus (ToMoLCV BR ON419947), tomato mottle leaf distortion virus (ToMoLDiV BR MW561191). Country abbreviations: AR (Argentina), BR (Brasil), CU (Cuba), Espanha (ES), Estados Unidos (EUA), Guatemala (GT), Uruguai (UY), Honduras (HN), México (MX), Venezuela (VE), Trinidad e Tobago (TT).