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Tomato severe rugose virus: Phylogeny, phylogeography, and identification of new natural hosts

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Advisor: Dr. Rita de Cássia Pereira Carvalho.

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Resumo Geral

PEREIRA-SILVA, JULIANA. Universidade de Brasília, março de 2021. **Tomato severe rugose virus: Filogenia, filogeografia e identificação de novas hospedeiras.** Orientadora: Profª Drª. Rita de Cássia Pereira-Carvalho.

O tomateiro (*Solanum lycopersicum*) é severamente afetado por diversos begomovírus (família *Geminiviridae*) que apresentam DNA de fita simples (ssDNA) com um (= monopartidas) ou dois (= bipartidas) componentes genômicos. A transmissão dos begomovírus ocorre por meio de vetores aleirodídeos do complexo *Bemisia tabaci*. O uso de variedades portadoras de genes de resistência é a estratégia mais eficiente para o manejo de doenças ocasionadas por begomovírus. No Brasil, os genes *Ty*-1 e *Ty*-3 têm sido os mais amplamente utilizados nos cultivos do tomateiro. Dentre os 21 begomovírus já formalmente descritos em tomateiro no país, tomato severe rugose virus (ToSRV) é o mais amplamente disseminado pelas principais regiões produtoras. ToSRV apresenta hospedeiros naturais e experimentais alternativos dentro das famílias Solanaceae, Fabaceae, Amaranthaceae (= Chenopodiaceae) e Malvaceae. No presente trabalho, caracterizações moleculares de isolados de ToSRV de potenciais novas hospedeiras **(Capítulo 2)** foram conduzidas por meio de ensaios de PCR usando primers universais e específicos para regiões dos dois componentes genômicos (DNA–A e DNA–B) e em combinação com sequenciamento via Sanger. Essa estratégia permitiu a caracterização de quatro isolados de ToSRV em quatro novas hospedeiras (*Pachyrhizus erosus*, *Oxalis latifolia*, *Solanum betaceum* e *S. torvum*) coletadas em condições naturais de campo. Os resultados expandem o círculo de plantas hospedeiras do vírus e reforçam a noção de que a ampla gama de hospedeiros de ToSRV pode desempenhar papéis biológicos e epidemiológicos relevantes, permitindo a ampla dispersão geográfica e grande frequência deste vírus em lavouras de tomate no Brasil. O ToSRV apresenta uma alta capacidade adaptativa e prevalência em condições de campo, especialmente no Centro-Oeste e Sudeste do Brasil. No entanto, até o momento não há relato oficial de ToSRV infectando tomateiros nas regiões Norte, Nordeste e na maioria dos estados do Sul do Brasil. Noventa e nove das 120 sequências de isolados ToSRV com DNA–A completo disponíveis no GenBank, correspondem a isolados coletados em tomateiro provenientes das regiões Centro-Oeste (15 isolados) e Sudeste (84 isolados), correspondendo aos Biomas Cerrado e Mata Atlântica. Além disso, 12 isolados foram coletados em plantas hospedeiras alternativas. Com intuito de fornecer novas informações sobre a diversidade genômica, distribuição geográfica e o potencial impacto do emprego de genes de resistência na prevalência de determinadas populações de ToSRV, trabalhos de caracterização molecular foram conduzidos por meio de ensaios de PCR com primers específicos para ToSRV em combinação com sequenciamento via Sanger de 105 novos isolados coletados em amostras sintomáticas de tomateiro provenientes das regiões Norte (1 isolado), Nordeste (2 isolados), Centro-Oeste (36 isolados), Sudeste (46 isolados) e Sul (20 isolados). Análises filogeográficas foram realizadas **(Capítulo 3),** visando analisar a distribuição geográfica e dispersão desta espécie nas diferentes regiões/biomas brasileiros utilizando os isolados caracterizados no presente trabalho (n=105). Foram realizadas, concomitantemente, análises para a verificação da presença em plantas contendo os genes de resistência *Ty*-1 e *Ty*-3 em associação com os isolados ToSRV. Em nosso levantamento, ToSRV foi identificado em 48 cidades em 11 estados e no Distrito Federal (DF). Os resultados indicam os primeiros relatos da presença de ToSRV nas regiões Norte (Tocantins), Nordeste (Ceará e Pernambuco) e Sul do Brasil (Santa Catarina

e Rio Grande do Sul). Na região Centro-Oeste são apresentados aqui, os primeiros relatos para cinco cidades do estado de Góias (GO) e três regiões produtoras no DF. Na região Sudeste, ToSRV foi registrado pela primeira vez em sete cidades do estado de Minas Gerais (MG) e em oito cidades de São Paulo (SP). Esses resultados indicam que ToSRV é uma espécie com ampla adaptação ao tomateiro e com um padrão de dispersão não regionalizado. ToSRV está atualmente se disseminando para outras regiões com considerável capacidade adaptativa a diferentes condições climáticas, em contraste com outras espécies endêmicas de begomovírus reportadas infectando o tomateiro em algumas regiões do Brasil.

PALAVRAS-CHAVE: *Geminiviridae, Begomovirus, Tomato severe rugose virus*

General Abstract

PEREIRA-SILVA, JULIANA. Universidade de Brasília, março de 2021. **Tomato severe rugose virus: Phylogeny, phylogeography, and identification of new natural hosts.** Advisor: Dr. Rita de Cássia Pereira-Carvalho.

The tomato (*Solanum lycopersicum*) crop is severely affected by several single-stranded DNA (ssDNA) begomoviruses (family Geminiviridae) that may have either one (= monopartite) or two (= bipartite) genomic components. The transmission of begomoviruses is done by members of the *Bemisia tabaci* complex. The use of resistant varieties is the most efficient strategy for the management of diseases caused by begomovirus. In Brazil, the *Ty*-1 and *Ty*-3 genes have been the most widely deployed in tomato crops. Among the 21 begomoviruses already formally described infecting tomato in the country, tomato severe rugose virus (ToSRV) is the most widely disseminated. ToSRV presents alternative natural and experimental hosts within the Solanaceae, Fabaceae, Amaranthaceae (= Chenopodiaceae) and Malvaceae families. In the present work, molecular characterizations of potential new ToSRV isolates from four putatively novel hosts (**Chapter 2**) were conducted via PCR assays using universal as well as specific primers targeting regions of their genomic components (DNA–A and DNA–B) in combination with Sanger dideoxy sequencing. This strategy allowed the characterization of four ToSRV isolates in four new hosts (viz. *Pachyrhizus erosus*, *Oxalis latifolia*, *Solanum betaceum*, and *S. torvum*) collected in natural field conditions. The results expand the host range of this virus and reinforce the notion that the wide range of ToSRV hosts can play relevant biological and epidemiological roles explaining, at least in part, its wide geographical dispersion and high frequency of this virus in tomato crops across many Brazilian regions. ToSRV has a high adaptive capacity and prevalence in field conditions, especially in the Midwest and Southeast regions. However, to date, there is no official report of ToSRV in tomatoes in the North and Northeast regions as well as in most of the states from the South region of Brazil. Ninety-nine out of the 120 sequences from ToSRV isolates with complete DNA–A available on GenBank, correspond to isolates from tomatoes collected in the Midwest (15 isolates) and Southeast (84 isolates) regions, encompassing areas belonging to the Cerrado and Atlantic Rain Forest Biomes. Also, 12 isolates were collected from alternative host plants. To provide new information on genomic diversity, geographic distribution, as well as the potential impact of the use of resistance genes on the prevalence of ToSRV populations, molecular characterization studies were conducted using PCR assays with virus-specific primers in combination with sequencing via Sanger of 105 new isolates. This virus collection was obtained from symptomatic tomato samples of the North (1 isolate), Northeast (2 isolates), Midwest (36 isolates), Southeast (46 isolates), and South (20 isolates) regions. Phylogeographic analyzes were performed **(Chapter 3)**, aiming to study the geographical distribution and dispersion of ToSRV across different Brazilian regions/biomes. Concomitantly, molecular analyzes were performed to verify the presence in plants containing the *Ty*-1 and *Ty*-3 resistance genes in association with the ToSRV isolates. In our survey, ToSRV was identified in 48 cities in 11 states and the Federal District (DF). Here, ToSRV was first reported in the North (Tocantins), Northeast (Ceará and Pernambuco) and South (Santa Catarina and Rio Grande do Sul) regions of Brazil. In the Midwest region, first reports of ToSRV were done in five cities in the state of Góias (GO) and three producing sectors in the Federal District (DF). In the Southeast region,

ToSRV was reported for the first time in seven cities in Minas Gerais (MG) state and eight cities in São Paulo (SP) state. These results indicate that ToSRV is a begomovirus with wide adaptation to tomatoes and displays a non-regionalized dispersion pattern. ToSRV is currently spreading to other regions with considerable adaptive capacity to different climatic conditions, in contrast to other endemic begomoviruses reported to infect tomato only in some regions of Brazil.

KEY-WORDS: *Geminiviridae*, *Begomovirus*, *Tomato severe rugose virus*

GENERAL INTRODUCTION

The tomato (*Solanum lycopersicum* L.) fruits are important sources of antioxidants, vitamins C and E as well as iron, phosphorous and carotenoid antioxidants such as β-carotene (also a vitamin A precursor) and lycopene. This vegetal crop is native from the western coast of South America, encompassing territories belonging to Ecuador, Peru, and the northern portion of Chile. After its domestication in Mexico, the tomato was introduced into Europe and subsequently disseminated to various regions of the globe (INCAPER, 2010). Tomato is considered as one of the main crops and one of the most consumed vegetables in the world, having also social and economic relevance in Brazil (IBGE 2020). Currently, the tomato production in Brazil is around 4.1 million tons per year covering ≈ 58170 hectares, placing the country in the 9th position in the world ranking (FAOSTAT 2020).

Many diseases are able to induce significant damage to the tomato crop in Brazil and worldwide (Lopes and Ávila 2005; Jones 2014; Ong et al. 2020). In relation to diseases of viral etiology, it is particularly difficulty to establish effective control strategies due to a series of biological features of this group of pathogens. In Brazil, members of the genus *Begomovirus* (Family: *Geminiviridae*) have been reported in tomato fields with incidence of up to 100% (Inoue-Nagata et al. 2016) and inducing losses in tomato of up to 100% (Lopes and Reis 2011). Begomoviruses are characterized by circular genomes, consisting of either one (= monopartite species) or two (= bipartite species), single-stranded DNA (ssDNA) molecules with size ranging from 2500 to around 2600 nucleotides (nts), encapsided in separated small particles (18-30 nm) (Rybicki et al. 2000; Rojas et al. 2018; ICTV 2021). The transmission of begomovirus under natural conditions is accomplished by insect members of the *Bemisia tabaci* (Genn.) (Family Aleyrodidae) cryptic species complex in a persistent, circulative, and non-propagative manner (Rybicki et al. 2000; Hogenhout et al. 2008; De Barro et al. 2010), even though alternative virus-vector relationships have been also proposed (Czosnek et al. 2017). Thus far, ≈160 begomoviruses have been characterized infecting tomatoes around the world (ICTV 2021), including the 21 described in Brazil (Matys et al. 1975; Ribeiro et al. 2007, 2003; Fernandes et al. 2006, 2008a; Calegario et al. 2007; Castillo-Urquiza et al. 2008; Albuquerque et al. 2012; Macedo et al. 2018; Rego-Machado et al. 2019; ICTV 2021).

Tomato severe rugose virus (ToSRV) is the most widespread tomato-infecting begomovirus across two major Brazilian biomes (Cerrado and Atlantic Rain Forest), displaying high adaptative capacity and prevalence under field conditions, especially in Central Brazil

(Reis et al 2020; Duarte et al. 2021). This virus has been also reported in Paraná state in the South region (Fernandes-Acioli et al. 2014) and the South-East region of the country (Fernandes et al. 2008a; Duarte et al., 2021). However, there is no official report of ToSRV infecting tomatoes in some of the Southern states (Rio Grande do Sul and Santa Catarina) as well as in states in the Northeast region and in the entire Northern region of the country (Fernandes et al. 2008a).

The introduction of the vector species *B. tabaci* Middle East Asia Minor 1 – MEAM1 (= *B. tabaci* biotype B) in the early 1990's allowed the gradual dissemination of novel begomoviruses across different Brazilian regions (Cotrim et al. 2007). The invasion of this vector is most likely the major biological event that contributed to the nationwide spread of ToSRV not only in tomato crops but also among alternative hosts such as beans (*Phaseolus vulgaris* L.) (Macedo et al. 2017a), soybeans (*Glycine max* L.) (Macedo et al. 2017b), *Nicandra physalodes* (L.) Gaertn. (Barreto et al. 2013), *Capsicum* species (Bezerra-Agasie et al. 2006), eggplants (*Solanum melongena* L.) (Moura et al. 2018), and *Physalis angulata* L. (Duarte et al. 2019). In addition, the polyphagous nature of this vector in association with genetic mechanisms able to generate variability in begomoviruses have been contributed to substantial emergence of novel tomato-infecting begomoviruses in Brazil and around the world (Seal et al. 2006; Duffy and Holmes 2008). In fact, the mutational dynamics due to the incorrect incorporation of nucleotides during viral replication and recombination events for distinct viral genomic components are considered to be the most important mechanisms generating diversity in begomovirus populations (Rocha et al. 2013; Lima et al. 2017).

The most efficient strategy for the management of diseases induced by begomovirus is the use of varieties carrying resistant genes/alleles. Currently, the following set of resistance genes is available for breeding purposes including the dominant genes *Ty*-1 (Zamir et al. 1994), *Ty*-2 (Hanson et al. 2006), *Ty*-3 (Ji et al. 2007b), *Ty*-4 (Ji et al. 2009) and *Ty*-6 (Gill et al. 2019) and also the recessive genes *tcm*-1 (Giordano et al. 2005), *tgr*-1 (Bian et al. 2007) and *ty*-5 (Anbinder et al. 2009). In Brazil, the *Ty*-1 gene was found to confer a tolerance response to several begomoviruses (Boiteux et al. 2007b; Reis et al. 2020). In fact, *Ty*-1 (sometimes in combination with the gene/allele *Ty*-3) has been widely used in novel fresh-market tomato cultivars due to its wide-spectrum action against a range of bipartite and monopartite species (Boiteux et al. 2007; Pereira-Carvalho et al. 2014). In this scenario, the growing employment of the dominant *Ty*-1 gene may represent a new selection factor that can be potentially able to

reshape the genetic structure of the begomovirus populations across the distinct Brazilian and global agroecosystems.

Studies on the geographical distribution and impact of the use of resistance genes on the prevalence of ToSRV throughout Brazil are yet scarce, but they will provide crucial information from the plant breeding standpoint, aiming to identify and to deploy genetic resistance factors with greater stability, durability, and efficiency. Phylogeographic data will also provide relevant information about viral species prevalence in space and time across the major tomato-producing regions. In fact, little is known about the patterns of ToSRV dissemination across the Brazilian territory as well as its microevolutionary history. Expanding the knowledge about the natural host of range of ToSRV is also a critical information for cultural management of the disease caused by this virus. Information related to these topics will be exposed in the present work.

HYPOTHESES

- A possible epidemiological advantage of ToSRV is due to its ability to infect a wider range of hosts, including species outside the Solanaceae family that occurs side-by-side with tomato across distinct Brazilian biomes;
- ToSRV is highly adapted to a wide range of environmental and crop management conditions and it is likely that this virus is already present across all Brazilian states, including the ones where it has not been detected thus far such as in the Southern region (Rio Grande do Sul and Santa Catarina) as well as in Northeast region and also in the entire Northern region of the country, and
- ToSRV is highly adapted to distinct genetic makeup of the currently employed tomato hybrids, being able to infect and cause either mild or severe symptoms even in plants carrying the resistant factors *Ty*-1 and *Ty*-3.

OBJECTIVES

General Objective

- To provide novel information on the natural host range, genomic diversity, geographical distribution, and potential impact of the use of resistance genes on the prevalence of ToSRV throughout distinct Brazilian biomes.

Specific Objectives

- To expand the amount of information about the natural host range of ToSRV isolates;
- To determine the range of environmental and cultural conditions where ToSRV is occurring across a multitude of Brazilian biomes, tomato-producing counties, and microclimatic conditions, and
- To investigate the impact of the resistant factors *Ty*-1 and *Ty*-3 on ToSRV diversity.

CHAPTER 1

LITERATURE REVIEW

1.1 The tomato crop: global and national importance

The tomatoes (*Solanum lycopersicum* L. and related wild species) are plant species native to the Andes, belonging to the family Solanaceae, genus *Solanum*, Section *Lycopersicon*. The optimal environmental conditions for cultivation of the domesticated tomatoes are those with mild temperatures. However, the modern and improved cultivars can be cultivated across a wide range of conditions, ranging from warm tropical climates (under lowland conditions) up to highland tropical and subtropical areas. The optimal temperatures for germination of tomato seeds are in the range of 20 to 25ºC (Embrapa 1993). The wild *Solanum* (section *Lycopersicon*) species are also originated in the Andean mountains of South America in areas encompassing Peru, Ecuador, and North region of Chile. More recently, wild tomatoes have been reported in other parts of the South America and Mexico (Naika et al. 2006). Tomatoes were more likely domesticated in pre-Columbian Mexico. Later on, this species was introduced to Europe in 1544 and afterwards it was spread across Europe and then to Southern and Eastern Asia, Africa, and the Middle East.

Tomato is one of the main vegetables worldwide (FAOSTAT 2020) with a world production of more than 182 million tons per year 2018. Brazil occupies a position among the top-ten tomato producers in the world ranking (FAOSTAT 2020). China is the largest global producer with more than 51.5 million tons in 2018, followed by India (16.4 million tons), United States (13.8 million tons), Turkey (11.6 million tons), Egypt (8.1 million tons), Italy (6 million tons), Iran (5.8 million tons), Spain (4.5 million tons), Brazil (4.1 million tons) and Mexico (3.4 million tons) (FAOSTAT 2020) **(Figure 1A)**.

According to IBGE (2020), the cultivated area in Brazil in the year 2020 was around 56.610 hectares (ha) with an average yield of 71.125 kilograms per hectare, reaching a total production of 4 million tons for fresh-tomato production. Goiás (GO) state was the largest producer, being responsible for 28.5% of the total in 2020, with a production of more than 1.3 million tons, followed by São Paulo (SP) state with 858.000 tons (19.8%), Minas Gerais (MG) state with 753.000 tons (12.8%), and Bahia (BA) state with 275.000 tons (6.4%) **(Figure 1B)**.

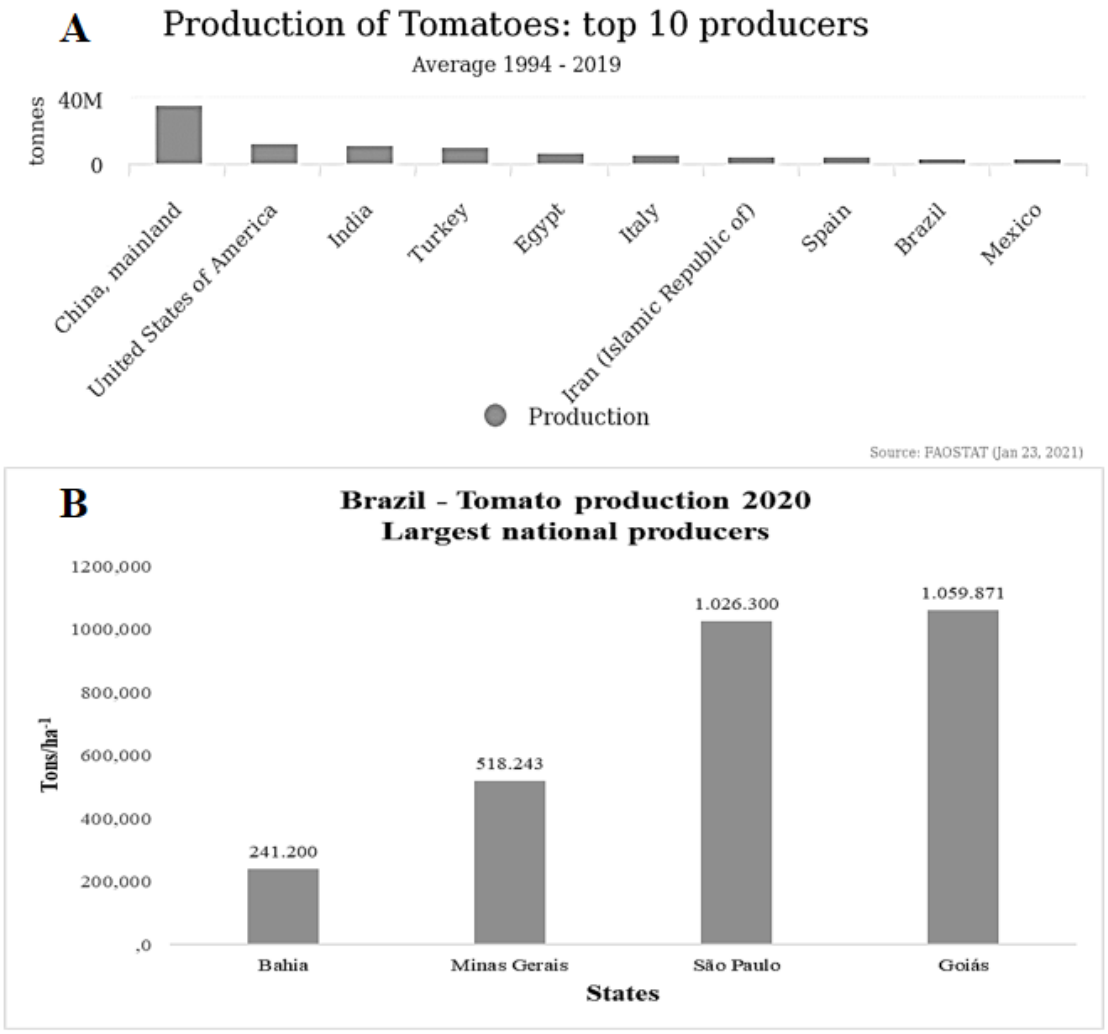

Figure 1: Tomato production in Brazil and around the globe. **Panel A:** the ten largest world tomato-producer countries. **Panel B:** the major tomato-producing Brazilian states. **Source**: IBGE (2020); FAOSTAT (2021).

The culinary versatility of the tomatoes contributes to its importance in the world. It may be consumed either raw or processed in the form of juice, sauce, paste, and dehydrated. Although 95% of the fruit content is water, it is a superior source of folic acid, vitamin C, and potassium. The most abundant phytonutrients in tomato fruits are carotenoids with lycopene being the most prominent antioxidant (Fontes and Nick 2019).

Tomato production in Brazil is based on two types of cultivation systems with different target markets. The growth habit of the tomato plants can be either determinate (for processing) or indeterminate (for fresh-market) (INCAPER 2010). Tomatoes for fresh consumption are

predominantly cultivated under open-field conditions and, in lesser extent, under greenhouse (protected) crop conditions. In these production systems, indeterminate varieties are employed trellised on bamboo stakes, where they are grown throughout the year by both small (commercial fields with less than 1,000 plants) and large farmers (production fields with more than 100,000 plants). Tomatoes for processing, on the other hand, are hybrids of determinate growth habit due to the presence of the recessive *sp* (*self-pruning*) gene. In Brazil, processing tomatoes are cultivated in large open-fields, which are predominantly irrigated by central pivot systems (Fernandes et al., 2008).

Tomato crop is an important economic activity in many countries around the world. Thus, efforts must be directed to minimize biotic and abiotic factors that might limit the overall tomato yield and quality. In this context, the breeding programs have developed and used a variety of modern and innovative techniques to improve local tomato germplasm, achieving expressive genetic gains, and developing superior varieties with resistance factors against both biotic and abiotic stresses (Nyaky and Danquah 2019).

1.2 Diseases that affect tomatoes

Diseases are often considered as one of the major limiting factors in the cultivation of tomato (Brahimi et al. 2017). As it is a host plant with a high number of phytopathogens, the occurrence of diseases both for the 'in natura' consumption segment and for the industry is one of the limiting factors in tomato production (Duval and Junior 2018). The most severe fungal diseases that affect tomato yields worldwide are the wilts caused by *Fusarium oxysporum* f. sp. *lycopersici*, root rot caused by *Fusarium solani*, damping off caused by *Rizoctonia solani* and root rot and damping-off caused by several *Pythium* species (Rashid et al. 2016). In Brazil, the black spot, which has the causative agent of the fungus *Alternaria solani*, is considered one of the most frequent and important diseases in the cultivation of tomatoes and other Solanaceae members (Talamini and Nunes 2018).

In greenhouse and open-field production, bacterial diseases are a serious problem. Five major bacterial pathogens are responsible for damages to the roots, stems, twigs, leaflets, leaves, buds, flowers and fruits: *Pseudomonas syringae* pv. tomato (causing bacterial speck); *Xanthomonas* species complex (causing bacterial spot); *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker); *Pseudomonas corrugata* (bacterial pith necrosis), and *Ralstonia* complex, causing bacterial wilt (Rashid et al. 2016; Lopes and Reis 2017). The

bacterial wilt is often found in protected crops in Brazilian regions, where crop rotation is difficult to be adopted (Lopes and Reis 2017).

Different species of nematodes are also described parasitizing the tomato crop. However, only the root-knot nematotes, belonging to the genus *Meloidogyne*, are considered the most relevant, from an economic point of view. In Brazil, the species *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. enterolobii* Yang and Eisenback and *M. arenaria* (Neal) Chitwood are the most frequent in tomato crops in different regions, causing damage ranging from mild to severe, depending on the cultivar used and environmental conditions (Gasparotto et al. 2019).

1.2.1 Diseases caused by virus in tomatoes

More than 40 viral species can infect tomato in Brazil (Reis 2020b). Among them, viruses in the genera *Cucumovirus* (Family *Bromoviridae*), *Potyvirus* (Family *Potyviridae*) and *Polerovirus* (Family *Luteoviridae*) reported by Cupertino et al. (1970); and *Tymovirus* (Family *Tymoviridae*) (Oliveira et al. 2013); *Begomovirus* (Family *Geminiviridae*), *Crinivirus* (Family *Closteroviridae*), *Orthotospovirus* (Family *Tospoviridae*), and *Tobamovirus* (Family *Virgaviridae*) (Ong et al. 2020).

Criniviruses – Members of genus *Crinivirus* cause significant diseases in economically important crops such as common beans, potatoes, lettuce, and tomatoes; mainly when occurring in mixed infections with other viruses (Tzanetakis et al. 2013). Members of the genus are transmitted by *B. tabaci* Genn. and *Trialeurodes vaporariorum* West. In Brazil, the first report of tomato chlorosis virus (ToCV) was done in 2008 (Barbosa et al. 2008). Other natural hosts were subsequently reported to be infected with ToCV (Fonseca et al. 2012; Boiteux et al. 2018). Later, isolates of this virus were reported infecting tomatoes in the Federal District (Nogueira et al., 2011) and in five states of Brazil (Barbosa et al. 2011). Symptoms observed in tomato plants infected with ToCV include chlorotic areas that evolve to bright interveinal yellowing, starting at the bottom leaves and gradually progressing to the top of the plant (Barbosa et al. 2008; Fiallo-Olivé and Navas-Castillo 2019).

Orthotospoviruses – According to Lima and Michereff Filho (2015) orthotospoviruses are better known by the Brazilian tomato growers by the denomination “vira-cabeça”. This is an important disease for the tomato crop due to the severity of the symptoms they cause in susceptible cultivars, as well as losses in fruit production and quality. The transmission occurs

by thrips (*Thrips* and *Frankliniella* species). The symptoms are diverse, in non-systemic hosts they are restricted to local lesions, chlorosis, and necrosis. At least four species occur naturally in tomato plants: tomato spotted wilt virus (TSWV), tomato chlorotic spot virus (TCSV), groundnut ringspot virus, and chrysanthemum stem necrosis virus (CSNV) (Lopes and Ávila 2005; Ong et al. 2020).

Potyviruses – These viruses also pose a constant threat to tomato crop. Susceptible plants display severe mosaic and leaf deformation, and for intolerant plants, it is common to develop a light mosaic and yellowing of apical leaves. Fruits show no symptoms, but they may have smaller sizes (Lopes and Ávila 2005). The most important potyviruses for tomato are potato virus Y and tobacco etch virus (Ong et al. 2020).

Begomoviruses – In Brazil, begomoviruses cause the highest economic damage to tomato crop. In the last decade, outbreaks of begomoviruses began to occur in all tomato-producing regions of Brazil, associated with the introduction of *B. tabaci* biotype B (= Middle East Asian Minor 1 – MEAM1). In Brazil, *B. tabaci* MEAM1 predominates, infesting more than 1,000 plant species and transmitting more than 300 virus species around the world. Biological and molecular features of the genus *Begomovirus* and genera in the same family will be present in the next topic. A total of 113 begomoviruses are currently reported as having tomatoes as their primary hosts (Reis 2020b).

1.3 Family *Geminiviridae*

Members of the family *Geminiviridae* are composed of viruses with small, non-enveloped, with genomes that comprise one or two single-stranded circular DNA of 2.5 to 5.2 kb (kilobases) **(Figure 2)**. Geminiviruses are plant pathogens that cause economically important diseases mainly in tropical and subtropical regions of the world (Zerbini et al. 2017; ICTV 2021). Geminiviruses are transmitted by various insects (whiteflies, leafhoppers, aphids, and treehoppers) in four families of homopterans. *Begomovirus* isolates are transmitted by whiteflies, whereas viruses of the genera *Mastrevirus*, *Curtovirus*, *Becurtovirus*, and *Turncurtovirus* are transmitted by leafhoppers. A member of the genus *Capulavirus* is transmitted by aphid and members of a single *Topocuvirus* species is transmitted by treehoppers. Members the *Eragrovirus* and *Grablovirus* genera have unknown vectors (ICTV 2021).

Geminiviruses is the largest family infecting both monocotyledonous and eudicotyledonous (Ong et al. 2020). Viruses belonging to this family are suitable models for the study of the evolutionary and ecological aspects of viral emergence since it includes many recently reported agricultural pathogens of great importance that emerged in the last century (Claverie et al. 2018). Several crops of economic interest are affected by geminiviruses, including tomato (Cotrim et al. 2007; Santana et al. 2007; Reis et al. 2020), soybean (*Glycine max* L. Merril) (Coco et al. 2013), common bean (*Phaseolus vulgaris* L.) (Fernandes-Acioli et al. 2011), cotton (*Gossypium hirsutum* L.) (Leke et al. 2016; Zubair et al. 2017), potato (*S. tuberosum* L.) (Morales et al. 2001) and cassava (*Manihot esculenta* Crantz) (Fauquet and Fargette 1990).

Currently, the family *Geminiviridae* is composed of nine genera. The demarcation criteria within each genus include host range, type of vector, genomic organization, and phylogenetic relationships (Brown et al. 2015; Navas-Castillo and Fiallo-Olivé 2020; **Table 1**). The two viruses not assigned to any genera in the family are: citrus chlorotic dwarf associated virus (CCDaV) and mulberry mosaic dwarf associated virus (MMDaV) (Varsani et al. 2017). CCDaV is the causative agent of infections in citrus plants and has the is vectored the whitefly *Parabemisia myricae* Kuwana. Peculiar features of CCDaV such as genome size and structure, phylogenetic relationships with other family members do not allow their allocation in any of the genera within this family (Loconsole et al. 2012). MMDaV is the second species not assigned to any genus, infects blackberry plants (*Morus alba* L.) and its vector remains be identified. Comparisons with other viruses indicate the possibility that MMDaV might be the genomic component of a CCDaV-related geminivirus (Lu et al. 2015; Ma et al. 2015). The main characteristics of the genera in the family *Geminiviridae* will be described below.

Beet curly top Iran virus and *Spinach curly top Arizona virus* are the two species of the **genus *Becurtovirus***, which is differentiated from other members of the *Geminiviridae* family by the type of nonanucleotide "TAA**G**ATT**C**C" of the origin of replication. Members of this genus are transmitted to dicotyledonous plants by leafhoppers (Varsani et al. 2014b; Zerbini et al. 2017). The becurtovirus genome comprises three ORFs in the viral sense, which encode the coat protein (CP), the movement protein (MP), and the protein involved in DNA regulation codified by ORFs V1, V2, and V3 respectively; and, two ORFs in the complementary sense (C1 and C2) involved in viral replication (Varsani et al. 2014b).

The **genus *Capulavirus*** contains four species. The genus name derives from the species type *Euphorbia caput-medusae latent virus* (Varsani et al. 2017). Isolates of the alfalfa

leaf curl virus are transmitted by *Aphis craccivora* K. (Varsani et al. 2017; Zerbini et al. 2017). No other vectors have been identified for viruses of the other species of the genus (ICTV 2021).

The **genus *Curtovirus*** has three species, including beet curly top virus, an important pathogen in North America and Iran. Members of this genus infect dicotyledonous and are transmitted by leafhoppers (Varsani et al. 2014a; Zerbini et al. 2017).

Mastrevirus is another genus of in the *Geminiviridae* family comprising viruses able to infect monocotyledonous and dicotyledonous. Mastreviruses are transmitted by several species of leafhoppers. Maize streak virus and wheat dwarf virus species are the most commonly studied. The mastrevirus genome consists of a simple circular DNA strand, ranging in size from 2.6-2.8 kb (ICTV 2021).

The **genus *Eragrovirus*** has a single species, *Eragrostis curvula streak virus* (Varsani et al. 2014b; Zerbini et al. 2017). All known isolates of this species have been found to infect monocotyledonous species *Eragrostis curvula* (Schrad) Nees. (Varsani et al. 2014b). A group of closely related viruses has been discovered infecting vines in Canada, South Korea, and the USA. These viruses have been classified to the **genus *Grablovirus*** (Varsani et al. 2017). Isolates of grapevine red blotch virus, are probable transmitted by the treehopper *Spissistilus festinus* Say (Varsani et al. 2017; Zerbini et al. 2017). Two new species were reported in California, wild vitis latent virus in *Vitis* sp. (Perry et al. 2018) and prunus latent virus is report in *Prunus* sp. (Al Rwahnih et al. 2018).

The monotypic **genus *Topocuvirus***, is represented by tomato pseudo-curly top virus (TPCTV), transmitted by treehoppers (Zerbini et al. 2017). The TPCTV genome consists of a single 2.8 kb ssDNA component (ICTV 2021).

The **genus *Turncurtovirus*** has three species (ICTV 2021). All isolates are transmitted by leafhoppers and have been found infecting dicotyledonous plants such as *Brassica rapa* L. and *Raphanus sativus* L. in Iran (Varsani et al. 2014b; Zerbini et al. 2017; Hasanvand et al. 2018). A new species of turncurtovirus has been identified in Iran in the leafhopper *Circulifer haematoceps* Mulsant and Rey and in plants of *Sesamum indicum* L., where the name sesame curly top virus has been proposed. The same isolate shares 87.3% nucleotide identity with a virus recently identified in Pakistan, sesame yellow mosaic virus. These isolates represent two strains of a tentative new species of genus *Turncurtovirus* and the name sesame curly top virus (SeCTV) has been proposed (Hasanvand et al. 2018).

The characteristics of **genus *Begomovirus*** will be described in the next topic.

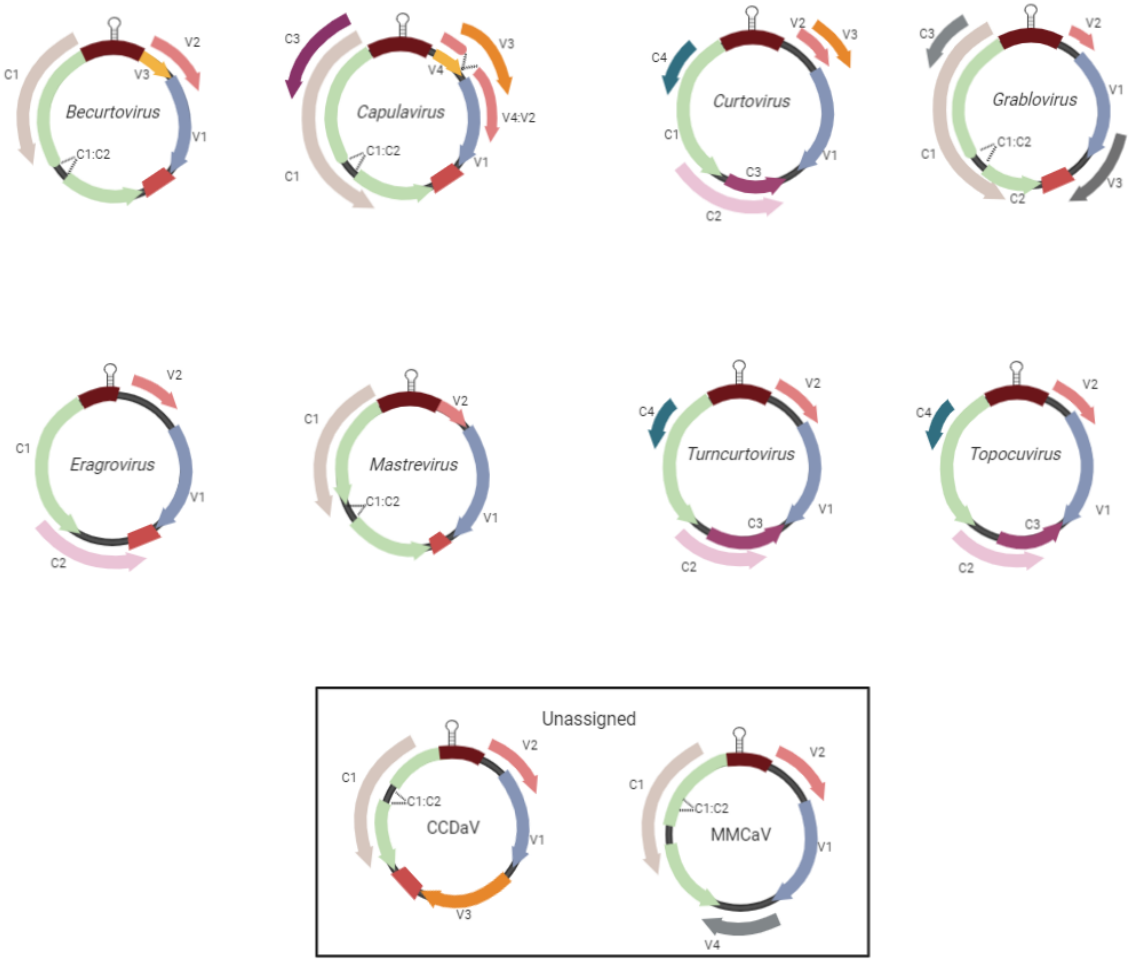

Figure 2: Genomic organization of viruses from the genera *Becurtovirus*, *Capulavirus*, *Curtovirus*, *Grablovirus*, *Eragrovirus*, *Mastrevirus*, *Turncurtovirus*, and *Topocuvirus*, and two unassigned species: *Citrus chlorotic dwarf associated virus* (CCDaV), and *Mulberry mosaic dwarf associated virus* (MMCaV), belonging to the family *Geminiviridae*. In the virion sense, ORF (Open Reading Frames) are indicated: V1 (Coat Protein – CP); V2 (Movement Protein – MP); V3 (Regulatory gene) and the complementary sense: ORFs C1 (Replication-associated protein – Rep); C2 (Transcriptional activator protein – Trap); C3 (Replication enhancer protein – REn) and C4 (Symptom-determining protein). In red: short intergenic region, and in reddish: long intergenic region. (Figure created with biorender.com).

Table 1: Current genera of the family *Geminiviridae* according to the International Committee on Taxonomy of Viruses (ICTV, 2021).

Genera[1,2]	***Begomovirus***	***Becurtovirus***	***Curtovirus***	***Eragrovirus***	***Capulavirus***	***Grablovirus***	***Mastrevirus***	***Topocuvirus***	***Turncurtovirus***
Type species	*Bean golden yellow mosaic virus*	*Beet curly top Iran virus*	*Beet curly top virus*	*Eragrostis curvula streak virus*	*Euphorbia caput-medusae latent virus*	*Grapevine red blotch virus*	*Maize streak virus*	*Tomato pseudo-curly top virus*	*Turnip curly top virus*
Number species	424	3	3	1	4	3	41	1	3
Vector	Whiteflies	Leafhoppers		----------	*Aphis craccivora*	treehoppers	leafhoppers	treehoppers	leafhoppers

[1]**Genome**: All genera show monopartite genome, except *Begomovirus*, which can present monopartite or bipartite genome. [2]**Hosts**: Monocotyledonous: *Curtovirus, Eragrovirus, Capulavirus, Mastrevirus* and Dicotyledonous: *Begomovirus, Becurtovirus, Capulavirus, Grablovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus.*

1.4 The genus *Begomovirus*

The **genus *Begomovirus*** is the largest in number of species within the *Geminiviridae* family with 424 species described (ICTV 2021). Begomoviruses play a crucial role as pathogens that cause serious economic impacts in crops of global importance (Zubair et al. 2017). Members in genus infect dicotyledonous and these viruses are characterized by their monopartite or bipartite genomes (Rojas et al. 2018). Begomoviruses can be classified into two lineages: Old World (OW) begomoviruses originated from Africa, Asia, and Europe; and, New World (NW) begomoviroses originated from Americas (Navas-Castillo and Fiallo-Olivé 2020).

The genome of bipartite begomoviruses consists of DNA–A and DNA–B components, with sizes of 2.5-2.7 kb **(Figure 3A)**. The genomes of monopartite begomoviruses resemble the bipartite component of DNA–A **(Figure 3B)**. Both components share approximately 200 nucleotides of the sequence in the long intergenic region sequence (LIR) that includes the origin of replication. DNA–A genomes have six open reading frames (ORFs), two in the virion sense (AV1/V1 and AV2/V2) and four in the complementary sense (AC1/C1 to AC4/C4). DNA–B encodes two proteins involved in the cell-to-cell movement: towards viral, the nuclear transport protein (NSP, BV1), and in the complementary sense, the movement protein (MP, BC1) (Jeske 2009; Zerbini et al. 2017; Navas-Castillo and Fiallo-Olivé 2020).

AV1/V1 ORFs encode the CP is considered a multifunctional protein, acting during virus vector transmission, nuclear transport, cell-to-cell and long distance movement in host plants (Jeske 2009). Studies with the CP gene show a varying degree of diversity revealing that NW viruses are more conserved in their CP gene sequences than viruses of the OW (Mondal et al. 2019). According to Cantu-Íris et al. (2019) several *Geminiviridae* genera show a existence of quasi-palindromic DNA segment with conserved ACTT-(N7)-AAGT core in the CP gene promoter. ORF AV2/V2 this ORF is found only in sequences of species from OW, and, is required for viral movement (Padidam et al. 1996). According to Sharma and Ikegami (2010) V2 has the potential to interact with the mechanisms of gene silencing and disease resistance in plants. Mutations in the C-terminal region of tomato leaf curl Java virus indicate that this protein interacted with the pathways of resistance of the virus.

The Rep is essential for rolling circle replication, and is involved in the modulation of gene expression (Haley et al. 1992). Studies by Nash *et al*. (2011) identified a conserved motive and showed that it is required to start viral replication. Others motive in Rep are proposed by

Arguello-Astorga et al. (2001), which identified a subdomain of the Rep whose primary structure varies among viruses that harbor different iterons, but which is highly similar between the same species. The identified protein region (referred to as Iteron-Related Domain, IRD) could be actually a component of the Rep domain involved in recognition of its cognate DNA elements. Beside this, Arguelo-Astorga et al. (2004) prove that the helix 4 motif, whose amino acid sequence is strongly conserved in geminivirus replication proteins and in the conserved leucine residue area, is part of an interface related to plant retinoblastoma protein (pRBR) in replication proteins of begomovirus.

The transcription-activating protein is coded by ORF AC2 in bipartite begomoviruses and C2 in monopartite begomoviruses. It is involved in the processes of gene activation, pathogenicity and suppression of silencing (Fondong 2013).

The REn is related to enhancer the symptoms in infected plants when compared to infections in wild species (Sung and Coutts 1995). Also, REn protein interacts with PCNA (Proliferating Cellular Nuclear Antigen), one of the main components of eukaryotic DNA chromosomal metabolism that works in a similar way to a slip ring, which ties DNA and modulates the interactions of other proteins with DNA and when interacting, REn directs the Rep protein to recruit the machinery necessary for viral DNA replication (Castillo et al. 2003).

AC4 encoding the less conserved protein of begomovirus, which is inserted entirely in the Rep protein (Kulshreshtha et al. 2019). This protein is involved in the development of symptoms during viral infection and in the regulation of the host cell cycle, recruiting factors from the host for virus replication (Krake et al. 1998; Park et al. 2010).

According to Briddon et al. (2010), the DNA–B components of bipartite begomoviruses are much more diverse than their DNA–A partners. Two ORFS are codified by DNA–B component in complementar (BC1) and viral sense (BV1). The ORF BV1 encodes a nuclear shuttle protein (NSP), which acts in intracellular virus movement involving the nucleus and, avirulence factor in some hosts (Garrido-Ramirez et al. 2000; Zhou et al. 2007). The ORF BC1, mediates cell-to-cell movement through plasmodesmas (Sanderfoot et al. 1996).

Members of *Begomovirus* employ the same general strategies for duplicating and expressing their genomes. They use a rolling circle replication system (RCR) to amplify their ssDNA genomes and produce dsDNA (Hanley-Bowdoin et al. 1999). The infection cycle begins with a viruliferous insect vector (whitefly), that feeds on the phloem of a healthy leaf,

and transmits virus particles into these cells. In the plant cell, the viral ssDNA is released and, since geminiviruses do not encode their own DNA polymerase, this ssDNA is copied by the host's DNA polymerases to generate double-stranded DNA (dsDNA). The dsDNA is transcribed by the host's RNA polymerase II, allowing the production of the Rep protein, the binding of Rep in almost every common region (CR) leads to a specific cut in the nonanucleotide region (5 'TAATATT ↓ AC 3': ↓ region of cut). This protein initiates viral replication, and the new circular ssDNA can be converted into dsDNA to enter into the replication cycle again and be encapsulated by the CP, and become available for acquisition by the vector (Pradhan et al. 2017; Navas-Castillo and Fiallo-Olivé 2020) or transported to neighboring cells through plasmodesma with the aid of the MP (Pradhan et al. 2017).

In Brazil, the first report of begomovirus occurred in 1955, infecting *Euphorbia* sp. (Flores et al. 1962), when symptoms of golden mosaic and chlorosis were observed and later characterized (Matys et al. 1975) as tomato golden mosaic virus (TGMV). Until 1990, reports of begomovirus infecting tomato crop were scarce and without apparent economic importance to producers. However, after the introduction of the biotype B of *B. tabaci* (=Middle East Asia Minor 1 – MEAM 1), there was a rapid and generalized increase in the number of reports of infections in the tomato crop (Ribeiro et al. 1994; Gotz et al. 2012).

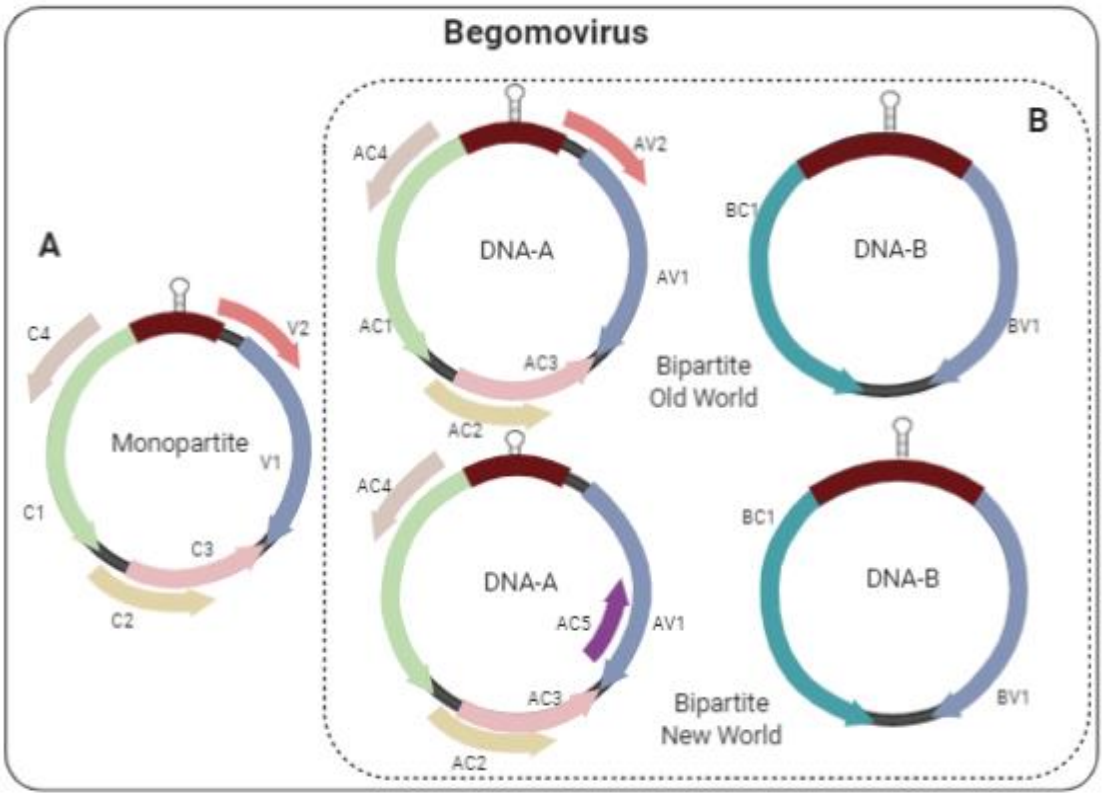

Figure 3: Genomic organization of the monopartite **(A)** and bipartite *Begomovirus* of the Old World and New World and **(B)**. The arrows denoted the position of the ORFs (Open Reading Frames) of DNA–A and DNA–B in the virion sense (V) and in the complementary sense (C) as indicated: **DNA–A:** AV1 (Coat Protein – CP); AV2 (Movement protein – MP); AC1 (Replication-associated protein – Rep); AC2 (Transcriptional activator protein – Trap); AC3 (Replication enhancer protein – REn); AC4 (Symptom-determining protein). **DNA–B**: BV1 (Nuclear shuttle protein BV1 – NSP) and BC1 (Movement Protein – MP). Segment in brown color represent the Common Region (CR). (Figure created with biorender.com)

1.5 Transmission of begomoviruses

The *Bemisia tabaci* (Family Aleyrodidade, Order Hemiptera) is a cosmopolitan and polyphagous complex of insects, which causes great damage to plants by injecting toxins during the phloem-feeding process. In addition, members of the *B. tabaci* complex are able to transmit more than 300 species of plant viruses classified in the genera *Begomovirus, Crinivirus, Carlavirus, Ipomovirus, Torradovirus,* and *Cytorhabdovirus* (Navas-Castillo et al. 2011; Verbeek et al. 2014; Gilbertson et al. 2015; Krause-Sakate et al. 2020; Pinheiro-Lima et al. 2020). It is estimated that about 90% of this total corresponds to *Begomovirus* species (Briddon et al. 1990). Transmission specificity of begomoviruses by the vector is mediated by CP.

Therefore, mutations in the CP gene can result in changes in the transmission of different begomoviruses by the vector (Pan et al. 2020).

According to Kanakala and Ghanim (2016), *B. tabaci* encompasses a complex of cryptic species, comprising at least 37 species, with variation in terms of molecular, transmission, host range, and susceptibility responses to insecticides (Tay et al. 2012; Lee et al. 2013; Boykin et al. 2013; Alemandri et al. 2015; Kanakala and Ghanim 2016). Among the cryptic species, four are present in Brazil, two of which are native to the country: New World 1 (NW1) and New World 2 (NW2) (Marubayashi et al. 2013) and two are invasive: Middle East Asia Minor 1 (MEAM1) and Mediterranean (MED) (Barbosa et al. 2015). *Bemisia tabaci* MEAM 1 is the prevalent species in Brazil and worldwide. Begomoviruses are persistent viruses, but it is yet controversial if their transmission is circulative, non-propagative (virus does not replicate in insect vector), or propagative (the virus replicates in the insect vector) (Fiallo-Olivé et al. 2020). There is accumulating evidence that at least the tomato yellow leaf curl virus (TYLCV) is able to replicate in *B. tabaci* (Pakkianathan et al. 2015).

An important step in the begomovirus infection cycle is the transmission between plants by the vector. For that, begomovirus needs first to go through the midgut wall to reach the hemolymph and then accumulate in the primary salivary glands, being then secreted in the saliva to infect new plants. The transport across midgut wall and through primary salivary glands are key steps determining differential transmission in different whitefly-begomovirus combinations (Navas-Castillo and Fiallo-Olivé 2020).

The spread of begomoviruses by *B. tabaci* has been facilitated by the introduction of the polyphagous *B. tabaci* MEAM1 of in many areas. This species is able to feed on a wide range of plants and raising probability of acquiring and transmitting a diversity of begomoviruses into potential new hosts (Navas-Castillo et al. 2011). *Bemisia tabaci* is widespread across many Brazilian regions (Krause-Sakate et al. 2020), including the recent report of *B. tabaci* MED in the South region (Moraes et al. 2017). In the Northeast region, the first reports of the whitefly in tomatoes occurred in 1995 in the São Francisco River Valley in areas of Juazeiro and Casa Nova (in Bahia-BA state), and Petrolina (Pernambuco-PE state). Climatic conditions and great diversity of host plants, favored the maintenance of whitefly populations throughout the year (Haji et al. 2005).

1.6 Genetic variability and evolution of begomoviruses

Genetic variability in begomoviruses occurs through three main mechanisms: mutation, recombination, and pseudo-recombination (Zhou et al. 1997; Garcia-Arenal et al. 2001; García-Arenal et al. 2003; Fernandes-Acioli et al. 2011; Rocha et al. 2013; Dallagnol 2018). Begomovirus populations have high levels of genetic variability driven by mutational dynamics, which allow them to adapt to a wide range of environments and hosts (Lima et al. 2017). Recombination can also contribute to the genetic diversification of these viral populations (Navas-Castillo and Fiallo-Olivé 2020). Recombination and pseudo-recombination do not occur without the simultaneous co-infection of different begomoviruses (Seal et al. 2006). In the recombination mechanism, segments of genetic information are exchanged among different genetic variants during the replication process (García-Arenal et al. 2001). The recombination among begomoviruses contributes to the increase and the emergence of new species (Bananej et al. 2004). As example, Vaghi Medina et al. (2014) characterized a new begomovirus, infecting tomato plants in Argentina (named as tomato mottle wrinkle virus) and performed recombination analyzes. Results showed that tomato mottle wrinkle virus is a recombinant species, with parental sequences belonging to the South American begomoviruses soybean blistering mosaic virus and tomato yellow vein streak virus. Pseudorecombination (or rearrangement) is observed in viruses that have more than one genomic component such as bipartite begomoviruses (Dallagnol 2018). These genetic rearrangements challenge the understanding of the specificity between the components of the genome in begomoviruses and provide mechanisms for rapid evolutionary change (Seal et al. 2006). Studies carried out with the recombinant cotton leaf crumple virus revealed sequences acquired from at least three different begomoviruses. Cotton leaf crumple virus also displayed evidences of intermolecular rearrangement (Idris and Brown 2004). Recent studies indicated that ToSRV has a high rate of molecular evolution, similar to an RNA virus, with different patterns of variation for DNA–A and DNA–B components, suggesting different evolutionary forces acting on each genomic component (Pinto et al. 2020).

1.7 Tomato severe rugose virus: history, spread and occurrence

Currently, tomato severe rugose virus (ToSRV) is the most widely disseminated begomovirus in tomato crop in Brazil, being predominant in the Southeast and Central regions

(encompassing the Cerrado and Atlantic Rain Forest biomes), showing high adaptive capacity and prevalence in the field (Cotrim et al. 2007; Fernandes et al. 2008a; Inoue-Nagata et al. 2016; Duarte et al. 2021; Reis et al. 2020). In addition, ToSRV has been reported infecting other host species, such as potatoes (*S. tuberosum* L.) (Souza-Dias et al. 2008), chili pepper (*Capsicum baccatum* var. *pendulum* L.) (Bezerra-Agasie et al. 2006), *Nicandra physalodes* L. (Barbosa et al., 2009), eggplant (*Solanum melongena* L.) (Moura et al. 2018), common beans (*P. vulgaris* L.) (Macedo et al., 2017), sweet-pepper (*Capsicum annum* L.) (Nozaki et al. 2006), soybeans (*Glycine max* L.) (Macedo et al. 2017b), *Sida* species (Lima et al. 2013), *Crotalaria juncea* L. (Barreto et al. 2013), and *Physalis angulata* (Duarte et al., 2019).

The high efficiency of begomoviruses such as ToSRV in triggering systemic infection in tomato plants was proven through assays with the vector *B. tabaci* MEAM 1. It was observed that tomato plants visited by viruliferous *B. tabaci* are systemically infected one day after inoculation. Additionally, a long incubation period of ToSRV was observed (≈ 18 days after inoculation), thus hindering the visual diagnosis of the disease and consequently contributing to the spread of the virus through asymptomatic seedlings into new planting areas (Favara et al. 2019). According to Freitas (2012), ToSRV is present in the leaf mesophyll cells, as is the case with other begomoviruses. The presence of the virus in the mesophyll can facilitate an instantaneous acquisition by the vector just after the penetration of the stylet into the leaf tissues since the insect is known to feed in the phloem vessels.

The first formal report of a tomato-infecting ToSRV isolate in Brazil done in Minas Gerais state in 1999. The first complete sequence of the DNA–A component was deposited in the GenBank in 2001 (accession no. AY029750) (Rezende et al. 2001, unpublished). Afterwards, several surveys detected ToSRV across many tomato-producing areas in Brazil (Ribeiro et al. 2003; Cotrim et al. 2007; Fernandes et al. 2008a; Rocha et al. 2010, 2013; González-Aguilera et al. 2012; Mituti et al. 2019; Duarte et al., 2021; Reis et al. 2020; Souza et al. 2020). Field surveys carried out by Ribeiro et al. (2003) from 1994 to 1999 in several states of Brazil revealed a quite remarkable diversity of begomoviruses associated with tomatoes, indicating the presence of seven new species, among them *Tomato rugose mosaic virus* (a recombinant species with ToSRV) and *Tomato chlorotic mottle virus*. Fernandes et al. (2008) carried out a characterization study of the begomoviruses associated with tomatoes in surveys done predominantly in fresh-market and processing tomato fields in Central Brazil in the period 2002-2004. It was observed the predominance of ToSRV in processing tomatoes and a substantial increase in ToSRV incidence in fresh-market tomato samples in 2004. Rocha et

al. (2010) carried out a survey of begomovirus in sweet-pepper and tomato crops in SP state from 2007 to 2008. Their work revealed the predominance of ToSRV in both sweet-peppers and tomatoes in all sampled regions. In addition, they also to identified high genetic variability among ToSRV isolates obtained from tomatoes.

The first molecular characterization of the DNA–A (Accession no. HQ606467) and DNA–B (Accession no. HQ606468) components of a ToSRV isolate was carried out in 2011 (Barbosa et al. 2011) with a sample collected in 2007 in Piracicaba (SP) and named as Pi 1. The symptoms associated with the Pi 1 isolate were severe rugose yellow mosaic. Phylogenetic analyzes indicated a close relationship of ToSRV with tomato rugose mosaic virus (ToRMV), displaying 87% nucleotide identity. The biological characterization of ToSRV Pi 1 was also performed using inoculation with viruliferous *B. tabaci* MEAM 1 adults. Thirty-three species from nine botanical families were evaluated as putative experimental hosts of this isolate. Of these, 20 species were not infected by ToSRV: *Gomphrena celosioides* Mart, *G. globosa* L. (Amaranthaceae); *Acanthospermum hispidum* DC, *Bidens pilosa* L., *Parthenium hysterophorus* L., *Porophyllum ruderale* Jacq. (Asteraceae); *Chenopodium amaranticolor* H.J.Coste & A. Reyn, *C. quinoa* Willd (Chenopodiaceae); *Commelina bengalensis* L. (Commelinaceae); *Macroptilium lathyroides* L. (Fabaceae); *Geranium molle* L. (Geraniaceae), *Malva parviflora* L., *Malvastrum coromandelianum* L. , *Sida pilosa* Retz, *S. santaremnensis* L. (Malvaceae); *Nicotiana benthamiana* L., *Physalis floridana* Rydb, *S. tuberosum* L. (Solanaceae) and *Urtica dioica* L. (Urticaceae). On the other hand, 13 species were susceptible to ToSRV infection, four of which were symptomatic: *S. lycopersicum* and *N. physalodes* (which developed yellow rugose leaf mosaic) and *Chenopodium album* and *Chenopodium ambrosioides* (which exhibited only yellow mosaic).

More extensive studies on the diversity, distribution, and genetic structure of ToSRV populations were performed by González-Aguilera et al. (2012). These analyses indicated that ToSRV population displayed a genetic variability similar to other tomato-infecting begomovirus populations described in Brazil. All ToSRV isolates, formed a group with other begomoviruses previously reported in Brazil, which diverged from another group of begomoviruses described from distinct American countries. Reis et al. (2020a) conducted metagenomics studies with begomoviruses infecting tomato cultivars with and without the *Ty*-1 resistance gene in Central Brazil. ToSRV was found as the predominant begomovirus in samples from Goiás state, with the majority of the samples presenting mixed infection (with the simultaneous presence of two to five viral species).

1.8 Resistance genes to begomovirus species

Cultivars carrying resistant genes are considered the most effective strategy to reduce losses caused by begomoviruses in tomatoes (Boiteux et al. 2012). The tomato breeding strategy is focused on the introgression of resistance alleles from wild germplasms into elite lines (Gill et al. 2019). The employment of tomato cultivars/hybrids without resistance factors to begomoviruses is a risky option, due to the wide dissemination and aggressiveness of these viruses to tomato (Hurtado et al. 2012). In fact, several accessions of wild *Solanum* (section *Lycopersicon*) are resistant to begomoviruses. Genetic studies allowed for the mapping resistance/tolerance gene/loci and these factors are now being explored in genetic resistance breeding programs (Verlaan et al. 2013).

Eight of these genetic factors against begomoviruses were characterized and mapped in the tomato genome. All of them, confer partial resistance and/or tolerance (*sensu* Cooper and Jones 1983), but not an immunity response. More details about these genes/loci will be presented. The most important tolerance gene to begomovirus is *Ty*-1. It was introgressed from the wild species accession *S. chilense* LA 1969 and currently is one of the most used resistance gene in breeding program. The *Ty*-1 locus was mapped on tomato chromosome 6 (Zamir et al. 1994; Verlaan et al. 2013). Fine mapping and functional analyses indicated that *Ty*-1 and *Ty*-3 genes are, in fact, alleles of the same gene and they code for an RNA-dependent RNA polymerase (Verlaan et al. 2013).

The tomato cultivars available in the Brazilian market carry mainly the *Ty*-1 gene, which confers high levels of tolerance to the monopartite begomovirus tomato yellow leaf curl virus (TYLCV) as well as tolerance to bipartite begomoviruses predominant in Brazil (Aguilera et al. 2011). Voorburg et al. (2020), showed that the *Ty*-1 gene also confers resistance to isolates of beet yellow curl virus (genus *Curtovirus*). However, their results also indicated a compromised resistance conferred by the *Ty*-1 gene with the simultaneous presence of co-infections with RNA viruses and the presence of beta-satellites, reinforcing the importance of monitoring the presence of other pathogens that encode RNAi suppressors and/or are able to interfere with the level of transcription gene silencing (TGS) during the cultivation of tomato plants with the *Ty*-1 gene. The *Ty*-2 gene was introgressed from *S. habrachoites* and mapped in chromosome 11 (Hanson et al. 2006). Tests carried out with plants carrying the *Ty*-2 gene inoculated with ToSRV, indicated high levels of resistance to this virus (Boiteux et al. 2007). Yamaguchi et al. (2018) identified two *Ty*-2 candidate leucine-rich repeat (LRR) and nucleotide

binding(NLR) genes as demonstrated by gene functional complementation. The Rep protein when co-expressed with the *Ty*-2 gene, triggered a hypersensitivity response, indicating that this is the avirulence (*Avr*) determinant of the *Ty*-2-based resistant response (Shen et al. 2020). The *Ty*-3 gene was mapped on chromosome 6, being introgressed from the accessions *S. chilense* LA 2779 and LA 1932. This gene has been used in breeding programs for begomovirus in Florida, to provide resistance to the monopartite TYLCV and to the bipartite tomato mottle virus (ToMoV) (Ji et al. 2007). A subgroup of resistant accessions from germplasm banks of the Embrapa Hortaliças and Universidade Federal de Viçosa displayed the *Ty*-3 gene in their genomes (Aguilera et al. 2011; Verlaan et al. 2013; Pereira-Carvalho et al. 2014). *Ty*-3 is very often found in cultivars carrying *Ty*-1 under field conditions in Brazil. The *Ty*-4 gene was mapped on chromosome 3, being introgressed from the accession *S. chilense* LA 1932. Studies of resistance variation and comparisons made with the *T*y-3 locus showed that the *Ty*-4 gene had a less prominent effect on the levels of resistance (Ji et al. 2009). The *Ty*-5 gene is located on chromosome 4 and it was introgressed from *S. peruvianum*, identified in the inbred line 'TY172' The resistance observed in 'TY172' is conferred by a dominant QTL (Quantitative Trait Loci) and other smaller QTLs (Anbinder et al. 2009). Studies of functional mapping and validation demonstrated that gene product present in 'TY172' is a recessive factor (named as *ty*-5) corresponding to a *pelota* (Protein pelota homolog) gene, which is a surveillance factor in the dissociation process of ribosomes into subunits (Lapidot et al. 2015; Wang et al. 2018). The *Ty*-6 gene provides resistance against both monopartite and bipartite begomoviruses and it was mapped on chromosome 10 of tomato (Gill et al. 2019). The best use of *Ty*-6 seems to be in combination with other begomovirus resistance genes such as *ty*-5 or *Ty*-3 (Scott et al. 2015; Gill et al. 2019). The *tcm*-1 was the first recessive begomovirus tolerance gene characterized. It is derived from *S. lycorpersicum* 'Tyking' and confers broad-spectrum resistance against begomovirus species (Giordano et al. 2005; Pereira-Carvalho et al. 2010; Pereira-cCarvalho et al. 2014). The *tcm*-1 gene is efficient against two bipartite begomoviruses from Brazil and one monopartite from Spain (Pereira-Carvalho et al. 2010). The *tgr*-1 gene, introgressed from the inbred line FLA653 (derived from a cross between *S chilense* LA 2779 and also 'Tyking'), conferred resistance to the monopartite TYLCV in Australia (Bian et al. 2007).

1.9 Phylogeography

Phylogeography deals spatial arrangements of genetic lineages, especially within and among closely related species. The development of modern molecular techniques and computer programs increased the efficiency and facilitated the implementation of phylogeographic studies. The phylogeographic studies also deal with the dissemination pattern of various populations over a given landscape (Avise 2009). The patterns of dispersion of distinct populations may occur due to their behavior and/or physical impediments in the landscape. Therefore, the ultimate goal in phylogeographic evaluations is to use gene trees to infer the historical data as well as contemporary forces that produced the current genealogical architecture of closely related populations and species (Avise 2009).

Genetic diversity is the natural variation transmitted from generation to generation among individuals within the same species, across species or across groups. Inferring genetic diversity in phylogeographical and population genetics studies is crucial to describe important demographic patterns, for instance, of organisms from a same species that occur throughout a given territory (Turchetto-Zolet et al. 2013). For phylogeographical studies, phylogenetic relationships of a given group of organisms are established. This relationship reconstruction can be done using DNA sequences for a given gene or from complete genomes, which is relatively easy to obtain from viruses with small genomes such as the begomoviruses. Analyses are then carried out aiming to identify clusters of individuals that occur in the sampled geographical area. It is also assessed whether groups of individuals with closer relationships occur in closer geographical areas. In other words, these studies seek to assess whether there is congruence between genetic lineages and their spatial distribution. These analyzes bring relevant data to the historical biogeography (Miyaki 2009).

In relation to plant viruses, Rakotomalala et al. (2019) investigated the patterns and dynamics of spatiotemporal spread of rice yellow mottle virus (RYMV) in Madagascar, comparing phylogeographic inferences about the dispersion routes of RYMV throughout the country, analyzing the determinants leading to RYMV invasion of new regions and migration within geographical areas. More recently, Duarte et al. (2021) described the viral diversity in the main tomato-producing regions of the Atlantic Rain Forest (Espírito Santo, RJ and MG) and carried out phylogeographical studies of the endemic begomovirus tomato common mosaic virus (ToCmMV). In this study, ToSRV displayed the highest prevalence in recent years followed by ToCmMV. However, according to our knowledge, the present work is the first to

provide novel information on the genomic diversity, geographical distribution and potential impact of tomato resistance genes on the prevalence of ToSRV throughout distinct Brazilian biomes.

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CHAPTER 2

Novel natural hosts of tomato severe rugose virus (ToSRV) in the Fabaceae, Solanaceae, and Oxalidaceae families.

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Abstract

Tomato severe rugose virus (ToSRV) is predominant among the more than 20 tomato-infecting begomoviruses reported in Brazil after the invasion of *Bemisia tabaci* Middle East-Asia Minor 1 (= biotype B) in the early 1990's. ToSRV is currently detected in association and/or displacing other begomoviruses in the major tomato-producing areas. The prevalence of ToSRV across many ecogeographical regions is yet elusive. This peculiar ToSRV feature can be partially explained by its large number of alternate hosts, including some not yet characterized. Here, isolates of ToSRV were characterized in four new natural hosts, including *Pachyrhizus erosus* (Fabaceae), *Solanum betaceum* (section *Pachyphylla*, Solanaceae), *S. torvum* (section *Torva*, Solanaceae), and *Oxalis latifolia* (Oxalidaceae family). These results reinforce the notion that the wide host range of ToSRV may play relevant biological and epidemiological roles in explaining the geographical dispersion and large frequency of this virus in tomato crops in the Neotropics.

Key words: *Pachyrhizus*, *Oxalis*, *Solanum betaceum*, *Solanum torvum*, *Begomovirus*, host range

Introduction

Geminiviridae is the largest plant-infecting family with almost 500 species described to date (Zerbini et al. 2017; Navas-Castillo and Fiallo-Olivé 2020). This family has currently nine genera, including the *Begomovirus*, which is composed of whitefly-transmitted viral species with one (= monopartite) or two (= bipartite) circular, single-stranded DNA component(s) with ≈ 2.6 kb. In bipartite begomoviruses, the DNA–A and DNA–B components are individually encapsulated into twinned particles formed by two incomplete icosahedrons (Zerbini et al. 2017; Navas-Castillo and Fiallo-Olivé 2020).

In Brazil, the numerous field surveys conducted after the invasion of the vector *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM 1 = biotype B) in the early 1990's uncovered an array of over 20 tomato-infecting begomoviruses (Reis et al. 2020). Thus far, the prevalent tomato-infecting begomoviruses are: tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato golden vein virus (TGVV), and tomato yellow vein streak virus (ToYVSV) (Reis et al. 2020, 2021). Among them, ToSRV is currently the most widespread begomovirus in Brazil, being present in association and/or displacing other viral species across major tomato-producing areas (Duarte et al. 2021; Reis et al. 2020, 2021).

The prevalence of ToSRV across many ecogeographic regions of Brazil is yet elusive. This peculiar viral feature can be partially explained by a wide array of epidemiological as well as specific ToSRV-host interactions, including the genetic makeup of the novel tomato hybrids (Reis et al. 2020), crop management strategies, ecological factors (Duarte et al. 2021), variability/adaptation of the vector populations as well as the presence of yet not characterized alternative hosts (both native and cultivated). Therefore, additional studies must be carried out in order to elucidate what major factor(s) is/are involved in this growing predominance of the ToSRV in tomatoes. In fact, isolates of ToSRV have been characterized under natural conditions infecting not only tomato, but also other Solanaceae and Fabaceae species. Tropical

weeds from the genera *Chenopodium* (Barbosa et al. 2011) and *Sida* (Lima et al. 2013) have been also detected as natural/experimental hosts of ToSRV. Here, isolates of ToSRV were characterized in four new natural hosts (including a new botanic family) in Brazil, expanding the host range of this economically important viral species.

Material and Methods

Foliar samples displaying mild apical chlorosis symptoms were obtained from *Solanum betaceum* Cav. (section *Pachyphylla*, Solanaceae), *S. torvum* Sw. (section *Torva*, subgenus *Leptostemonum*, Solanaceae) (Weese and Bohs 2007) and yam bean [*Pachyrhizus erosus* (L.) Urban; Fabaceae] plants growing in the vicinity of highly begomovirus-infected tomato fields. In addition, samples of leaves and bulbils of the subtropical weed *Oxalis latifolia* Kunht (Oxalidaceae) plants, exhibiting begomovirus-like symptoms were also collected. Bulbils were taken from ten symptomatic *O. latifolia* plants and transplanted (under protected greenhouse conditions) to 5L-pots filled with sterile substrate (**Figure 1**). Altogether, 57 foliar samples were obtained, being 28 samples from *O. latifolia* leaves exhibiting golden spots and leaf chlorotic sectors in two areas of the Rural sectors of Gama (Federal District–DF) and in Formosa (Goiás State–GO), Brazil from 2003 to 2015. Ten leaf samples of *P. erosus* showing mild apical chlorosis symptoms in Gama–DF (2015); ten leaf samples of *S. betaceum* with apical chlorosis in Pipiripau–DF (2013) and nine leaf samples of *S. torvum* exhibiting interveinal chlorosis collected in Gama-DF (2008) were also collected.

The total genomic DNA was extracted with 2X CTAB buffer and organic solvents according to Boiteux et al. (1999). The total purified DNA was initially used as template in rolling-circle amplification (RCA) assays (Inoue-Nagata et al. 2004). After that, the enriched ssDNA samples were used as templates in PCR assays using universal primers targeting conserved regions of the begomoviral DNA–A and DNA–B genomic components (Rojas et al. 1993)

(Supplementary Table 1). Internal primers were used to recover the complete genome sequence (DNA–A), employing a primer walking strategy as described (Duarte et al. 2021). PCR assays followed by Sanger dideoxy amplicon sequencing were also carried out with the maturase (*mat*K) (Wicke and Quandt 2009; Kar et al. 2015) and the ribulose 1,5 bisphosphate carboxylase/oxygenase (*rbc*L) genes (Levin et al. 2003) **(Supplementary Table 1)**, aiming to confirm the botanical identification of the host samples.

A phylogenetic tree was constructed using 99 ToSRV sequences available in GenBank (data retrieved in February, 2021). From this, 48 representative isolates of all clades and the accession DQ336550 (one tomato yellow spot virus isolate employed as outgroup) were selected and a Bayesian inference phylogenetic tree was constructed employing the software MrBayes v. 3.2.6 (Huelsenbeck et al. 2001) available in the Geneious package (V.11.1.5) (Kearse et al. 2012a). The GTR (General Time Reversible) was employed as the nucleotide substitution model and the analysis was performed for 10 million generations, excluding the first one million generations as burn-in. The trees were visualized in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator v.25.2.

Results

The botanical identification of the viral hosts was confirmed by morphological analyses and also by using the information from the *mat*K and *rbc*L gene sequences. These hosts were identified as being: *O. latifolia* (99% coverage and 99.49% *rbc*L gene identity; 99% coverage and 99.78% *mat*K gene identity), *P. erosus* (99% and 99% *rbc*L gene; 99% and 99.66% *mat*K gene), *S. betaceum* (99% and 100% *rbc*L gene; 100% and 99.86% *mat*K gene) and *S. torvum* sensu lato (99% and 100% *rbc*L gene; 99.48 to 99.66% *mat*K gene).

The bipartite nature of all begomovirus isolates associated with these hosts was confirmed using the PCR conditions and the degenerated universal primers described by Rojas et al. (1993). The

amplicons were directly sequenced by Sanger dideoxy sequencing using the same universal primers of Rojas et al. (1993). The partial DNA–A sequences (≈1.200 bp) displayed nucleotide identity ranging from 95-99% to a wide array of ToSRV isolates available in the GenBank database. Two out of the 28 *O. latifolia* field-collected leaf samples were found to be infected by ToSRV and this virus was also detected in leaves originated from transplanted bulbils. The remaining samples were found to be infected by sida micrantha mosaic virus (SiMMV; data not shown). All symptomatic leaf samples of *P. erosus*, *S. betaceum* and *S. torvum* were found to be infected by ToSRV. The complete sequences of the DNA–A components of isolates from these novel hosts (one isolate per host) were recovered by using the tool *De Novo Assemble* (Kearse et al. 2012a) and by employing a set of ToSRV-conserved internal primers (Duarte et al. 2021).

The complete DNA–A sequences of the four ToSRV isolates described here were deposited at the GenBank database as follows: *O. latifolia* (A DF_567 JP_LVV 208, accession MW560617), *P. erosus* (A DF_500 JP_LVV 207, accession MW560615), *S. betaceum* (DF–607, accession KY524458), and *S. torvum* (A DF_259 JP_LVV 206, accession MW560614). These isolates displayed nucleotide sequence identities ranging around 99% to a wide array of ToSRV isolates (**Supplementary Table 2**). We annotated (in the viral sense) the AV1 gene (coding the coat protein–CP gene), while in the complementary sense we annotated the following genes: AC1 (replication-associated protein–Rep), AC2 (transcription activator TrAP), AC3 (replication enhancer–REn), and AC4 (symptoms determinant).

The complete DNA–B of the isolate DF–607 was recovered (GenBank accession KY964449). Partial DNA–B sequences of the following begomovirus isolates were deposited at the GenBank database: *P. erosus* (B DF_500 JP_LVV 209, accession MW602393) and *O. latifolia* (B DF_567 JP_LVV 210, accession MW623052). A fragment of the DNA–B component

(≈1200 bp) from the other isolates were amplified and they displayed around 98% nucleotide identity with the DNA–B sequences from distinct ToSRV isolates (**Supplementary Table 2**).

Phylogenetic analyzes grouped the ToSRV isolates according to their geographic origin **(Figure 2)**. The DF–607 isolate (from *S. betaceum*) was grouped with ToSRV isolates previously reported infecting the following Fabaceae species: *Phaseolus vulgaris* L. and *Glycine max* L. (both isolates collected in Goiás (GO) state, also in Central Brazil). The isolates from *P. erosus* (A DF_500 JP_LVV 207) and *O. latifolia* (A DF_567 JP_LVV 208) clustered in the same clade, whereas the isolate of *S. torvum* (A DF_259 JP_LVV 206) grouped with ToSRV isolates from *S. lycopersicum* also reported in GO state.

Discussion

Isolates of ToSRV have been characterized infecting naturally tomatoes as well as a wide array of Solanaceae hosts such as chili pepper (*Capsicum baccatum* var. *pendulum* L.) (Bezerra-Agasie et al. 2006), sweet-pepper (*C. annuum* L.) (Nozaki et al. 2006), potatoes (*S. tuberosum* L.) (Souza-Dias et al. 2008), *Nicandra physalodes* L. (Barbosa et al. 2009), eggplants (*S. melongena* L.) (Moura et al. 2018), and *Physalis angulata* (Duarte et al. 2019). In addition, the spiny solanum accessions *S. stramoniifolium* Jacq., *S. asperolanatum* Ruiz & Pav., *S. jamaicense* Mill., from the subgenus *Leptostemonum* (Solanaceae) were also reported as asymptomatic experimental hosts of ToSRV as well as *S. mammosum* L., which displayed mild apical chlorosis (Michereff-Filho et al. 2012). However, according to our knowledge, this is the first worldwide report of ToSRV naturally infecting *S. torvum* as well as *S. betaceum* (tamarillo). *Solanum torvum* and closely related species are semi-perennial, non-cultivated spiny *Solanum*, which are originated from the West Indies and nowadays are distributed across many tropical and subtropical areas (Knapp 2011). Accessions of *S. torvum* have been used in intensive agriculture as rootstocks, especially for eggplants and tomatoes (Cutti and Kulczynski

2017). *Solanum torvum* was recently reported in natural habitats in Europe (Calabria region of Italy), more likely as a result of an 'escape' event from agricultural areas that are employing accessions of this species as rootstocks (Musarella 2020). *Solanum torvum* has been previously reported as a natural host of groundnut bud necrosis virus (GBNV) from the genus *Orthotospovirus* in India (Kumari et al. 2019). In addition, related spiny *Solanum* species such as *S. sisymbriifolium* Lam., *S. americanum* Mill., and *S. aethiopicum* L., and eggplant have been also reported as natural hosts of tomato chlorosis virus (genus *Crinivirus*, family *Closteroviridae*) in South America (Arruabarrena et al. 2015; Fonseca et al. 2015). The tamarillo or tree-tomato, *S. betaceum* (section *Pachyphylla*) is also a semi-perennial *Solanum* species that has been employed as a specialty crop in some South America countries (Ramírez and Kallarackal 2019). Tamarillo has been also evaluated as an economic alternative for vegetable farmers in Brazil. Therefore, *Solanum* species of the sections *Torva* and *Pachyphylla* may potentially function as year-round sources of virus inoculum in tropical areas for many important viruses of tomatoes as well as other Solanaceae crops, including ToSRV.

Fabaceae species have been also detected as natural and/or experimental ToSRV hosts such as common beans (*P. vulgaris*) (Macedo et al. 2017a), soybeans (*G. max*) (Macedo et al. 2017b), and *Crotalaria juncea* L. (Barreto et al. 2013). Here, yam bean (*P. erosus*) was confirmed as a new Fabaceae host of ToSRV. Interestingly, Fabaceous hosts have been previously reported as either non-symptomatic such as commom beans (Macedo et al 2017a) or expressing mild symptoms. The same reaction was observed here in yam bean, which displayed only mild apical chlorosis symptoms. To our knowledge, the present work is the first report of *P. erosus* as a natural host of ToSRV.

Some tropical weeds from the genera *Sida* (Malvaceae) and *Chenopodium* (Amaranthaceae) have been also detected as either natural or experimental hosts of ToSRV (Barbosa et al. 2011; Lima et al. 2013). Like *Sida* and *Chenopodium* species, Oxalidaceae weeds such as *O. latifolia* and *O. oxyptera* are very often found infesting tomato fields in subtropical regions. *Oxalis oxyptera* has been reported as a host of begomoviruses associated with the disease "infectious chlorosis of Malvaceae" since the 1970's when only the whitefly vector *B. tabaci* biotype A was present in Brazil (Fontenele et al. 2018). The ornamental species *O. debilis* was also reported as a host of a new begomovirus in the USA (Herrera et al. 2015). After the invasion of *B. tabaci* MEAM 1, sida micrantha mosaic virus (SiMMV) was reported as the predominant *Oxalis*-infecting begomovirus under Brazilian conditions (Fontenele et al. 2018). However, our results suggest that *O. latifolia* might play a role as an alternative ToSRV reservoir under field conditions. The control of *Oxalis* species via cultural management and by herbicides is usually of low efficiency due to the presence of aerial stolons as well as subterranean (primary and secondary bulbils) organs. Under such circumstances, it is most likely that this weed might represent a persistent source of inoculum of ToSRV and SiMMV to tomato as well other host crops under natural conditions. In addition, the presence of begomovirus-infected bulbils may also represent a vehicle for large geographical dispersion of these viruses since these structures may be present in infested soils and may also remain intact in the cow manure, which is largely employed as fertilizer in the vegetable-producing sector in Brazil. To our knowledge, the present work is also the first report of a member of the Oxalidaceae family as a host of ToSRV.

ToSRV is hitherto the predominant virus among the more than 20 begomoviruses reported infecting tomatoes in Brazil after the invasion of *B. tabaci* MEAM 1 in the early 1990's, being present in mixed infections and/or displacing other viral species across major tomato-producing regions (Duarte et al. 2021; Reis et al. 2020). The prevalence of ToSRV in many

ecogeographical regions across the country remains to be elucidated. However, its wide geographic distribution and presence in alternative hosts may represent major factors contributing to this gradual predominance of ToSRV in the Central and Southeast regions of Brazil. Here, we reported four new natural alternative hosts of ToSRV in members of the Fabaceae, Solanaceae, and Oxalidaceae families. These results reinforce the notion that the wide host range of ToSRV may play relevant biological and epidemiological roles, allowing for the wide geographical dispersion and large frequency of this virus in tomato crops in the Neotropics.

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Compliance with ethical standards: yes.

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

Ethical approval: This research does not contain any studies with human participants or animals performed by any of the authors.

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Figure 1. Symptoms induced by tomato severe rugose virus (ToSRV) in *Oxalis latifolia* Kunht plants. (**A**) Asymptomatic *O. latifolia* plant (in the left) and symptomatic plant (in the right), (**B**) Details of *O. latifolia* plant displaying begomovirus-like symptoms as observed under field conditions.

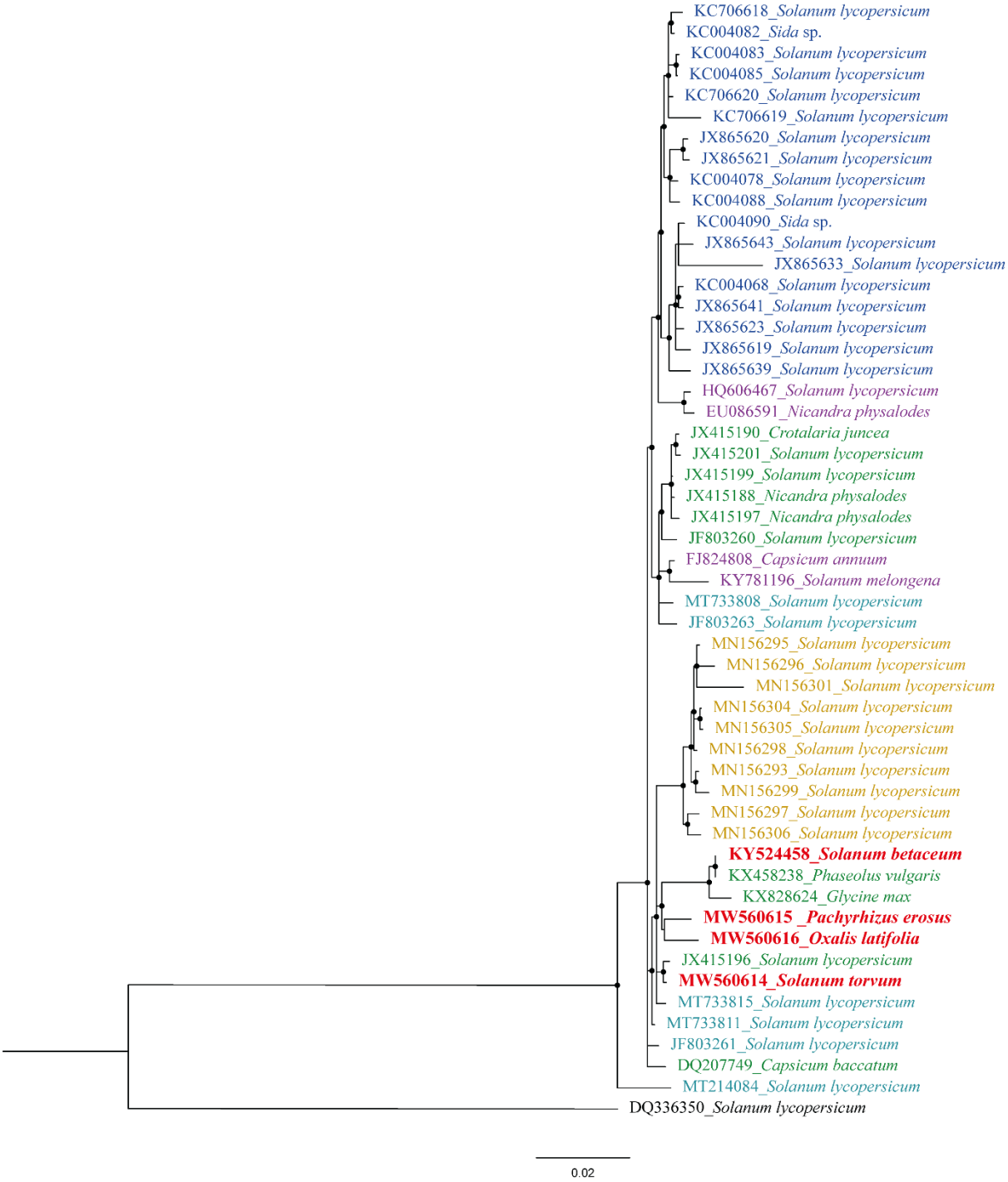

Figure 2. Bayesian phylogenetic tree based upon the complete DNA–A genomic sequence from 48 tomato severe rugose virus (ToSRV) isolates and one nucleotide sequence from tomato yellow spot virus (employed as outgroup; GenBank accession number: DQ336350). Sequences were aligned with MAFFT. The black circles are representing the nodes with posterior probability values ≥ 79 %. The scale bar indicates pairwise nucleotide distance. The sequences of the four new ToSRV hosts are displayed in red color. Isolates from São Paulo (SP) state (*n*= 4) are displayed in purple, whereas isolates from Goiás (GO) state (*n*=10) are displayed in green, isolates from Minas Gerais (MG) state (*n*=18) are in dark blue, isolates from the Federal District-DF (*n*=6) are in blue, isolates from Espírito Santo (ES) state (*n*=10) are in yellow. The outgroup, a tomato yellow spot virus isolate from MG state (*n*=1) is displayed in black color.

Supplementary materials

Supplementary Table 1 – Primer sequences used for PCR detection and Sanger sequencing of tomato severe rugose virus, and botanical classification of four putative tomato severe rugose virus (ToSRV) hosts.

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

Supplementary Table 2: Information about 48 tomato severe rugose virus (ToSRV) isolates used in the phylogenetic analysis. In bold are the isolates characterized in the present study.

Accession	Host	Sequence Length (nts)	Local of collection
FJ824808	*Capsicum annuum*	2591	Brazil-SP
DQ207749	*Capsicum baccatum*	2593	Brazil-GO
JX415190	*Crotalaria juncea*	2591	Brazil-GO
KX828624	*Glycine max*	2593	Brazil-GO
JX415188	*Nicandra physalodes*	2591	Brazil-GO
JX415197	*Nicandra physalodes*	2591	Brazil-GO
EU086591	*Nicandra physalodes*	2590	Brazil-SP
MW560616	***Oxalis latifolia* (A_DF-567 JP_LVV 208)**	2593	**Brazil-DF**
MW560615	***Pachyrhizus* erosus (A_DF-500 JP_LVV 207)**	2593	**Brazil-DF**
KX458238	*Phaseolus vulgaris* cv. Carioca	2593	Brazil-GO
KC004082	*Sida* sp.	2593	Brazil-MG
KC004090	*Sida* sp.	2593	Brazil-MG
KY524458	***Solanum betaceum* (DF-607)**	2593	**Brazil-DF**
DQ336350	*Solanum lycopersicum*	2674	Brazil-MG
KC004085	*Solanum lycopersicum*	2592	Brazil MG
JX865619	*Solanum lycopersicum*	2594	Brazil MG
MN156297	*Solanum lycopersicum*	2592	Brazil-ES
MN156306	*Solanum lycopersicum*	2592	Brazil-ES
JX415196	*Solanum lycopersicum*	2591	Brazil: DF
JF803261	*Solanum lycopersicum*	2591	Brazil-DF
MN156298	*Solanum lycopersicum*	2592	Brazil-ES
MN156304	*Solanum lycopersicum*	2592	Brazil-ES
MN156305	*Solanum lycopersicum*	2592	Brazil-ES

JX415201	*Solanum lycopersicum*	2591	Brazil-GO
JX415199	*Solanum lycopersicum*	2591	Brazil-GO
MN156293	*Solanum lycopersicum*	2592	Brazil-ES
MN156299	*Solanum lycopersicum*	2592	Brazil-ES
MN156295	*Solanum lycopersicum*	2592	Brazil-ES
MN156296	*Solanum lycopersicum*	2592	Brazil-ES
MN156301	*Solanum lycopersicum*	2592	Brazil-ES
MT733811	*Solanum lycopersicum*	2592	Brazil-DF
MT733815	*Solanum lycopersicum*	2592	Brazil-DF
KC004078	*Solanum lycopersicum*	2593	Brazil-MG
KC706618	*Solanum lycopersicum*	2593	Brazil-MG
KC706619	*Solanum lycopersicum*	2594	Brazil-MG
KC004083	*Solanum lycopersicum*	2592	Brazil-MG
KC706620	*Solanum lycopersicum*	2592	Brazil-MG
KC004088	*Solanum lycopersicum*	2593	Brazil-MG
KC004068	*Solanum lycopersicum*	2593	Brazil-MG
JX865620	*Solanum lycopersicum*	2594	Brazil-MG
JX865621	*Solanum lycopersicum*	2593	Brazil-MG
JX865623	*Solanum lycopersicum*	2594	Brazil-MG
JX865633	*Solanum lycopersicum*	2593	Brazil-MG
JX865639	*Solanum lycopersicum*	2593	Brazil-MG
JX865639	*Solanum lycopersicum*	2593	Brazil-MG
JX865643	*Solanum lycopersicum*	2593	Brazil-MG
HQ606467	*Solanum lycopersicum*	2592	Brazil-SP
MW560614	***Solanum torvum* (A_DF-259 JP_LVV 206)**	**2593**	**Brazil-DF**
KY781196	*Solanum melongena*	2595	Brazil-SP
JF803260	*Solanum lycopersicum*	2591	Brazil-DF

JF803263	*Solanum lycopersicum*	2591	Brazil-DF
MT214084	*Solanum lycopersicum*	2593	Brazil-DF
MT733808	*Solanum lycopersicum*	2592	Brazil-DF

*Brazilian region acronyms: SP = São Paulo State, GO = Goiás State, DF= the Federal District, MG = Minas Gerais State and ES = Espírito Santo. State.

Supplementary Table 3 – Information of the four tomato severe rugose virus (ToSRV) isolates reported infecting four new host species: *Solanum torvum* Sw., *Pachyrhizus erosus* (L). Urban, *Oxalis latifolia* Kunht and *Solanum betaceum* Cav.

Isolates*	Botanical species	BLASTn (% identity and isolate name/host)	
		DNA–A component	DNA–B component
A DF_259 JP_LVV 206	*S. torvum*	JX415196.1 (99.77) BR780 Tom3:08/tomato	MT215002 (95.87) GO-247 LR_LVV 27
A DF_500 JP_LVV 207	*P. erosus*	MT733811.1 (99.15) BR:62/tomato	MT215002 (98.54) GO-247 LR_LVV 27
A DF_567 JP_LVV 208	*O. latifolia*	MT733811.1 (99.07) BR:62/tomato	MT215002 (98.24) GO-247 LR_LVV 27
DF–607	*S. betaceum*	KX458238.1 (99.88) BRITA 127:14/common bean	HQ606468.1 (99.88) Pi-1/tomato

* DNA–A complete sequences were recovered from the isolates: A DF_259 JP_LVV 206, A DF_500 JP_LVV 207, A DF_567 JP_LVV 208 and DF–607. DNA–B complete sequence was recovered for DF–607 and partial DNA–B sequences were recovered from the following isolates: A DF_259 JP_LVV 206, A DF_500 JP_LVV 207, A DF_567 JP_LVV 208 isolates.

Supplementary File 1

Sequences DNA–A and DNA–B isolates of tomato severe rugose virus (ToSRV) found infecting four new host species.

>A DF_259 JP_LVV 206 [organism=Tomato severe rugose virus] [isolate=A DF_259 JP_LVV 206] segment DNA A, complete sequence

ACCGGATGGCCGCGCGATTTTTCACCCCTTTAGTTTCAATTAAAGTAAAG
TGATTGTCTGTGACCCAATCATATTGGGCCTGTCGAGCTTAGATATTTGT
AACAACTTAAGGCCCAAGTTGTTAAACGGCTATAAATTGAACATACACT
TTACTTTTGCTTTAATTCAAAATGCCTAAGCGTGATGCCCCATGGCGTTT
AACGGCGGGAACTTCAAAGGTTTCCCGCTCTGTCAATTATTCTCCCCGTG
CAGGATATGGACCCAAATATAACAAGGCCGCTGAGTGGGTGAACAGGC
CCATGTACAGGAAGCCCAGGATCTACCGTACTTTGAGAGGCCCAGATGT
TCCTAGAGGCTGTGAAGGGCCTTGTAAGGTTCAGTCTTACGAGTCTCGT
CATGATGTTTCCCATGTCGGGAAGGTGATTTGTGTGTCTGACGTTACACG
TGGTAACGGTATTACTCACCGTGTTGGTAAGCGTTTCTGCGTGAAGTCTG
TATATATTTTAGGGAAGGTATGGATGGACGAGACCATCAAGTTGAAGAA
TCACACAAATAGTGTGATGTTCTGGTTGGTTAGAGATCGGAGACCTTAT
TCCTCACCTATGGATTTTGGCCAGGTGTTCAACATGTTTGACAACGAGCC
TAGCACTGCAACTGTTAAGAACGATCTTCGGGATCGTTTTCAGGTCATG
CACAAGTTTTATGCCAAGGTTACTGGTGGACAGTATGCCAGTAATGAGC
AGGCATTAGTGAAGCGCTTTTGGAAGGTCAACAACAACGTAGTCTACAA
CCATCAGGAGGCAGGGAAATACGAGAATCATACTGAGAACGCCTTGCT
ATTGTATATGGCATGTACTCATGCCTCTAACCCCGTGTATGCTACATTGA

AAAATTCGGATCTATTTTTATGATTCGATTACTAATTAATAAAATTTAAA
TTTTATTGAATGATTTTCCAGTACAGCATTTACATATGATTTGTCTGTTGC
GAAACGAACAGCTCTGATTACATTATTAATGGAAATAACGCCTAATCGA
TCTAGATACAGTAAGACTAAATATTTAAATCTAGTTAAATATGTCGTCCC
AGAAGCTGTCAGTGAAGTCGTCCATATCTGGAAGTTGAGGAAGCTCTTG
TGGAGATGCAATGCTCTCCTCAGGTTGTGGTTGAACCGTATTTGGACGT
GGTAGACTCTGCTCGCGGTGTACATTGGATCCTCCACTTTGTTTATCTTG
AAATAGAGGGGATTTGATATCTCCCAAATATAGACGCCATTCTCTGCCT
GACGTGCAGTGATGAGTTCCCCTGTGCGTGAATCCATGTCCTCTGCAGTC
GATGTGTACGTAAATAGAGCACCCGCACTCTATATCAATTCGTCGTCTCC
TGATTCCTCGTTTTTTAGCAGCTCTGTGTTGTACCTTGATAGAGGGGGGT
GTTAAGGAAGACGAATTTCGCATTGTGCTTTGTCCAATTATTTAGACTTG
CATTTTCTTCTTTGTCGAGGAAACATTTATAGCTGGCCCCCCTCGCCAGG
ATTGCAAAGCACGATGCATGGGATACCACCTTTAATTTGAACTGGCTTT
CCGTATTTGCAGTTTGATTGCCAATCCTTTTGGGCCCCAAGCAATTCTTT
CCAGTGCTTTAACTTTAGATAGTGCGGTGCGATGTCATCAATGACGTTAT
ATTCAACATGATTTGAATAAACCCTAGGATTGAAATCTAGGTGTCCACT
CAAATAATTATGGGCCCCTAATGCACGTGCCCACATCGTCTTTCCCGTTC
GAGAATCACCTTCAATGATGATACTAATAGGTCGTTCCGGCCGCGCAGC
GGCACCTCTCCCAAAATAGTCATCTGCCCAAGCTTGCATCTCTCTCGGTA
CGCTAGTGAAAGAGGAGAGGGGAAACGTAGGGGCCCATGGCTCCGGAG
CCTTTGTAAATATCCTATCTAAATTACTATTTAGATTGTGAAACTGAAAT
AAGAACTTTTCAGGCAGCTTCTCACGGATTATCTGCAGGGCGACGTCTTT
GGAAGGTGCGTTCAAGGCTTCTGCGGCAGCGTCGTTAGCTGTCTGGCAA
CCGCCTCTAGCACTTCTTCCGTCGATTTGGAATTCCCCCCACTCGATAGT
ATCTCCGTCCTTGTCGACATAGGATTTGACGTCGGACGATGACTTAGCTC
TCTGAATGTTCGGATGGAAATGTGTTGACCTTGTTGGGGAGACCAGGTC
GAAAAATCGTTGATTTTGGCAGCAGTAGTGGCCCTCAAATTGAAGAAGC
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TGAACTTCTTATTTGTAGGAGTGTTTAGGGTTTTTAATTGGGAAAGTGCT
TCTTCTTTCCGGATAATGAGCATGTGGGATATGTGAGGAAATAGTTCTTC
GCTTTTATTTGAAAGCGCTTTGGAGCTGATGGCATATTTGTAAATATGAC
CCTTACTACCAATTGGTAGCTGCTCTAAAACTCATATGAATTGGTAGTTA
TGGTAGCTCTTATATAGTAGAAGTTCTTTAAGGAGATTGCTACACGTGG
CGGCCATCCGTTATAATATT

>A DF_500 JP_LVV 207 [organism=Tomato severe rugose virus] [isolate=A DF_500 JP_LVV 207] segment DNA A, complete sequence

ACCGGATGGCCGCCCGATTTTTCACCCCTTTAGTTTCAATTAAAGTAAAG
TGATTTACTGTGGCCCAATCATATTGGGCCTGTCGAGCTTAGATATTTGT
AACAACTTAAGGCCCAAGTTGTTAAACGGCTATAAATTGAACATACACT
TTACTTTTGCTTTAATTCAAAATGCCTAAGCGTGATGCCCCATGGCGTTT
AACGGCGGGAACTTCAAAGGTTTCCCGCTCTGTCAATTATTCTCCCCGTG
CAGGATATGGACCCAAATATAACAAGGTCGCTGAGTGGGTCAACAGGC
CCATGTACAGGAAGCCCAGGATCTACCGTACTTTGAGAGGCCCAGATGT
TCCTAGAGGCTGTGAAGGGCCTTGTAAGGTTCAGTCTTACGAGTCTCGT
CATGATGTTTCCCATATCGGGAAGGTGATTTGTGTGTCTGACGTTACACG

TGGTAACGGTATTACTCACCGTGTTGGTAAGCGTTTCTGCGTGAAGTCTG
TATATATTTTAGGGAAGGTATGGATGGACGAGACCATCAAGTTGAAGAA
TCACACAAATAGTGTGATGTTCTGGTTGGTTAGAGATCGGAGACCTTAT
TCGTCACCCATGGATTTTGGCCAGGTGTTCAACATGTTTGACAACGAGC
CTAGCACTGCAACTGTTAAGAACGATCTTCGGGATCGTTTTCAGGTCAT
GCACAAGTTTTATGCTAAGGTTACTGGTGGACAGTATGCCAGTAATGAG
CAGGCATTAGTGAAGCGCTTTTGGAAGGTCAACAACAACGTAGTCTACA
ACCATCAGGAGGCAGGGAAATACGAGAATCATACTGAGAACGCCTTGC
TATTGTATATGGCATGTACTCATGCCTCTAACCCCGTGTATGCTACATTG
AAAATTCGGATCTATTTTTATGATTCGATTACTAATTAATAAAATTTAAA
TTTTATTGAATGATTTTCCAGTACAGCATTTACATATGATCTGTCTGTTG
CGAAACGAAACAGCTCTGATTACATTATTAATGGAAATAACGCCTAATC
GATCTAGATATAATAAGACTAAATATTTAAATCTAGTTAAATATGTCGT
CCCAGAAGCTGTCAGTGAAGTCGTCCATATCTGGAAGTTGAGGAAGCTC
TTGTGGAGATGCAATGCTCTCCTCAGGTTGTGGTTGAACCGTATTTGGAC
GTGGTAGACTCTGCTCGCGGTGTACATTGGATCCTCCACTTTGTTTATCT
TGAAATAGAGGGGATTTGATATCTCCCAAATATAGACGCCATTCTCTGC
CTGACGTGCAGTGATGAGTTCCCCTGTGCGTGAATCCATGTCCTCTGCAG
TCGATGTGTACGTAAATAGAGCACCCGCACTCTATATCAATTCGTCGTCT
CCTGATTCCTCGTTTTTTAGCAGCTCTGTGTTGTACCTTGATAGAGGGGG
GTGTTAAGGAAGACGAATTTCGCATTGTGCTTTGTCCAATTATTTAGACT
TGCATTTTCTTCTTTCTCGAGGAAACATTTATAGCTGGCCCCCTCGCCAG
GATTGCAAAGCACGATGCATGGGATACCACCTTTAATTTGAACTGGCTT
TCCGTATTTGCAGTTTGATTGCCAATCCTTTTGGGCCCCAAGCAATTCTT
TCCAGTGCTTTAACTTTAGATAGTGCGGTGCGATGTCATCAATGACGTTA
TATTCAACATGATTTGAATAAACCCTAGGATTGAAATCTAGGTGTCCAC
TCAAATAATTATGGGCCCCTAATGCACGTGCCCACATTGTCTTTCCAGTT
CGAGAATCACCTTCAATGATGATACTAATAGGTCGTTCCGGCCGCGCAG
CGGCACCTCTCCCAAAATAGTCATCTGCCCAAGCTTGCATCTCTCTCGGT
ACGCTAGTGAAAGAGGAGAGGGGAAACGTAGGGGCCCATGGCTCCGGA
GCCTTTGTAAATATCCTATCTAAATTACTATTTAGATTGTGAAACTGAAA
TAAGAACTTTTCAGGCAGCTTCTCACGGATTATCTGCAGGGCGACGTCTT
TGGAAGGTGCGTTCAAGGCTTCTGCGGCAGCGTCGTTAGCTGTCTGGCA
ACCGCCTCTAGCACTTCTTCCGTCGATTTGGAATTCCCCCCACTCGATAG
TATCTCCGTCCTTGTCGACATAGGATTTGACGTCGGACGATGACTTAGCT
CTCTGAATGTTCGGATGGAAATGTGTTGACCTTGTTGGGGAGACCAGGT
CGAAGAATCGTTGATTTTGGCAGCAGTAGTTGCCCTCAAATTGAAGAAG
CACGTGGAGATGAGGCTCCCCATTTTCATGGAGCTCTCTGCAAATCTTG
ATGAACTTCTTATTTGTAGGAGTGTTTAGGGTTTTTAATTGGGAAAGTGC
TTCTTCTTTCGATAATGAGCATTTGGGATATGTGAGGAAATAATTCTTCG
CTTTTATTTGAAAGCGCTTTGGAGCTGATGGCATATTTGTAAATATGACC
CTTACTACCAAATGGTAGCTGCTCTAAAACTCATATGAATTGGTAGTTAT
GGTAGCTCTTATATAATAGAAGTTCTCTAAGGAGATTGCTACACGTGGC
GGCCATCCGTTATAATATT

>A DF_567 JP_LVV 208 [organism=Tomato severe rugose virus] [isolate=A DF_567 JP_LVV 208] segment DNA A, complete sequence

ACCGGATGGCCGCGCGATTTTTCACCCCTTTATTTTCAATTAAAGTAAAG
TGATTGTCTGTGGCCCAATCATATTGGGCCTGTCGAGCTTAGATATTTGT
AACAACTTAAGGCCCAATTTGTTAAACGGCTATAAATTGAACATACACT
TTACTTTTGCTTTAATTCAAAATGCCTAAGCGTGATGCCCCATGGCGTTT
AACGGCGGGAACTTCAAAGGTTTCCCGCTCTGTCAATTATTCTCCCCGTG
CAGGATATGGACCCAAATATAACAAGGCCGCTGAGTGGGTGAACAGGC
CCATGTACAGGAAGCCCAGGATCTACCGTACTTTGAGAGGCCCAGATGT
TCCTAGAGGTTGTGAAGGGCCTTGTAAGGTTCAGTCTTACGAGTCTCGTC
ATGATGTTTCCCATATCGGGAAGGTGATTTGTGTGTCTGACGTTACACGT
GGTAACGGTATTACTCACCGTGTTGGTAAGCGTTTCTGTGTTAAGTCTGT
ATATATTTTAGGGAAGGTATGGATGGACGAGACCATCAAGTTGAAGAAT
CACACAAATAGTGTGATGTTCTGGTTGGTTAGAGATCGGAGACCTTATT
CGTCACCTATGGATTTTGGCCAGGTGTTCAACATGTTTGACAACGAGCCT
AGCACTGCAACTGTTAAGAACGATCTTCGGGATCGTTTTCAGGTCATGC
ACAAGTTTTATGCCAAGGTTACTGGCGGACAGTATGCCAGTAATGAGCA
GGCATTAGTGAAGCGCTTTTGGAAGGTCAACAACAACGTAGTTTACAAC
CATCAGGAGGCAGGGAAATACGAGAATCATACTGAGAACGCCTTGCTA
TTGTATATGGCATGTACTCATGCCTCTAACCCCGTGTATGCTACATTGAA
AATTCGGATCTATTTTTATGATTCGATTACTAATTAATAAAATTTAAATT
TTATTGAGTGGTTTTCCAGTACAGCATTTACATATGATTTGTCTGTTGCG
AAACGAACAGCTCTGATTACATTATTAATGGAAATTACGCCTAATCGAT
CTAGATACAATAAGACTAAATATTTAAATCTATTTAAATATGTCGTCCCA
GAAGCTGTCAGTGAAGTCGTCCATATCTGGAAGTTGAGGAAGCTCTTGT
GGAGATGCAATGCTCTCCTCAGGTTGTGGTTGAACCGTATTTGGACGTG
GTAGACTCTGCTCGCGGTGTACATTGGATCCTCCACTCTGTTTATCTTGA
AATAGAGGGGATTTGATATCTCCCAAATATAGACGCCATTCTCTGCCTG
ACGTGCAGTGATGAGTTCCCCTGTGCGTGAATCCATGTCCTCTGCAGTCG
ATGTGTACGTAAATAGAGCACCCGCACTCTATATCAATTCGTCGTCTCCT
GATTCCTCGTTTTTTAGCAGCTCTGTGTTGTACCTTGATAGAGGGGGGTG
TTAAGGAAGACGAATTTCGCATTGTGCTTTGTCCAATTATTTAGACTTGC
ATTTTCATCTTTGTCGAGGAAACATTTATAGCTGGCCCCCTCGCCAGGAT
TGCAAAGCACGATGCATGGGATACCACCTTTAATTTGAACTGGCTTTCC
GTATTTGCAGTTTGATTGCCAATCTTTTTGGGCCCCAATCAATTCTTTCC
AGTGCTTTAACTTTAGATAGTGCGGTGCGATGTCATCAATGACGTTATAT
TCAACATGATTTGAATAAACCCTAGGATTGAAATCTAGGTGTCCACTCA
AATAATTATGGGCCCCTAATGCACGTGCCCACATCGTCTTTCCCGTTCGA
GAATCACCTTCAATGATGATACTAATAGGTCGTTCCGGCCGCGCAGCGG
CACCTCTCCCAAAATAGTCATCTGCCCAAGCTTGCATCTCTCTCGGTACG
CTAGTGAAAGAGGAGAGGGGAAACGTAGGGGCCCATGGCTCCGGAGCC
CTTGTAAATATCCTATCTAAATTACTATTTAGATTGTGAAACTGAAATAA
GAACTTTTCAGGCAGCTTCTCACGGATTATCTGCAGGGCGACCTCTTTGG
AAGGTGCGTTCAAGGCTTCTGCGGCAGCGTCGTTAGCTGTCTGGCAACC
GCCTCTAGCACTTCTTCCGTCGATTTGGAATTCCCCCCACTCGATAGTAT
CTCCGTCCTTGTCGACATAGGATTTGACGTCGGACGATGACTTAGCTCTC
TGAATGTTCGGATGGAAATGTGTTGACCTTGTTGGGGAGACCAGGTCGA
AGAATCGTTGATTTTGGCAGCAGTAGTTGCCTTCAAATTGAAGAAGCAC
GTGGAGATGAGGCTCCCCATTTTCATGGAGCTCTCTGCAAACCTTGATG
AACTTCTTATTTGTAGGAGTGTTTAGGGTTTTTAATTGGGAAAGTGCTTC
TTCTTTTGATAATGAGCATTTGGGATATGTGAGGAAATAATTCTTCGCTT
TTATTTGAAAGCGCTTTGGAGCTGATGGCATATTTGTAAATATGACCCTT

ACTACCAAATGGTAGCTGCTCTAAAACTCATATGAATTGGTAGTTATGG
TAGCTCTTATATAGTAGAAGTTCTATCTAAGGAGATTGCTACACGTGGC
GGCCATCCGCTATAATATT

>B DF_259 JP_LVV 206 [organism=Tomato severe rugose virus] [isolate=A DF_259 JP_LVV 206

ACCGGATGGCCGCGCGATTTTTCTTAGCTGCTACGTGGCGAAATCGTGT
ACGTTGCCTCACGCTTTACATTTTAATTGAGCGCTTTTTTGAAGTCCGCG
AAATGAGTTAATTATCTTTTTGAAATCCGCTGCTTGTGAATCACCTTTAA
TTTGAATTAAAGGTTGGATAGTTCATATTGATCAATCATTTCGCTGGTTT
ATTTCATGTCGTGGTGTAATTACAACCGTTCGTTAAAAATATAAGATTGG
TACGATGTGGACCGTCTAAATTTCATCTACATTGTTAATTTGACAATTTA
ATTCATATTTAAACTCTGTTTTTGTGTGGGTTTACACCACGTCTATACAA
ATTGTCCAGGGAAGTTGGTATGAGCATAATTTTTATTTTGTCTTATCATA
TTATCTGAACATGTTTCCCATTAAGTATAGACGTGGAATGTTGTTTAATC
ATCGACGAGGTTACTCATCGACTATAACTGAACCTACGGCGTTAATTGG
TACCTGTTGTCTGTATTCTATTACGCACTGGGCGATTTTCATACAGCTAC
GACTAACCCTTGCGCTTAATTGAGACGCCGTTGAAGGGAATTGAAGCAT
TATCTCGGAAAGATCATGTTAAAGGGGATATTCATCACGCTTAGATTCT
ATATAATTAAATGCGTTGGGAGGATTCACAACTTGGGATTCCAAAATTG
AAAATAGGGAGCGCAGCGACAATGTTTGAGGAAAGTTAATAAGGAAAG
ATGATAATATTTTCGTCAACTGAATATATTACAAGAAAATTGTCTGTTGA
TCCAATTGGGAAATTGGAGATGATAATAACTAAAAAACGAGGAATCAG
GAGACGACGAATTGAGAGGAGATATTGTCTATTTTCGGTGTAATTGATA
AATACCCCTCTATTTGCTCTTTAAATAGAATTTATAAGGCAAGGGCATAT
TTGTAAATATGACCCTTACTACCAAATGGTAGCTGCTCTAAAACTCATAT
GAATTGGTAGTTATGGTAGCTCTTATATAATAGAAGTTCTATCTAAGGA
GATTGCTACACGTGGCGGCCATCCGTTATAATATT

>B DF_500 JP_LVV 207 [organism=Tomato severe rugose virus] [isolate=A DF_500 JP_LVV 207] segment DNA B, partial sequence

ACCGGATGGCCGCGCGATTTTTCTTACCTGCTACGTGGCGAAATCGTGT
ACGTTGCCTCGCGCTTTCCATTTAATTGAGCGCTTTTTTGAAGTCCGCGA
AATGAGTTAATTGTCTTTTTGAAATCCGCTATTTGTGAATCACCTTTAAT
TTGAATTAAAGGTTGGATAGTTCATATTGATCAATCATTTCGCTGGTTTA
TTTCCTGTCGTGGTGTAATTACAACCGTTCGTTAAAAATATAAGAAATTT
ACGACGTGGACTGTCTAAATTTCATCTACATAGTTAATTTGACAAATGA
ATGCATATTTAAACTCTGCTTTCGTGTGGGTTTATACCACGTCTATACAT
ATTGTCCAGGTTATTTTGTATAAATATAATTTTTATTTTGTCTTATCTAAT
TATATGAACATGTATCCCATTAAGTATAGACGTGGAATGTTGTTTAATCA
TCGACGAGGTTACTCATCTAATCCCGTATTTAAGCGTTTACACGGAGCG
AAACGAAGTGATTTCAAGCGCCGTTCGAGTAATCAGAATCTGATAGGAA
GGTCCACGACGCCTGTAGTGATTCATTGTCCGTCATTCTTCTGTCATGAA
TTTCGACTATAACTGAACCTACGGCGTTAATTGGTACCTGTTGTCTGTAT
TCTATTACGCAATGGTCGATTTTCATACAGCTACGACTAAGCCTTGCGCT
TAATTGAGACGCCGTTGAAGGGAATTGAAGCATTATCTCGGTTAGATCA
TGGGAAAGCTGATATTCATCACGCTTAGATTCTATATAATTAAATGCGTT
GGGAGGATTCACAAGCTGAGATTCCATTATTGAAAATAGGGAGCGCAG

CGACAATGTTTCAGGAAAGTTAATAAGGGAAGATGATAATATTTTCGTC
AACTGAATATATGACAAGAAAATTGTCTGTTGATCCAATTGGGAAATTG
GAGATGATAATAACTAAAAAACGAGGAATCAGGAGACGACGAATTGAG
AGGAGATATTGTCTATTTTCGGTGTAATTGATAAATACCCCTCTATTTGC
TCTTTAAATAGAATTTTTAAGGCAAGGGCATATTTGTAAATATGACCCTT
ACTACCAAATGGTAGCTGCTCTAAAACTCATATGAATTGGTAGTTATGG
TAGCTCTTATATATTAGAAGTTCTTTCTAAGGAGATTGCTACACGTGGCG
GCCATCCGTTATAATATT

>B DF_567 JP_LVV 210 [organism=Tomato severe rugose virus] [isolate=A DF_567 JP_LVV 210] segment DNA B, partial sequence

ACCGGATGGCCGCGCGATTTTTCTTATCTGCTACGTGGCGAAATCGTGTA
CGTTGCCTCGCGCTTTCCATTTTAATTGAGCGCTTTTTTGAAGTCCGCGA
AATGAGTTAATTGTCTTTTTGAAATCCACTATTGTGAATCACCTTTAATT
TGAATTAAAGGTTGGATAGTTCATATTGATCAATCATTTCGCTGGTTTAT
TTCCTGTCGTGGTGTAATTACAACCGTTCGTTAAAAATATAAGAAATTTA
CGACGTGGACTGTCTAAATTTCATCTACATAGTTAATTTGACAAATGAAT
GCATATTTAAACTCCGCTTTCGTGTGGGTTTACACCACGTCTATACATAT
TGTACAGGTTATTTTGTATAAGTATAATTTTTTTTTGTCTTATCTTATTAT
ATGAACATGTATCCCATTAAGTATAGACGTGGAATGTTGTTTAATCATC
GACGAGGTTACTCATCTAATCCCGTATTTAAGCGTTTACACGGAGCGAA
ACGAAGTGATTTCAAGCGTCGTTCGACTAATCAGTCTATGTTACATCTGA
TAGGAAGGTCCACGACGCCTGTAGTGATTCATTATCCGTCATTCTTCTGT
CATGAATTTCGACTATAACTGAACCTACGGCGTTAATTGGTACCTGTTGT
CTGTATTCTATTACGCAATGGTCGATTTTCATACAGCTACGACTAAGCCT
TGCGCTTAATTGAGACGCCGTTGAAGGGAATTGAAGCATTATCTCGGTT
AGATCATGGGAAAGCTGATATTCATCACGCTTAGATTCTATATAATTAA
ATGCGTTGGGAGGATTCACAAGCTGAGATTCCATTATTGAAAATAGGGA
GCGCAGCGACAATGTTTGAGGAAAGTTAATAAGGGAAGATGATAATAT
TTTCGTCAACTGAATATATGACAGGAAAATTGTCTGTTGATCCAATTGG
GAAATTGGAGATGATAATAAGTAAACAACTAGGAATCAGGAGACGACG
AATTGAGAGGAGATATTGTCTATTTTAGGTGTAATTGATAAATACCCCTC
TACTTGCTCTTTAAATAGTATTTTCAAGCCAAGGGCATATTTGTAAATAT
GACTCTTACTACCAAATGGTAGCTGCTCTAAAACTCATATGAATTGGTA
GTTATGGTAGCTCTTATATAGTAGAAGTTCTTTTCAAGGAGATTGCCACA
CGTGGCGGCCATCCGTTATAATATTACCGGATGGCCGCGCGATTTTTCTT
ATCTGCTACGTGGCGAAATCGTGTACGTTGCCTCGCGCTTTCCATTTTAA
TTGAGCGCTTTTTTGAAGTCCGCGAAATGAGTTAATTGTCTTTTTGAAAT
CCACTATTGTGAATCACCTTTAATTTGAATTAAAGGTTGGATAGTTCATA
TTGATCAATCATTTCGCTGGTTTATTTCCTGTCGTGGTGTAATTACAACC
GTTCGTTAAAAATATAAGAAATTTACGACGTGGACTGTCTAAATTTCAT
CTACATAGTTAATTTGACAAATGAATGCATATTTAAACTCCGCTTTCGTG
TGGGTTTACACCACGTCTATACATATTGTACAGGTTATTTTGTATAAGTA
TAATTTTTTTTTGTCTTATCTTATTATATGAACATGTATCCCATTAAGTAT
AGACGTGGAATGTTGTTTAATCATCGACGAGGTTACTCATCTAATCCCGT
ATTTAAGCGTTTACACGGAGCGAAACGAAGTGATTTCAAGCGTCGTTCG
ACTAATCAGTCTATGTTACATCTGATAGGAAGGTCCACGACGCCTGTAG

TGATTCATTATCCGTCATTCTTCTGTCATGAATTTCGACTATAACTGAAC
CTACGGCGTTAATTGGTACCTGTTGTCTGTATTCTATTACGCAATGGTCG
ATTTTCATACAGCTACGACTAAGCCTTGCGCTTAATTGAGACGCCGTTGA
AGGGAATTGAAGCATTATCTCGGTTAGATCATGGGAAAGCTGATATTCA
TCACGCTTAGATTCTATATAATTAAATGCGTTGGGAGGATTCACAAGCT
GAGATTCCATTATTGAAAATAGGGAGCGCAGCGACAATGTTTGAGGAA
AGTTAATAAGGGAAGATGATAATATTTTCGTCAACTGAATATATGACAG
GAAAATTGTCTGTTGATCCAATTGGGAAATTGGAGATGATAATAAGTAA
ACAACTAGGAATCAGGAGACGACGAATTGAGAGGAGATATTGTCTATTT
TAGGTGTAATTGATAAATACCCCTCTACTTGCTCTTTAAATAGTATTTTC
AAGCCAAGGGCATATTTGTAAATATGACTCTTACTACCAAATGGTAGCT
GCTCTAAAACTCATATGAATTGGTAGTTATGGTAGCTCTTATATAGTAGA
AGTTCTTTTCAAGGAGATTGCCACACGTGGCGGCCATCCGTTATAATATT

Supplementary file 2

Sequences *mat*K and *rbc*L isolates of tomato severe rugose virus (ToSRV) found infecting four new host species

>DF_259_rbcL partial sequence

TCCCCCCCCCAACAGAGACTAAAGCAAGTGTTGGATTCAAAGCTGGTGT
TAAAGAGTACAAATTGACTTATTATACTCCTGAGTACCAAACCAAGGAT
ACTGATATATTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCAC
CTGAAGAAGCAGGGGCCGCGGTAGCTGCCGAATCTTCTACTGGTACATG
GACAACTGTATGGACCGATGGACTTACCAGTCTTGATCGTTACAAAGGG
CGATGCTACCGCATCGAGCGTGTTGTTGGAGAAAAAGATCAATATATTG
CTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTTCCGTTACCAAT
ATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAGCCCTGCGCGC
TCTACGTCTGGAAGATCTGCGAATCCCTCCTGCTTATGTTAAAACTTTCC
AAGGTCCGCCTCATGGGATCCAAGTTGAAAGAGATAAATTGAACAAGT
ATGGTCGTCCCCTGTTGGGATGTACTATTAAACCTAAATTGGGGTTATCT
GCTAAAAACTACGGTAGAGCTGTTTATGAATGTCTTCGTG

>DF_500_rbcL partial sequence

TATGTCACCCCCAAACAGAGACTAAAGCTAGTGTTGGGTTCAAAGCTGG
TGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGAAACCAAA
GATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCC
GCCTGAAGAAGCAGGTGCCGCGGTAGCCGCCGAATCTTCTACTGGTACA
TGGACAACTGTGTGGACCGATGGGCTTACCAGTCTTGATCGTTACAAAG
GACGATGCTACCACCTCGAACCTGTTGCTGGGGAAGAAAATCAATATAT
TGCTTATGTAGCTTATCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTCGGTAATGTATTTGGGTTCAAGGCCCTGCGC
GCTCTACGTCTGGAGGATTTGCGAATCCCTACTTCTTATATTAAAACTTT
CCAAGGTCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAACAAG

TATGGTCGTCCCCTATTAGGATGTACTATTAAACCTAAATTGGGGTTATC
CGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTTCGTGGTGGATTG
GGATTTTACA

>DF_567_rbcL partial sequence

TATGTCACCCCCAAACAGAGACTAAAGCAAGTGTTGGATTCAAGGCCGG
TGTTAAAGATTATAAATTGACTTATTATACTCCTGAATATGAAACCAAA
GATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCC
CCCTGAGGAAGCCGGGGCAGCGGTAGCTGCTGAATCTTCTACTGGTACA
TGGACAACTGTGTGGACCGATGGGCTTACCAATCTTGATCGTTACAAAG
GACGATGCTACCACATCGAGCCTGTTGCTGGAGAAGAAAGTCAATTTAT
TGCTTATGTAGCTTACCCCTTAGACCTTTTTGAAGAAGGTTCTGTTACTA
ACATGTTTACTTCCATTGTGGGTAATGTGTTTGGGTTCAAGGCGCTGCGG
GCTCTACGTCTAGAGGATTTGCGAATCCCTGTTGCTTATGTTAAAACTTT
TCAAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAATTGAATAAG
TATGGCCGTCCCCTATTGGGATGTACTATTAAACCTAAATTAGGGTTATC
CGCTAAGAATTATGGTAGAGCAGTTTATGAATGTCTCCGTGGT

>DF_607_rbcL partial sequence

TTGGATTCAAAGCTGGTGTTAAAGAGTACAAATTGACTTATTATACTCCT
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AATCTTCTACTGGTACATGGACAACTGTATGGACCGATGGACTTACCAG
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AGAAGGTTCCGTTACCAATATGTTTACTTCCATTGTAGGTAATGTATTTG
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TGCTTATATTAAAACTTTCCAAGGTCCGCCTCATGGGATCCAAGTTGAA
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ATGTCTTCGTG

>DF_259_matK partial sequence

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TAGGTATGAAGGAAAATCAATTCTGGCTTCAAAAGGGACGTTTCTTTTG
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TATGTACTTTCACACAGGAAGGATCCATATAAACCAATTATCCAACCAT
TCCCGTGACTTTATGGGCTATCGTTCAAGTGTGCGACTAAATCATTCAAT
GGTACGTAGTCAAATGTTCGAAAATTCATTTCTAATCAATAATCCAATTA

AGAAATTCGATACCCTTGTTCCAATTATTCCTTTGATTGGATCATTAGCT
AAAGCACACTTTTGTACCGTATTAGGGCATCCCATTAGTAAACCGGTTT
GGTCCGATTTATCAGATTCTGATATTATTGACCGATTTGGGCGTATATGC
AGAATCT

>DF_500_matK partial sequence

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AAAATCTTGATTTCTACTTTTTTTTCAAAAAGTAATCCAAGATTTTTCTTG
TTCCTATATAATTTATATGTACGGGAATATGAATCTATCTTTCTTTTTCTA
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GCGAATTTTTTTCTATGAAAAAATAGAACATCTTGGACAAGTATCTGTTA
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TAAAGCAAAATTTTGTAATGTATTTGGTCATCCCATTAGTAAGCCGGTTT
GGGCCAATTTATCTTATTTTGATATTATTTGACCGGTTTTTGCGGATATG
CAGAATCT

>DF_567_matK partial sequence

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AAGAAACTCGATACGATAATTCCGATTATTCCTTTGATTGGATCATTGGC
AAAATCGAAATTTTGTAACACATTGGGCTATCCCATTAGTAAGCCGACT
TGGGCGGATTTGTCGGATTCTGATATTATGGACCGATTTGTGCGTATATG
CAGAAAT

>DF_607_matK partial sequence

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TATATAATTCTTATGTATATGAATGCGAATCCACTTTCGTCTTTCTACGG

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CHAPTER 3

Original Article

DNA–A genomic diversity, phylogeography, and spatiotemporal dynamics of tomato severe rugose virus lineages across the Brazilian Neotropics

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Work to be submitted for Virus Genes

Abstract

Phylogeographic studies dealing with plant viruses are still scarce in comparison to other groups of viral pathogens, especially for some viral species of economical and biological relevance such as the tomato severe rugose virus (ToSRV). This virus was initially reported as one member of the complex of begomoviruses infecting tomatoes in Brazil detected after the invasion of the vector *Bemisia tabaci* Middle East-Asia Minor 1 (= biotype B) in the 1990's. ToSRV is hitherto the most widely disseminated tomato-infecting begomovirus in Brazil with high prevalence under field conditions. However, extensive studies on the overall diversity and patterns of geographical distribution of ToSRV across the Brazilian territory are yet scarce. In this context, our major objective was to study the diversity of ToSRV isolates and to investigate the spatiotemporal dynamics of viral populations across a wide range of macro- and micro-ecogeographical regions of Brazil. In our survey, the complete DNA-A genome of 105 tomato-infecting ToSRV isolates was characterized from samples scattered across 48 cities belonging to 11 states and the Federal District (DF). This collection of isolates was obtained from plants with and without the tolerance resistance factors *Ty*-1 and *Ty*-3. In this study, ToSRV was reported for the first time in commercial tomato fields established in areas encompassing the Amazon Rain Forest (North), Caatinga (Northeast), and Pampa (South) biomes. Phylogeographical analyses indicated that the ancestral ToSRV population emerged more likely in 1983 in the region of Pimenta in Minas Gerais (MG) state (Southeast Brazil). The first event of migration occurred to Cascalho Rico (MG) region and then a migration network was observed to the processing tomato fields in highland areas of Central Brazil as well as to fresh-market tomato areas in the Southeast region. Multiple events of migration among and within all five macro-regions have occurred since then. Viral diversity was mainly organized according to the geographic area with no clustering observed for isolates obtained from plants with either *Ty*-1 or *Ty*-3 genes. The present study represents the largest phylogeographic and evolutionary analysis reported for ToSRV, which can be considered as the most important tomato-infecting begomovirus in the Neotropics.

Keywords: begomovirus, tomato, spread, *Bemisia tabaci* MEAM1, distribution

Introduction

The reconstruction of the phylogeographic history has been increasingly used to study the evolutionary relationships and geographical distribution of viral species, and has been widely applied to human viruses (Messina et al. 2014; Njoto et al. 2018; Ehichioya et al. 2019; Biswas and Majumder 2020) and animal viruses (Carnieli et al. 2011; Vieira et al. 2013; Miranda and Thompson 2016). Phylogeographic studies on plant viruses are still scarce when compared to other groups of viral pathogens (Rakotomalala et al. 2019) especially for some economically and biological relevant viral species such as tomato severe rugose virus; ToSRV (genus *Begomovirus*, family *Geminiviridae*). This family has currently nine genera, including the *Begomovirus*, which is composed of whitefly-transmitted viral species with one (= monopartite) or two (= bipartite) circular, single-stranded DNA component(s) with ≈ 2.6 kb (Ribeiro et al. 2003; Cotrim et al. 2007; Fernandes et al. 2008b; Rocha et al. 2010, 2013; González-Aguilera et al. 2012; Mituti et al. 2019; Reis et al. 2020; Souza et al. 2020; Duarte et al. 2021).

ToSRV was initially reported as one member of the complex of begomoviruses infecting tomatoes in Brazil after the invasion of the vector *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM 1 = biotype B) in the 1990's. Currently, ToSRV is the most widely disseminated begomovirus in tomato crops in Brazil, being predominant in the Southeast and Central regions, showing high prevalence under field conditions (Reis et al. 2020). The first formal report of ToSRV in Brazil occurred in 1999, in Minas Gerais, affecting tomato crops. Afterwards, several surveys detected ToSRV isolates across many tomato-producing areas in Brazil (Ribeiro et al. 2003; Cotrim et al. 2007; Fernandes et al. 2008b; Rocha et al. 2010, 2013; González-Aguilera et al. 2012; Mituti et al. 2019; Reis et al. 2020; Souza et al. 2020; Duarte et al. 2021). The first full-sequence of the ToSRV DNA–A component was deposited in the GenBank database in 2001 (accession no. AY029750) (Rezende et al. 2001, yet unpublished). Isolates of ToSRV have also been characterized infecting naturally tomatoes as well as a wide array of Solanaceae and Fabaceae species.

The first formal report of a tomato-infecting ToSRV isolate in Brazil done in Minas Gerais state in 1999. The first complete sequence of the DNA–A component was deposited in the GenBank in 2001 (accession no. AY029750) (Rezende et al. 2001, unpublished). Afterwards, several surveys detected ToSRV across many tomato-producing areas in Brazil (Ribeiro et al. 2003; Cotrim et al. 2007; Fernandes et al. 2008a; Rocha et al. 2010, 2013; González-Aguilera et al. 2012; Mituti et al. 2019; Duarte et al., 2021; Reis et al. 2020; Souza et al. 2020). Field surveys carried out by Ribeiro et al. (2003) from 1994 to 1999 in several states of Brazil revealed a quite remarkable diversity of begomoviruses associated with tomatoes, indicating the presence of seven new species, among them *Tomato rugose mosaic virus* (a recombinant species with ToSRV) and *Tomato chlorotic mottle virus*. A survey of begomoviruses associated with fresh-market and processing tomato fields in Central Brazil in the years 2002-2004 indicated the predominance of ToSRV in both production systems (Fernandes et al. 2008b). A survey begomovirus in the sweet-pepper and tomato fields in São Paulo state during the years 2007 and 2008 indicated ToSRV as the predominant begomovirus in all sampled

regions in both crops (Rocha et al. 2010). In addition, they also to identified high genetic variability among ToSRV isolates obtained from tomatoes.

The first complete molecular characterization of both DNA–A (Accession no. HQ606467) and DNA–B (Accession no. HQ606468) components of ToSRV was carried out in 2011 (Barbosa et al. 2011) with a virus collected infecting tomatoes in 2007 in Piracicaba (SP) and named as Pi1 isolate. The symptoms associated with ToSRV Pi1 isolate were severe rugose mosaic. Phylogenetic analyzes indicated a close relationship of ToSRV with tomato rugose mosaic virus (ToRMV), displaying 87% nucleotide identity. The biological characterization of ToSRV Pi 1 was also performed indicated 13 species were susceptible to ToSRV infection, four of which were symptomatic: *S. lycopersicum* and *N. physalodes* developed yellow rugose leaf mosaic and *Chenopodium album* and *C. ambrosioides* exhibited only yellow mosaic.

More extensive studies on the diversity, distribution, and genetic structure of ToSRV populations were performed by González-Aguilera et al. (González-Aguilera et al. 2012). In this study, analysis of variability descriptors indicated that the ToSRV population displayed a genetic variability similar to other tomato-infecting begomovirus populations described in Brazil. All ToSRV isolates, formed a group with begomoviroses previously reported in Brazil, which diverged from another group of begomoviroses described from other American countries. Reis et al. (Reis et al. 2020) performed metagenomics studies with begomovirus species that infect tomato cultivars with and without the *Ty*-1 resistance gene in Central Brazil. The most prevalent tomato-infecting begomoviruses were tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato golden vein virus (TGVV), and tomato yellow vein streak virus (ToYVSV) (Reis et al. 2020, 2021). Among them, ToSRV is currently the most widespread begomovirus in Brazil, being present in association and/or displacing other viral species across major producing areas (Reis et al. 2020, 2021; Duarte et al. 2021).

Even though ToSRV is one of the most important tomato-infecting begomovirus in Brazil, no comprehensive studies on the overall diversity and patterns geographical distribution of ToSRV across the Brazilian territory are available. In this context, our major objective was to study the diversity of ToSRV isolates/lineages and to investigate the spatiotemporal dynamics of viral populations across a wide range of macro- and micro-ecogeographical regions of Brazil. These studies will provide crucial information from the plant breeding standpoint, aiming to identify and to deploy genetic resistance factors with greater stability, durability, and efficiency. Furthermore, the phylogeographic data will provide relevant information about viral species prevalence in space and time across the major tomato-producing regions.

Material and Methods

Detection and partial characterization of tomato severe rugose virus in a begomovirus collection (CNPH-Begomovirus Tomato Collection) – Isolates used in this study were selected from the CNPH-Begomovirus Tomato Collection, which has 1.500 begomovirus isolates from symptomatic tomato samples, collected from 2001 until the present (**Fig. 1a**), in tomato-producing areas of the five

Brazilian regions. Samples showed typical symptoms induced by begomovirus-infections, with chlorosis, leaf apical deformation, epinasty and overall growth reduction (**Fig. 1a**). Total DNA was extracted, using CTAB 2X buffer and organic solvents (Boiteux et al. 1999) followed by rolling circle amplification (RCA) previously described by Inoue-Nagata et al. (Inoue-Nagata et al. 2004). Primers PAL1v1978 / PARc496 (DNA–A 1.200 bp) and pBL/cRC1 (DNA–B 400 bp) were used (Rojas 1993). Amplicons were purified from agarose gel using Promega Kit and sequenced at the Genomic Analysis Laboratory (CNPH), with ABI PRISM 3100 sequencer using the BigDye® Terminator Cycle Sequencing Ready Reaction Kit version 3.1 protocol (Applied Biosystems). The sequences obtained were analyzed using the BLASTn algorithm (Altschul et al. 1997) and evaluated in the public database NCBI (www.ncbi.nim.nih.gov). For recovery the complete genome sequence, 105 isolates were selected [Northeast (n = 2 isolates), North (n = 1), Midwest (n = 36), Southeast (n = 46), and South (n = 20)] based on identity of more than 95% in a partial sequence (Brown et al. 2015) compared with tomato severe rugose virus sequences available in GenBank **(Supplementary Table 1).**

Design of specific primer for tomato severe rugose virus – A specific primer pair for recovering the complete genome (~2600 bp) sequence of tomato severe rugose virus 'ToSRVF' (5'-TAT TGG GCC TGT CGA GCT TAG AT ATT T-3') and 'ToSRVR' (5'-CCA ATA TGA TTG GGC CAC AGA CAA-3') was designed using Geneious program (Kearse et al. 2012b). Firstly, this primer set was validated using total DNA obtained from four tomato samples, previously confirmed as positive to ToSRV, and in addition total DNA samples extracted from tomato infected, each with five begomoviruses **Supplementary Figure 1.** The amplicons from agarose gel were purified using Ludwig Biotechnology and sent to sequencing as describe above. The PCR conditions for the ToSRV-specific primer set were adapted for annealing temperature and the other conditions were in according to Reis et al. (Reis 2020b). The annealing temperature was 61.2 ºC during 3 minutes. The other primers and conditions were used according to Duarte et al. (Duarte et al. 2021). These primers were able to amplify fragments of the DNA–A component that partially overlap, thus allowing the generation / obtaining for the complete information of the DNA–A component of these isolates.

Analysis of the common region and conserved elements of Rep and CP protein from ToSRV isolates – Common region (CR) analyzes were performed after alignment of A MG_387 JP_LVV 211 (accession number: MW653951, DNA–A) and B MG_387 JP_LVV 212 (accession number: MW653952, DNA –B]. For analysis of motifs in the DNA–A component, 204 ToSRV tomato isolates [99 sequences obtained from public database NCBI (www.ncbi.nim.nih.gov), **Supplementary Table 2**, and 105 recovered in this work] were aligned. Comparisons of Rep proteins of these ToSRV isolates were performed in order to identify the Iteron-Related Domain (IRD) and the motif I according to Arguello-Astorga et al. (Argüello-Astorga and Ruiz-Medrano 2001). We also examined the structural motif of the Helix 4, conserved among the members of the family *Geminiviridae* (Arguello-Astorga et al. 2004). In addition, analysis were carried out to identify the almost palindrome segment of DNA–A [ACTT- (N7) -AAGT], a structural element conserved among promoters of the CP gene of geminiviruses (Cantú-Iris et al. 2019).

Verification of the presence of the *Ty*-1 and *Ty*-3 resistance genes – The total DNA obtained from 105 tomato plants were subjected to PCR – CAPS (Cleaved Amplified Polymorphic Sequences) assays. Amplicons were obtained using the primers' UWTy1F (5'-ATA AGC ATT TCA TGT CAG ATG TCT A-3') and 'UWTy1R (5'-CTA GAT CTT CAA TAG C-3') according to Ferro (Ferro 2013). PCR products were then subjected to cleavage with the restriction enzyme *Taq*-1 to identify presence or absence of the alleles. The cleaved products were submitted to electrophoresis on 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. Samples were also subjected to SCAR (Sequence Characterized Amplified Regions) assays, using the primers 'FLUW25F' (5'-CAA GTG TGC ATA TAC TTC ATA (T/G) TC ACC-3') and 'FLUW25R' (5'-CCA TAT ATA ACC TCT GTT TCT ATT TCG AC-3') in according to Ji et al. (Ji et al. 2007a).

Sequencing and analysis of sequences – Sequencing of PCR positive the samples (after analyses with the five ToSRV-specific primer pairs) was carried out at the Genomic Analysis Laboratory of Embrapa Vegetable Crops (CNPH), with ABI PRISM 3100 sequencer using the BigDye® Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems) protocol. The sequences obtained were analyzed using the program Geneious 11.0.5 (https://www.geneious.com.br). Initially, *De Novo* Assembly analysis was performed aiming to obtain the complete sequences of DNA–A components isolate. After assembling the contigs and evaluating their quality, they were analyzed with the BLASTn tool (Altschul et al. 1997). This tool was used to compare the sequences obtained with the sequences deposited in the public data bank GenBank (http://www.ncbi.nlm.nih.gov), aiming to identify the viral species **(Supplementary Table 1)**. The assembled sequences were aligned with the reference genome of the highest percentage of identity obtained in BLASTn, and from these, the annotation of the assembled genomes was performed. ORF annotation was performed in Geneious program [38].

Phylogeographical analyses – To reconstruct the geographical origin and spatial distribution of ToSRV isolates during diversification, sequences of 105 ToSRV-isolates obtained were in the Relaxed Random Walk (RRW) model (Lemey et al. 2009, 2010) implemented in the BEAST 1.8.4 software (Drummond et al. 2012). RRW infers routes of colonization of strains in a continuous phylogeographic diffusion and simultaneously reconstructs evolutionary history through a Bayesian approach. It was employed the HKY replacement model with the Akaike weight of evidence (wAIC) provided by JMODELTEST 2 (Darriba et al. 2012); symmetrical substitution model with variable Bayesian stochastic search variable selection (BSSVS); strict molecular clock; Coalescent tree model - Bayesian Skyline; and reconstruction of the ancestral state in all ancestors. Finally, phylogenetic trees were calculated using the Bayesian Markov Chain Monte Carlo (MCMC) in chains of 10,000,000 generations, with a sampling frequency of 1,000, in the BEAST program. The convergence of the chains was carried out in the TRACER program v1.7.1 (Rambaut et al. 2018) to evaluate the calculation of the effective sample size (Effective Sample Size - ESS), after a 10% burn-in, being accepted only results with values of ESS ≥ 200. The summarization of the trees generated in a tree of maximum credibility (Maximum Clade Credibility - MCC) was performed in the TreeAnnotator v1.8.4 program (also include in the BEAST package) and, finally, we performed the space-time

reconstruction using SPREAD3 (Bielejec et al. 2011) for viewing the distribution trajectories of the ToSRV isolates.

Results

Complete DNA–A genome sequences and molecular characterization of tomato severe rugose virus – In this study, PCR assays using 'ToSRV F'/ 'ToSRV R' primer pair were performed to recover the complete DNA–A sequences of 105 isolates/lineages from ToSRV obtained from of tomato plants displaying typical begomovirus-like symptoms, including, apical chlorosis deformation, epinasty, and overall growth reduction (**Fig. 1a**). The complete sequences of the DNA–A and DNA–B components were obtained via Sanger for the isolate A MG_387 JP_LVV 211 (accession number: MW653951) and B MG_387 JP_LVV 212 (accession number: MW653952). The MG_387 isolate was the ToSRV representative isolate for illustration of iterons and common region (CR). In addition, infectious clones were already obtained for this isolate (work in preparation). The DNA–A component of A MG_387 JP_LVV 211 has 2,593 nucleotides (nts) with genome organization typical of begomovirus found in the New World, containing one ORF (Open reading frame) in the viral sense (AV1) and four ORFs in the complementary sense (AC1, AC2, AC3, and AC4). The DNA–B component has 2,571 nts codifying two ORFs, one in the viral sense (BV1) and the other in the complementary sense (BV1) (**Fig. 1b**). The CR (166 nts), was identified by comparing the DNA–A and DNA–B sequences. In CR, the hairpin a containing stem-loop, the repeated sequences of the GGTAG iterons and Rep IRD = MPSAPKR**FQIK** (Argüello-Astorga and Ruiz-Medrano 2001) were identified, as well as the complementary reverse sequence of the same, and the conserved nonanucleotide TAATATT 'AC (**Fig. 1c**). In all 204 sequences of ToSRV isolates analyzed in the present study [99 from GenBank [(**Supplementary Table 2**) plus 105 from our collection)] the structural motif of Helix 4 (**Fig. 1d)**, conserved in all geminiviruses, was examined and it was found to be conserved among them (Arguello-Astorga et al. 2004). All isolates showed the amino acid sequence SKDVALQIIREK. In addition, the DNA–A quasi-palindromic [ACTT–(N7)–AAGT] segment, which is conserved among the CP promoters of the members of the *Geminiviridae* family, was confirmed among all the isolates **(Supplementary Figure 1)**.

Geographical distribution of ToSRV isolates – In our survey, the complete DNA–A genome of 105 ToSRV tomato-infecting isolates were characterized scattered across 48 counties belonging to 11 states and the Federal District (DF). ToSRV was reported by the first time in commercial tomato fields established in areas encompassing the Amazon Rain Forest (North), Caatinga (Northeast), and Pampa (South) biomes. In the North region, ToSRV was found in Araguaína (Tocantins – TO); in the Northeast it was detected in Ibiapina (Ceará – CE) and Chã Grande (Pernambuco – PE); in South Brazil isolates were found infecting tomatoes in Florianópolis (Santa Catarina – SC), Santo Amaro da Imperatriz (SC), Tijuca (SC), Cruzmaltina (Paraná – PR), Barro Branco (PR), Marilândia do Sul (PR), Faxinal (PR), Torres (Rio Grande do Sul – RS), and Caxias (RS). In the Central Brazil region (encompassing the Cerrado biome), ToSRV was reported for the first time in five counties in Góias (GO) (Morrinhos, Corumbá de Góias, Bonfinópolis, Planaltina de Góias, and São João D'Aliança) and

three producing regions in DF (Taquara, Café sem Troco, and Gama). In the Southeast region, ToSRV was registered for the first time in seven counties in the state of MG (Serra Azul, Cascalho Rico, Araguari, Pouso Alegre, Pimenta, Ituitaba, and Mateus Leme) and eight counties in São Paulo – SP state (Capão Bonito, Mogi Guaçu, Monte Mor, Quadra, Araçoiaba da Serra, Itapeva, Sebastianópolis do Sul, and Estiva Gerbi) comprising transition areas of the Cerrado and Atlantic Rain Forest biomes.

Evaluation of the presence of *Ty*-1 and *Ty*-3 resistance genes – The results of the analyzes to verify the presence or absence of the *Ty*-1 and *Ty*-3 resistance genes are show in **Fig. 2**. Our data indicate that 25 out of the 105 samples checked as positive for *Ty*-1 and five for *Ty*-3 **(Supplementary Table 1)**. Most of the branches that confer the presence of the *Ty*-1 gene (16 isolates) come from the Midwest region (GO and DF). Only five isolates [three isolates from GO (GO_496_41_2011, GO_497_41_2011 and GO_599_17_2013), one from DF (DF_228_42_2007) and one from MG (MG_387_40_2015)] were positive for the presence of both genes.

Phylogenetic and phylogeographical analysis of ToSRV – Phylogenetic analysis based on an alignment of the complete nucleotide sequences of DNA–A components of 105 ToSRV sequences is shown in **Fig. 2**. ToSRV isolates formed five distinct groups. Group I include (the isolate from the CE and isolates from the DF and GO collected between 2002 and 2005). In group II, the isolates from the Midwest (DF and GO) are grouped together with isolates from the PE and the RS, from collections between 2007 and 2016. Group III consisted of isolates from the Southeast region (MG, ES, RJ and SP) collected between the years 2010 to 2017. In group IV are the isolates collected in the South region (SC, PR and RS) grouped with isolates from MG and SP, from collections from 2006 to 2016. In group V, isolates from the Midwest grouped together with the isolate collected in TO. The phylogeographical reconstruction analysis allowed us to identify that this viral and the city of Pimenta–MG as its most plausible place of origin (**Fig. 2**). The spatiotemporal dynamics of ToSRV (performed according to the analyzes made by BEAST) is illustrated in **Fig. 3**. Our results indicate that the ancestral population of ToSRV emerged in 1983 in the county of Pimenta–MG, in 2002, the first migration events took place to Cascalho Rico–MG and for the northeastern region, county of Ibiapina–CE. Subsequently, in 2003, migration events took place in the central-west region, reaching the counties of Luziânia – GO, Morrinhos–GO and in the producing region of Planaltina–DF. From Pimenta–MG migration events to Marilândia do Sul–PR and Faxinal–PR were observed in 2004. In 2006, ToSRV was introduced in the counties of Cruzmaltina–PR, Santo Amaro da Imperatriz–SC, and Florianópolis–SC. In the following year (2007), ToSRV was introduced in Torres–RS, concluding the migration events to the South region. Soon after the introduction of the virus in Mogi Guaçu–SP and Capão Bonito–SP (2008) it spread quickly to neighboring areas, including the southern region of MG state. That same year, the virus was introduced in the counties of Chã Grande–PE and Araguaína–TO. The ToSRV lineage of Pimenta–MG detected in 1983 is the more likely original source of viral population that disseminated to other regions of Brazil.

Discussion

Tomato severe rugose virus is considered the most prevalent virus in Central Brazil [16, 17, 42]. Here, we presented complete information about genomic organization and conserved motifs found in DNA–A [A MG_387 JP_LVV 211 (accession number: MW653951)] and DNA–B [B MG_387 JP_LVV 212 (accession number: MW653952)] components related to virus replication [31], interactions between virus and host proteins [32] and promoter region of coat protein (CP). Even though performed for a wide array of bipartite begomoviruses, it is the first set of extensive analyses carried out for ToSRV (**Fig 2 b-d**).

The present study represents the largest phylogeographic and evolutionary analysis reported for ToSRV, which can be considered as the most important tomato-infecting begomovirus in the Neotropics. Here, we presented a widespread distribution map of ToSRV isolates in tomato crops across different Brazilian regions and biomes, confirming the high viral plasticity in adapting to the great biological, environmental and climatic diversity of tropical and subtropical regions (Coutinho 2006). In fact, ToSRV is currently identified only in Brazil, infecting both cultivated and non-cultivated plants (Bezerra-Agasie et al. 2006; Nozaki et al. 2006; Souza-Dias et al. 2008; Barbosa et al. 2009; Barreto et al. 2013; Macedo et al. 2017b; Moura et al. 2018; Duarte et al. 2019).

The first formal report of ToSRV was done in MG state tin 1999 (accession no. AY029750) (Rezende et al. 2001, which has not yet been published). Our data, however, show that ToSRV was already present in this region, at least 16 years before its first report in Brazil. The first molecular characterization of the DNA–A and DNA–B components, however, was only carried out in 2011 (Barbosa et al. 2011). Afterwards, several survey studies have detected ToSRV across many tomato-producing areas especially in Central and Southeast Brazil (Ribeiro et al. 2003; Cotrim et al. 2007; Fernandes et al. 2008b; González-Aguilera et al. 2012; Rocha et al. 2013; Mituti et al. 2019; Reis et al. 2020; Souza et al. 2020; Duarte et al. 2021). Studies carried out between 2007 and 2008, in tomato and pepper crops in the SP state, indicated the prevalence of ToSRV in both crops (Rocha et al. 2010). Our analyzes show that in these same years, ToSRV was already dispersed in several producing regions of SP and neighboring states like RJ, corroborating with the data from this previous study.

Our studies show that the ToSRV isolates presents a non-regionalized dispersion pattern, and the isolates is clustered according to the geographic location, with a few exceptions. Similar results were found in a previous study, which evaluated the genetic structure of begomovirus populations, making possible to verify the same grouping of ToSRV populations by locality (Rocha et al. 2013). Analyzes carried out showed that these populations are evolving in an advanced, non-neutral manner, with a tendency towards generalized purifying selection operating at nucleotide and protein levels. A complex interaction between adaptation to various plant species and vector biotypes, may reflected in the genetic structure, geographic distribution, and dispersion patterns of begomovirus populations (Rocha et al. 2013). Our data also indicated that ToSRV is more recently spreading to other regions

displaying an adaptive plasticity to different environmental conditions, which seems to be in sharp contrast with other species of the complex of begomoviruses reported in tomato in Brazil. For instance, it seems that tomato mottle leaf curl virus is more adapted to warm conditions in the Northeast region, whereas tomato common mosaic virus endemic to regions encompassing the Atlantic Rain Forest biome in the states of ES, RJ, and MG (Inoue-Nagata et al. 2016).

ToSRV was previously reported as one of the predominant begomoviruses in Central and in the Southeast regions of Brazil (Cotrim et al. 2007; Fernandes et al. 2008b; Inoue-Nagata et al. 2016; Reis et al. 2020; Duarte et al. 2021). In recent studies carried out in the ARF region, it was show the prevalence of this virus in comparison to other begomoviruses, being predominant in the states of ES and RJ (Duarte et al. 2021). In this work, we present the first formal report of ToSRV in the to the North and North-East regions of Brazil. Although the reports of ToSRV in the Central and Southeast regions are numerous, we find here in this study, the first formal detection of this virus in 29 municipalities in the Central, South-East, and South regions. These results indicate that ToSRV is a viral species with wide adaptation to tomato and with a non-regionalized, nationwide dispersion pattern.

The use of tomato varieties carrying resistance genes is one of the most effective management strategies for diseases of viral etiology. In Brazil, cultivars carrying the *Ty*-1 gene have been more intensively used, mainly across producing regions in Central Brazil (Boiteux et al. 2012). Our data are is agreement with these previous results, showing that the majority of the positive samples for the presence of *Ty*-1 and *Ty*-3 come from the Central Brazilian region. Previous study employing the next-generation sequencing approach evaluated the diversity of single-stranded DNA viruses in tomato samples having or not having have the *Ty*-1 begomovirus tolerance gene. It was show that ToSRV had a large number of reads, indicating their relative predominance in tomato samples with the *Ty*-1 gene (Reis et al. 2020). The ability of ToSRV isolates to replicate (even in lower levels) in plants with the *Ty*-1 gene can be considered as an additional factor that explains the general prevalence of this virus under Brazilian conditions (Reis et al. 2020). Nonetheless, several additional factors may be associated with a rapid dispersion of this species across tomato crops, such as a high efficiency in triggering systemic diseases (Favara et al. 2019), a transmission efficiency of the vector *B. tabaci* MEAM 1 (Toloy et al. 2018), transport of contaminated seedlings to other regions, wide range of host species that can function as a reservoir of the virus.

Conclusions

The results described here, based on sequences of more than 100 sequences of DNA–A component of samples collected in the five Brazilian regions, provide additional support for the hypothesis that the isolates of tomato severe rugose virus presents a non-regionalized dispersion pattern, adapting itself to different environmental conditions of temperature and altitude, as well as to the distinct genetic composition of the tomato hybrids currently employed, being able to infect and cause mild or severe symptoms even in plants carrying the *Ty*-1 and *Ty*-3 resistance factors.

Author Contributions: JPS, MENF, LSB, and RCPC conceived and designed the experiments; JPS performed the laboratory bench experiments; JPS, RCPC, MCS and WSF, contributed to perform the computational analysis of the phylogeography and phylogeny; JPS, RCPC, MENF, FMBN and IAO provided the assemble of the genomes and deposit of the sequences. JPS, RCPC wrote the paper; LSB, MENF and RCPC reviewed the manuscript that was posterior reviewed by all the authors.

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Compliance with ethical standards: yes.

Conflict of Interest: All the authors declare that they have not conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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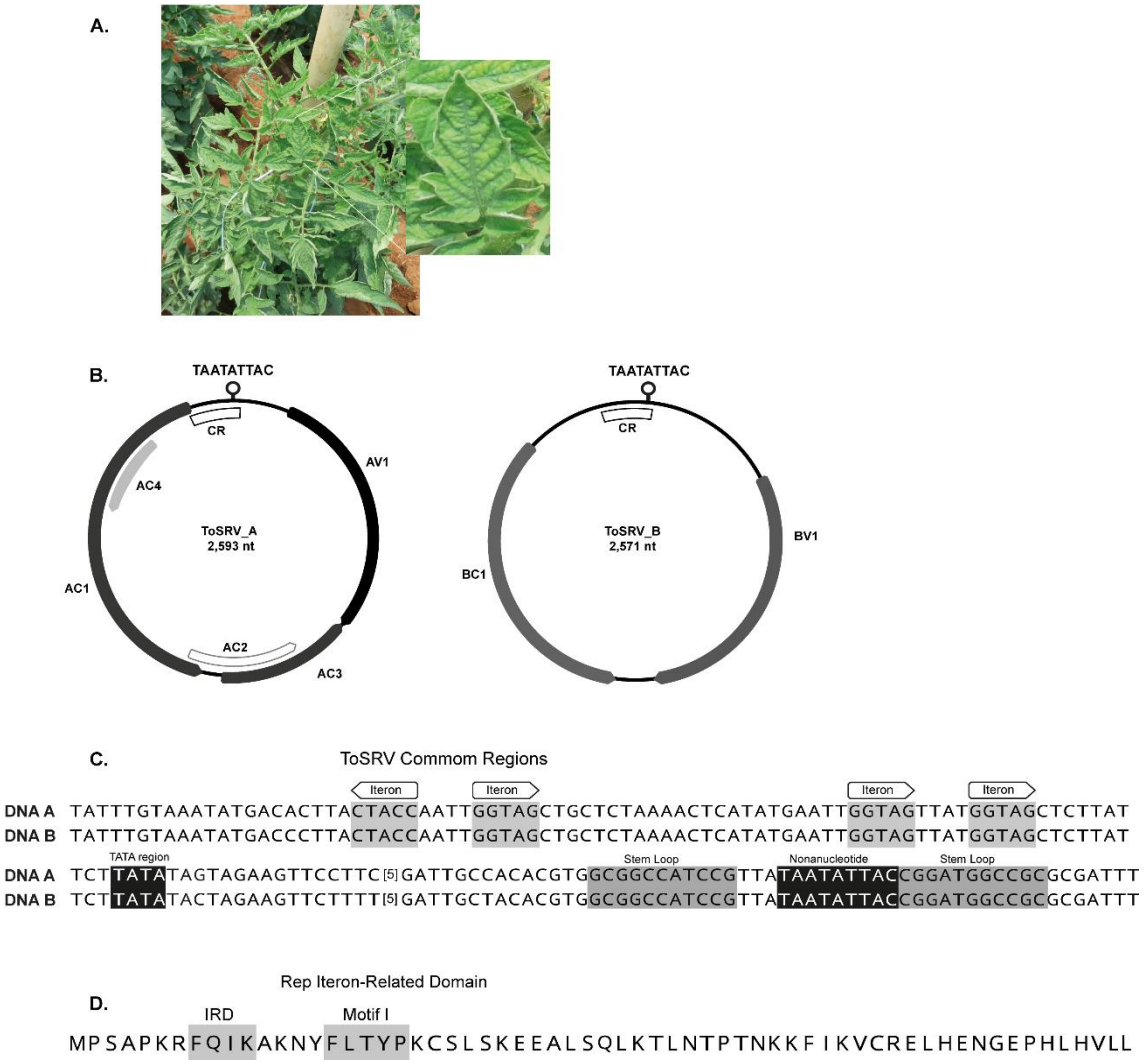
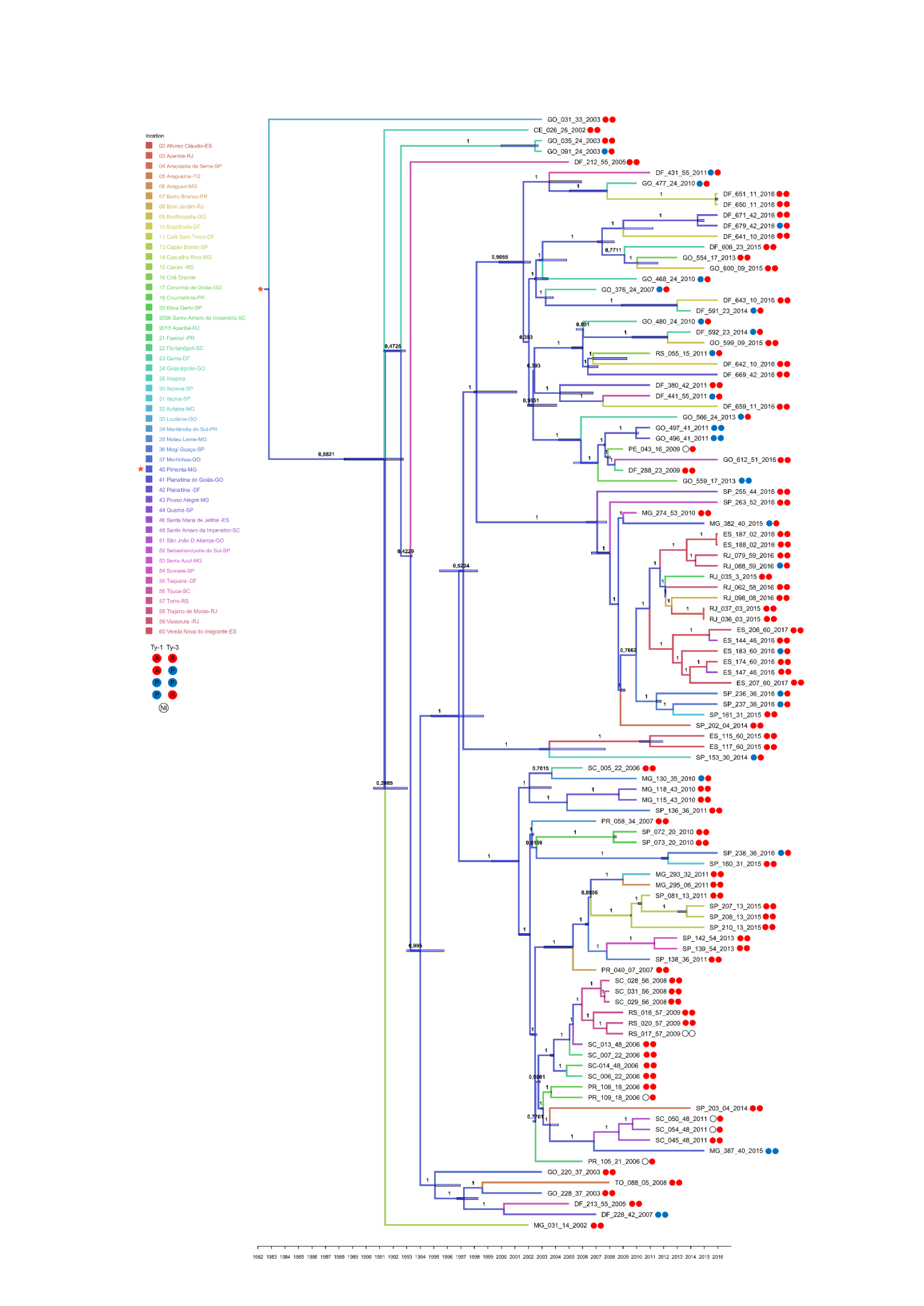


Fig.1 Typical symptoms induced by tomato severe rugose virus (ToSRV) in tomato and viral genomic organization. **a** Symptoms induced by begomovirus in tomato plants, chlorosis, leaf apical deformation, epinasty and overall growth reduction. **b** Genome organization of ToSRV [A MG_387 JP_LVV 211 (accession number: MW653951) and B MG_387 JP_LVV 212 (accession number: MW653952), including the common region (CR) and the stem loop. DNA–A component encodes for: in complementary sense Rep/AC1, replication-associated protein; TrAp/AC2, transcriptional activator protein; REn, replication enhancer; AC4, symptom-determining protein; and viral sense encodes for: CP/AV1, coat protein. DNA–B component encodes in the viral sense for: MP/BV1, movement protein, and in the complementary sense encodes for: NSP/BC1, nuclear shuttle protein. **c** Common region (CR) showing the TATA region, the iteron GGTAG, stem loop and nonanucleotide TAATATTAC is indicated **d** Rep protein sequence showing Iteron-Related Domain (IRD) and Motif I


location
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03 Aperibé-RJ
04 Araçoiaba da Serra-SP
05 Araguaína-TO
06 Araguari-MG
07 Barro Branco-PR
08 Bom Jardim-RJ
09 Bonfinopolis-GO
10 Brazlândia-DF
11 Café Sem Troco-DF
13 Capão Bonito-SP
14 Cascalho Rico-MG
15 Caxias -RS
16 Chã Grande
17 Corumbá de Goiás-GO
18 Cruzmaltina-PR
20 Etiva Gerbi-SP
2006 Santo Amaro da Imperatriz-SC
2015 Aperibé-RJ
21 Faxinal -PR
22 Florianópoli-SC
23 Gama-DF
24 Goianápolis-GO
26 Ibiapina
30 Itapeva-SP
31 Itapira-SP
32 Ituitaba-MG
33 Luziânia-GO
34 Marilândia do Sul-PR
35 Mateu Leme-MG
36 Mogi Guaçu-SP
37 Morrinhos-GO
40 Pimenta-MG
41 Planaltina de Goiás-GO
42 Planaltina -DF
43 Pouso Alegre-MG
44 Quadra-SP
46 Santa Maria de Jetibá -ES
48 Santo Amaro da Imperatriz-SC
51 São João D Aliança-GO
52 Sebastianópolis do Sul-SP
53 Serra Azul-MG
54 Sumaré-SP
55 Taquara -DF
56 Tijuca-SC
57 Torre-RS
58 Trajano de Morae-RJ
59 Vassoura -RJ
60 Venda Nova do Imigrante-ES
Ty-1 Ty-3
A A
A P
P P
P A
NI
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0,9351
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1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016

Fig. 2 Phylogenetic dendrogram based on alignment of the complete nucleotide sequences of the DNA–A components of 105 tomato severe rugose virus (ToSRV) isolates obtained in the present work. These isolates were collected in 48 counties of 11 states and the Federal District (DF). The red dots indicate the isolates with the absence of the *Ty*-1 and *Ty*-3 resistance genes. The blue dots indicate the isolates with the presence of the *Ty*-1 and *Ty*-3 genes. The origin of the virus population is indicated in the figure by a yellow star. The branches are colored based on the most probable state location of the descendent nodes. The scale at the bottom of the tree represents time in years.

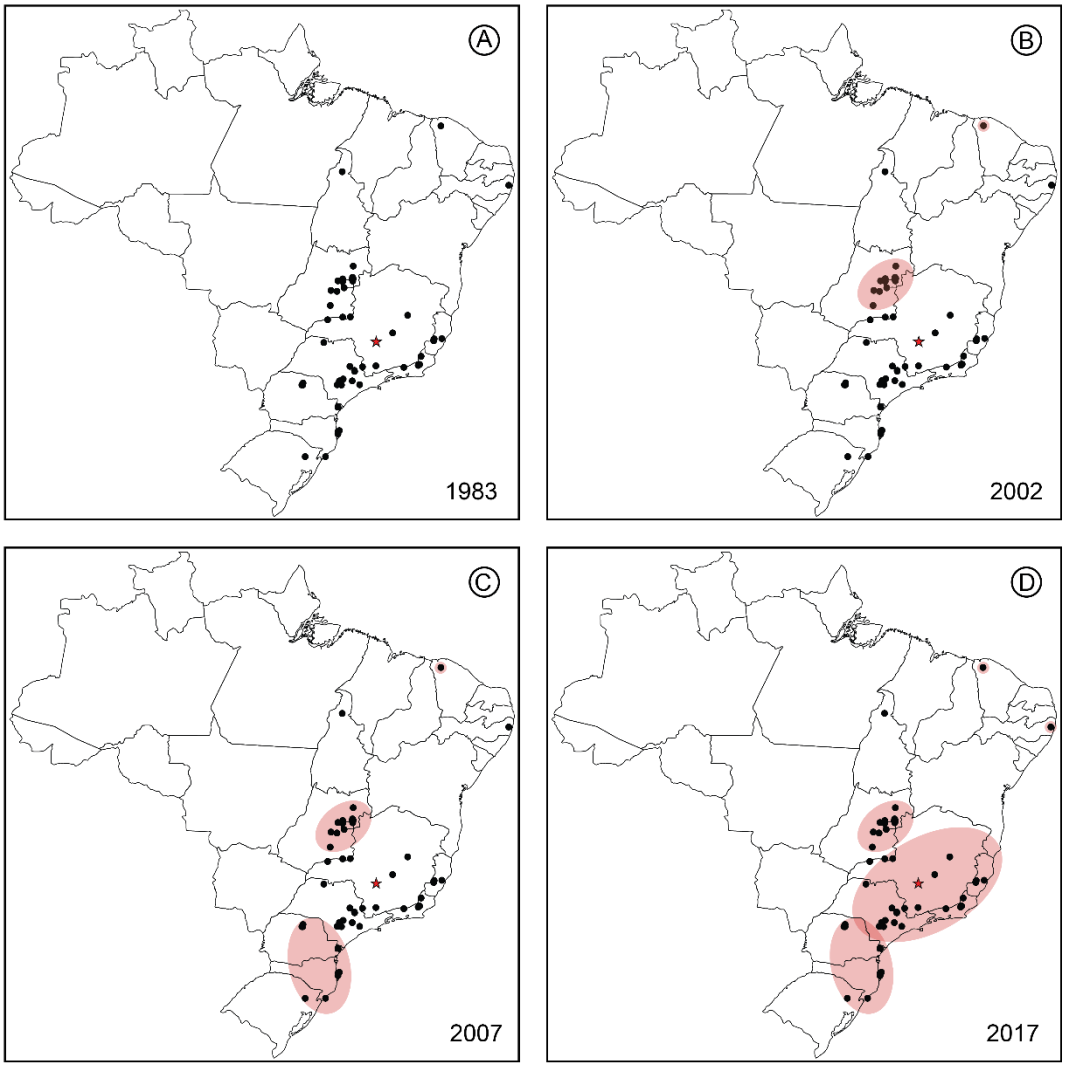
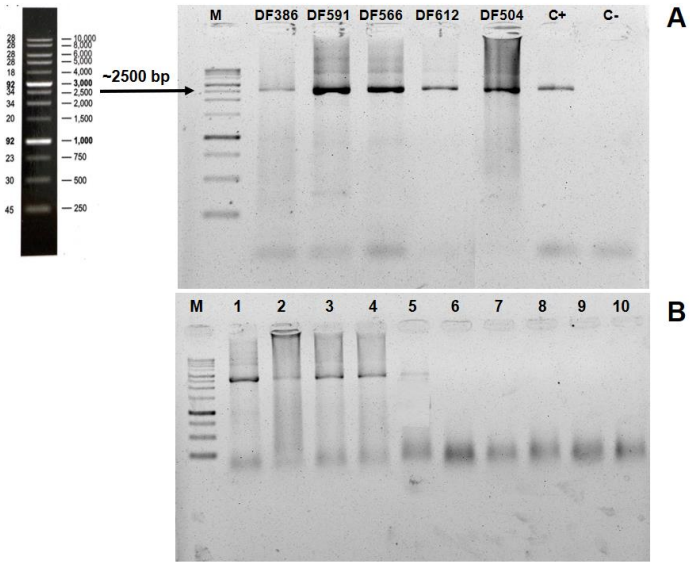


Fig. 3 Spatiotemporal dynamics of tomato severe rugose virus (ToSRV), based on phylogeographic analyses, generated by SPREAD3 program. Snapshots of dispersal patterns for 1983, 2002, 2007 and 2017 were provided. The location of ToSRV isolates is represented by black dots. The red star represents place of origin (Pimenta–MG) of ToSRV reported in 1983. The pink circles indicate dispersal patterns/routes.

Supplementary materials



Supplementary Figure 1. Electrophoresis in 1% agarose gel for the specific primer for tomato severe rugose virus (ToSRV). **a** PCR with ToSRV specific primer pair, generating amplicon with ~ 2600bp. Five total DNA from tomato samples that previously tested positive for ToSRV (DF386, DF591, DF566, DF612, and DF504), and confirmed by Sanger sequencing were used as positive controls. Water was a template for the negative control. **b** ToSRV primer validation test. ToSRV isolates: 1-MG387, 2-CE26, 3-DF441, 4-DF380 and 5-MG383 and isolates previously confirmed as different species of begomovirus: 6-T01 (tomato leaf curl virus – ToLCV), 7- T02 (bean golden mosaic virus – BGMV), 8-GO305(tomato yellow vein streak virus – ToYVSV), 9-T03 (sida micranta mosaic virus – SiMMV) and 10-ES07 (tomato commom mosaic virus – ToCMV, GenBank Access: MN156311.1). All these samples were confirmed, and none were in mixed infected. The molecular marker used was Kasvi.

Supplementary Table 1. Information associated with the one-hundred and five (105) tomato-infecting isolates of tomato severe rugose virus (ToSRV) with complete DNA–A sequences obtained in the present work.

N.	ISOLATES	GENBANK ACCESS	CITY - STATE	YEAR	GEOGRAPHIC COORDENATIONS		*Ty*-1	*Ty*-3	REGION	BIOMES	
					LATITUDE (S)	LONGITUDE (W)					
1	A CE_026 JP_LVV 02	MW596547	Ibiapina-CE	2002	35532	405331	A	A	Northeast (2)	Caatinga	**A=ABSENCE of resistance genes**
2	A PE_043 JP_LVV 04	MW596530	Chã Grande-PE	2009	81503	352816	N.I	A			**P=PRESENCE of resistance genes**
3	A TO_088 JP_LVV 06	MW596591	Araguaína-TO	2008	71033	481544	A	A	North (1)	Amazônia	**N.I= No information**
4	A DF_212 JP_LVV 07	MW596579	Taquara-DF	2005	153803	473122	A	A	Midwest (37)	Cerrado	
5	A DF_213 JP_LVV 08	MW596577	Taquara-DF	2005	153803	473122	A	A			
6	A DF_431 JP_LVV 10	MW596563	Taquara-DF	2011	153803	473122	P	A			
7	A DF_441 JP_LVV 12	MW596548	Taquara-DF	2011	153803	473122	P	A			
8	A DF_641 JP_LVV 13	MW596545	Brazlândia-DF	2016	154008	481218	A	A			
9	A DF_642 JP_LVV 14	MW596539	Brazlândia-DF	2016	154008	481218	A	A			
10	A DF_643 JP_LVV 15	MW596560	Brazlândia-DF	2016	154008	481218	A	A			
11	A DF_650 JP_LVV 16	MW596570	Café Sem Troco-DF	2016	155624	473514	A	A			
12	A DF_651 JP_LVV 17	MW573991	Café Sem Troco-DF	2016	155624	473514	A	A			
13	A DF_659 JP_LVV 18	MW602389	Café Sem Troco-DF	2016	155624	473514	A	A			
14	A DF_228 JP_LVV 19	MW596562	Planaltina-DF	2007	154208	474058	P	P			

15	A DF_380 JP_LVV 20	MW596543	Planaltina-DF	2011	154208	474058	A	A			
16	A DF_669 JP_LVV 23	MW596578	Planaltina-DF	2016	154208	474058	A	A			
17	A DF_671 JP_LVV 24	MW573992	Planaltina-DF	2016	154208	474058	A	A			
18	A DF_679 JP_LVV 25	MW596538	Planaltina-DF	2016	154208	474058	P	A			
19	A DF_288 JP_LVV 26	MW596542	Gama-DF	2009	155948	481240	A	A			
20	A DF_591 JP_LVV 27	MW596541	Gama-DF	2014	155948	481240	P	A			
21	A DF_592 JP_LVV 28	MW596549	Gama-DF	2014	155948	481240	P	A			
22	A DF_606 JP_LVV 30	MW596540	Gama-DF	2015	155948	481240	A	A			
23	A GO_031 JP_LVV 33	MW573981	Luziânia-GO	2003	160838	480143	A	A			
24	A GO_228 JP_LVV 37	MW596564	Morrinhos-GO	2003	174532	490753	A	A			
25	A GO_220 JP_LVV 38	MW596582	Morrinhos-GO	2003	174532	490753	A	A			
26	A GO_035 JP_LVV 40	MW596571	Goianápolis-GO	2003	162957	490149	A	A			
27	A GO_091 JP_LVV 41	MW596555	Goianápolis-GO	2003	162957	490149	A	A			
28	A GO_376 JP_LVV 42	MW596536	Goianápolis-GO	2007	162957	490149	P	A			
29	A GO_477 JP_LVV 43	MW596565	Goianápolis-GO	2010	162957	490149	P	A			
30	A GO_480 JP_LVV 44	MW596535	Goianápolis-GO	2010	162957	490149	P	A			
31	A GO_468 JP_LVV 45	MW653950	Goianápolis-GO	2010	162957	490149	P	A			
32	A GO_566 JP_LVV 46	MW596583	Goianápolis-GO	2013	162957	490149	P	A			
33	A GO_554 JP_LVV 52	MW596550	Corumbá de Goiás-GO	2013	155506	484905	A	A			

34	A GO_559 JP_LVV 54	MW596534	Corumbá de Goiás-GO	2013	155506	484905	P	P			
35	A GO_496 JP_LVV 55	MW596544	Planaltina de Goiás-GO	2011	152712	473648	P	P			
36	A GO_497 JP_LVV 56	MW573982	Planaltina de Goiás-GO	2011	152712	473648	P	P			
37	A GO_599 JP_LVV 58	MW596556	Bonfinopolis-GO	2015	163614	485713	A	A			
38	A GO_600 JP_LVV 59	MW596551	Bonfinopolis-GO	2015	163614	485713	A	A			
39	A GO_612 JP_LVV 61	MW596572	São João D Aliança-GO	2016	144133	473058	A	A			
40	A ES_147 JP_LVV 73	MW596553	Santa Maria de Jetibá-ES	2016	200123	404529	A	A	Southeast (48)	Cerrado and Mata Atlântica	
41	A ES_144 JP_LVV 74	MN156293	Santa Maria de Jetibá-ES	2016	200123	404529	A	A			
42	A ES_115 JP_LVV 75	MW596537	Venda Nova do Imigrante-ES	2015	201916	410826	A	A			
43	A ES_117 JP_LVV 76	MN156291	Venda Nova do Imigrante-ES	2015	201916	410826	A	A			
44	A ES_174 JP_LVV 78	MW573980	Venda Nova do Imigrante-ES	2016	201916	410826	A	A			
45	A ES_183 JP_LVV 80	MW596554	Venda Nova do Imigrante-ES	2016	201916	410826	P	A			
46	A ES_206 JP_LVV 81	MN156299	Venda Nova do Imigrante-ES	2017	201916	410826	A	A			
47	A ES_207 JP_LVV 82	MW596581	Venda Nova do Imigrante-ES	2017	201916	410826	A	A			
48	A ES_188 JP_LVV 84	MW596580	Afonso Cláudio-ES	2016	200310	410527	A	A			
49	A ES_187 JP_LVV 85	MW574002	Afonso Cláudio-ES	2016	200310	410527	A	A			
50	A MG_274 JP_LVV 91	MW596561	Serra Azul-MG	2010	182056	430933	A	A			
51	A MG_031 JP_LVV 93	MW596533	Cascalho Rico-MG	2002	183424	475238	A	A			
52	A MG_295 JP_LVV 97	MW573983	Araguari-MG	2011	183603	481224	A	A			

53	A MG_115 JP_LVV 101	MW596532	Pouso Alegre-MG	2010	221303	455517	A	A			
54	A MG_118 JP_LVV 102	MW596531	Pouso Alegre-MG	2010	221303	455517	A	A			
55	A MG_382 JP_LVV 104	MW596584	Pimenta-MG	2015	202141	455340	P	A			
56	A MG_293 JP_LVV 107	MW596552	Ituitaba-MG	2011	185605	492842	A	A			
57	A MG_130 JP_LVV 110	MW596566	Mateus Leme-MG	2010	195752	442636	P	A			
58	A MG_387 JP_LVV 211	MW653951	Pimenta-MG	2015	202141	455340	P	P			
59	A RJ_062 JP_LVV 113	MW573984	Trajano de Moraes-RJ	2016	220030	421823	A	A			
60	A RJ_098 JP_LVV 118	MW573986	Bom Jardim-RJ	2016	220802	422804	A	A			
61	A RJ_079 JP_LVV 119	MW596567	Vassoura-RJ	2016	222242	434015	A	A			
62	A RJ_088 JP_LVV 121	MW573985	Vassoura-RJ	2016	222242	434015	P	A			
63	A RJ_035 JP_LVV 125	MW602390	Aperibé-RJ	2015	213817	420709	A	A			
64	A RJ_036 JP_LVV 126	MW596574	Aperibé-RJ	2015	213817	420709	A	A			
65	A RJ_037 JP_LVV 127	MW596557	Aperibé-RJ	2015	213817	420709	A	A			
66	A SP_081 JP_LVV 130	MW596522	Capão Bonito-SP	2011	235916	482211	A	A			
67	A SP_207 JP_LVV 133	MW596587	Capão Bonito-SP	2015	235916	482211	A	A			
68	A SP_208 JP_LVV 134	MW596588	Capão Bonito-SP	2015	235916	482211	A	A			
69	A SP_210 JP_LVV 135	MW596594	Capão Bonito-SP	2015	235916	482211	A	A			
70	A SP_142 JP_LVV 140	MW596576	Sumaré-SP	2013	224834	472106	A	A			
71	A SP_139 JP_LVV 141	MW596558	Sumaré-SP	2013	224834	472106	A	A			

72	A SP_236 JP_LVV 142	MW596590	Mogi Guaçu-SP	2016	221826	465926	P	A			
73	A SP_237 JP_LVV 143	MW596589	Mogi Guaçu-SP	2016	221826	465926	P	A			
74	A SP_238 JP_LVV 144	MW596559	Mogi Guaçu-SP	2016	221826	465926	P	A			
75	A SP_136 JP_LVV 145	MW596568	Monte Mor-SP	2011	225339	471947	A	A			
76	A SP_138 JP_LVV 147	MW596585	Monte Mor-SP	2011	225339	471947	A	A			
77	A SP_255 JP_LVV 150	MW596593	Quadra-SP	2016	231329	480801	A	A			
78	A SP_202 JP_LVV 151	MW602391	Araçoiaba da Serra-SP	2014	232851	473759	A	A			
79	A SP_203 JP_LVV 152	MW596586	Araçoiaba da Serra-SP	2014	232851	483759	A	A			
80	A SP_153 JP_LVV 154	MW596521	Itapeva-SP	2014	235714	485427	P	A			
81	A SP_160 JP_LVV 158	MW596595	Itapira-SP	2015	235714	222412	A	A			
82	A SP_161 JP_LVV 159	MW596546	Itapira-SP	2015	235714	222412	A	A			
83	A SP_263 JP_LVV 162	MW596592	Sebastianópolis do Sul-SP	2016	203235	495918	A	A			
84	A SP_072 JP_LVV 167	MW596523	Estiva Gerbi-SP	2010	221547	475725	A	A			
85	A SP_073 JP_LVV 168	MW574001	Estiva Gerbi-SP	2010	221547	475725	A	A			
86	A PR_109 JP_LVV 171	MW596525	Cruzmaltina-PR	2006	235906	512707	N.I	A	South (20)	Mata Atlântica and Pampa	
87	A PR_108 JP_LVV 172	MW596526	Cruzmaltina-PR	2006	235906	512707	A	A			
88	A PR_040 JP_LVV 174	MW596529	Barro Branco-PR	2007	252749	484950	A	A			
89	A PR_058 JP_LVV 178	MW596528	Marilândia do Sul-PR	2007	234412	511833	A	A			
90	A SC_005 JP_LVV 187	MW573994	Florianópolis-SC	2006	272145	484846	A	A			

91	A SC_006 JP_LVV 188	MW573995	Florianópolis-SC	2006	272145	484846	A	A			
92	A SC_007 JP_LVV 189	MW573996	Florianópolis-SC	2006	272145	484846	A	A			
93	A SC_013 JP_LVV 191	MW573997	Santo Amaro da Imperatriz-SC	2006	273946	484801	A	A			
94	A SC_014 JP_LVV 192	MW573998	Santo Amaro da Imperatriz-SC	2006	273946	484801	A	A			
95	A SC_045 JP_LVV 193	MW573999	Santo Amaro da Imperatriz-SC	2011	273946	484801	A	A			
96	A SC_050 JP_LVV 194	MW574000	Santo Amaro da Imperatriz-SC	2011	273946	484801	A	A			
97	A SC_054 JP_LVV 195	MW596575	Santo Amaro da Imperatriz-SC	2011	273946	484801	N.I	A			
98	A SC_028 JP_LVV 196	MW573989	Tijuca-SC	2008	271241	483756	A	A			
99	A SC_029 JP_LVV 197	MW573990	Tijuca-SC	2008	271241	483756	A	A			
100	A SC_031 JP_LVV 198	MW573993	Tijuca-SC	2008	271241	483756	A	A			
101	A RS_016 JP_LVV 199	MW573987	Torres-RS	2009	291415	494403	A	A			
102	A RS_017 JP_LVV 200	MW596569	Torres-RS	2009	291415	494403	A	A			
103	A RS_020 JP_LVV 201	MW573988	Torres-RS	2009	291415	494403	A	A			
104	A PR_105 JP_LVV 203	MW596527	Faxinal-PR	2006	235911	511927	N.I	A			
105	A RS_055 JP_LVV 204	MW596524	Caxias-RS	2011	291454	510113	P	A			

Supplementary Table 2. Information on ninety-nine (99) tomato-infecting isolates of tomato severe rugose virus (ToSRV) with complete DNA–A sequences available at GenBank

N.	GENBANK ACCESS	GEOGRAPHIC COORDENATIONS		CITY - STATE	YEAR	ISOLATE	SIZE	REGION	Biomes
		LATITUDE (S)	LONGITUDE (W)						
1	JF803263	154413	480930	Alexandre Gusmão-DF	2004	DF:BR:PADFM:04	2591	Midwest (37)	Cerrado
2	JF803260	153608	473955	Planaltina-DF	2003	MG:BR:Pip1696:03	2591		
3	JF803261	153608	473955	Planaltina-DF	2003	MG:BR:Pip1792:03	2591		
4	MT733808.1	153536	473211	Planaltina-DF	2003/2005	ToSRV: BR: G1	2592		
5	MT733811.1	154.310	471922	Planaltina-DF	2009/2011	ToSRV: BR: G2	2592		
6	MT733815.1	153907	473111	Planaltina-DF	2014/2016	ToSRV: BR: G3	2592		
7	MT214084.1	155409	474634	São Sebastião-DF	2016	DF-667 LR_LVV 22	2593		
8	JF803262	165816	504257	Indiara-GO	2003	MG:BR:Ind2857:04	2591		
9	JX415193.1	162829	480011	Luziânia-GO	2008	BR: 768Tom8b: 08	2591		
10	JX415196.1	162829	480011	Luziânia-GO	2008	BR: 780Tom3: 08	2591		
11	JX415198.1	163020	490117	Goianópolis-GO	2008	BR: 1646Tom4a: 08	2591		

12	MT215001.1	163020	490117	Goianópolis-GO	2003	GO-247 LR_LVV 27	2592		
13	JX415199.1	163020	490117	Goianópolis-GO	2008	BR: 1646Tom4b: 08	2591		
14	JX415201.1	172337	502225	Acreúna-GO	2009	BR: 3539Tom8a: 09	2591		
15	JX415202.1	172337	502225	Acreúna-GO	2009	BR: 3539Tom8b: 09	2591		
16	JX865615.1	204514	425253	Viçosa-MG	2009	BR:Vic01:09	2593	Southeast (48)	Cerrado and Mata Atlântica
17	JX865616.1	204514	425253	Viçosa-MG	2009	BR:Vic02:09	2593		
18	JX865617.1	204514	425253	Viçosa-MG	2009	BR:Vic03:09	2593		
19	JX865618.1	204514	425253	Viçosa-MG	2009	BR:Vic04:09	2593		
20	JX865619.1	204514	425253	Viçosa-MG	2009	BR:Vic05:09	2594		
21	JX865620.1	204514	425253	Viçosa-MG	2010	BR:Vic06:10	2594		
22	JX865621.1	204514	425253	Viçosa-MG	2010	BR:Vic07:10	2593		
23	JX865622.1	204514	425253	Viçosa-MG	2010	BR:Vic08:10	2595		
24	JX865623.1	204514	425253	Viçosa-MG	2010	BR:Vic09:10	2594		
25	JX865624.1	204514	425253	Viçosa-MG	2010	BR:Vic010:10	2593		
26	JX865625.1	204514	425253	Viçosa-MG	2010	BR:Vic11:10	2593		
27	JX865626.1	204514	425253	Viçosa-MG	2009	BR:Vic12:09	2593		
28	JX865627.1	204514	425253	Viçosa-MG	2009	BR:Vic13:09	2593		
29	JX865628.1	204514	425253	Viçosa-MG	2009	BR:Vic14:09	2593		
30	JX865629.1	204514	425253	Viçosa-MG	2009	BR:Vic15:09	2594		
31	JX865630.1	204514	425253	Viçosa-MG	2010	BR:Vic17:10	2592		
32	JX865631.1	204514	425253	Viçosa-MG	2010	BR:Vic18:10	2594		
33	JX865632.1	204514	425253	Viçosa-MG	2010	BR:Vic19:10	2593		
34	JX865633.1	204514	425253	Viçosa-MG	2010	BR:Vic20:10	2593		
35	JX865634.1	204514	425253	Viçosa-MG	2009	BR:Vic21:09	2593		

36	JX865635.1	204514	425253	Viçosa-MG	2009	BR:Vic22:09	2593		
37	JX865636.1	204514	425253	Viçosa-MG	2010	BR:Vic23:10	2592		
38	JX865637.1	204514	425253	Viçosa-MG	2010	BR:Vic24:10	2593		
39	JX865638.1	204514	425253	Viçosa-MG	2010	BR:Vic25:10	2594		
40	JX865639.1	204514	425253	Viçosa-MG	2009	BR:Vic26:09	2593		
41	JX865640.1	204514	425253	Viçosa-MG	2009	BR:Vic27:09	2593		
42	JX865641.1	204514	425253	Viçosa-MG	2009	BR:Vic28:09	2593		
43	JX865642.1	204514	425253	Viçosa-MG	2010	BR:Vic29:10	2593		
44	JX865643.1	204514	425253	Viçosa-MG	2010	BR:Vic30:10	2593		
45	JX865644.1	204514	425253	Viçosa-MG	2010	BR:Vic31:10	2593		
46	JX865645.1	204514	425253	Viçosa-MG	2010	BR:Vic32:10	2593		
47	JX865646.1	204514	425253	Viçosa-MG	2010	BR:Vic33:10	2594		
48	JX865647.1	204514	425253	Viçosa-MG	2010	BR:Vic34:10	2593		
49	JX865648.1	204515	425254	Viçosa-MG	2010	BR:Vic35:10	2593		
50	JX865649.1	204514	425253	Viçosa-MG	2010	BR:Vic36:10	2593		
51	JX865650.1	204514	425253	Viçosa-MG	2010	BR:Vic40:10	2593		
52	KC004068.1	152026	434039	Jaíba-MG	2008	BR:Jai125:08	2593		
53	KC004069.1	152026	434039	Jaíba-MG	2008	BR:Jai127:08	2593		
54	KC004070.1	195318	442555	Florestal-MG	2008	BR:Flo165:08	2594		
55	KC004071.1	195318	442555	Florestal-MG	2008	BR:Flo202:08	2593		
56	KC004072.1	195318	442555	Florestal-MG	2008	BR:Flo203:08	2593		
57	KC004073.1	195318	442555	Florestal-MG	2008	BR:Flo206:08	2598		
58	KC004074.1	195318	442555	Florestal-MG	2008	BR:Flo208:08	2593		
59	KC004075.1	205703	434809	Carandaí-MG	2008	BR:Car214:08	2592		
60	KC004076.1	205703	434809	Carandaí-MG	2008	BR:Car218.1:08	2593		
61	KC004077.1	205703	434809	Carandaí-MG	2008	BR:Car219.10:08	2593		

62	KC004078.1	205703	434809	Carandaí-MG	2008	BR:Car220:08	2593		
63	KC004079.1	205703	434809	Carandaí-MG	2008	BR:Car224:08	2593		
64	KC004080.1	205703	434809	Carandaí-MG	2008	BR:Car226.3:08	2593		
65	KC004081.1	205703	434809	Carandaí-MG	2008	BR:Car227:08	2593		
66	KC004083.1	205703	434809	Carandaí-MG	2008	BR:Car230:08	2592		
67	KC004084.1	205703	434809	Carandaí-MG	2008	BR:Car232:08	2592		
68	KC004085.1	205703	434809	Carandaí-MG	2008	BR:Car233:08	2592		
69	KC004086.1	205703	434809	Carandaí-MG	2008	BR:Car235:08	2592		
70	KC004087.1	205703	434809	Carandaí-MG	2008	BR:Car236.1:08	2592		
71	KC004088.1	205703	434809	Carandaí-MG	2008	BR:Car237.6:08	2593		
72	KC004089.1	205703	434809	Carandaí-MG	2008	BR:Car238:08	2593		
73	KC706617.1	210603	434159	Carandaí-MG	2008	BR:Car218.3:08	2594		
74	KC706618.1	210603	434159	Carandaí-MG	2008	BR:Car221:08	2593		
75	KC706619.1	210603	434159	Carandaí-MG	2008	BR:Car226.5:08	2594		
76	KC706620.1	210603	434159	Carandaí-MG	2008	BR:Car237:08	2592		
77	AY029750	184919	482637	Uberlândia-MG	1999	Minas Gerais	2588		
78	MN156291.1	201939	410809	Venda Nova do Imigrante-ES	2016	ES117	2592		
79	MN156292.1	201939	410809	Venda Nova do Imigrante-ES	2015	ES120	2592		
80	MN156295.1	201939	410809	Venda Nova do Imigrante-ES	2016	ES173	2592		
81	MN156301.1	201939	410809	Venda Nova do Imigrante-ES	2017	ES271	2592		
82	MN156296.1	201939	410809	Venda Nova do Imigrante-ES	2016	ES183	2592		
83	MN156293.1	200131	404439	Santa Maria de Jetibá-ES	2016	ES144	2592		

84	MN156300.1	200131	404439	Santa Maria de Jetibá-ES	2017	ES255	2592		
85	MN156306.1	200444	410722	Afonso Claudio-ES	2016	ES202	2593		
86	MN156297.1	200444	410722	Afonso Claudio-ES	2016	ES187	2592		
87	MN156294.1	202142	403934	Domingos Martins-ES	2016	ES146	2592		
88	MN156298.1	202142	403934	Domingos Martins-ES	2016	ES196	2592		
89	MN156302.1	202142	403934	Domingos Martins-ES	2018	ES286	2592		
90	MN156303.1	202142	403934	Domingos Martins-ES	2018	ES287	2592		
91	MN156304.1	202142	403934	Domingos Martins-ES	2018	ES288	2592		
92	MN156305.1	202142	403934	Domingos Martins-ES	2018	ES291	2592		
93	MN156307.1	202142	403934	Domingos Martins-ES	2018	ES285	2593		
94	MN156308.1	202142	403934	Domingos Martins-ES	2018	ES289	2593		
95	MN156309.1	202142	403934	Domingos Martins-ES	2018	ES290	2593		
96	MN156310.1	202142	403934	Domingos Martins-ES	2018	ES292	2593		
97	MN156299.1	204025	410035	Vargem Alta-ES	2017	ES206	2592		
98	HQ606467	224400	473850	Piracicaba-SP	2007	ToSRV: Pi 1	2592		
99	MG837738.1	231133	492639	Pirajú-SP	2005	BR-Pir1-05	2591		