CAROLINA DA SILVA ROCHA

VARIABILIDADE E ESTRUTURA GENÉTICA DE POPULAÇÕES DE BEGOMOVÍRUS EM TOMATEIRO E PLANTAS DANINHAS EM SEIS LOCALIDADES DO SUDESTE BRASILEIRO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

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BIOGRAFIA

Carolina da Silva Rocha, filha de Alaerte Adão da Rocha e Joana D'Arc da Silva Rocha, nasceu no dia oito de outubro de 1979, em Viçosa, MG.

Em março de 2000, ingressou no curso de Agronomia, na Universidade Federal de Viçosa, MG, graduando-se como Engenheira Agrônoma em Julho de 2005.

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ROCHA, Carolina da Silva, D.Sc. Universidade Federal de Viçosa, julho de 2011. **Variabilidade e estrutura genética de populações de begomovírus em tomateiro e plantas daninhas em seis localidades do sudeste brasileiro.** Orientador: Francisco Murilo Zerbini Júnior. Co-orientadores: Claudine Marcia Carvalho e Poliane Alfenas Zerbini.

A incidência de begomovírus aumentou drasticamente no Brasil desde a década de 1990, após a introdução do biótipo B da mosca-branca *Bemisia tabaci*. Acredita-se que o inseto vetor transferiu vírus nativos infectando hospedeiros silvestres para o tomateiro. Após um rápido processo evolutivo, novas espécies adaptadas ao novo hospedeiro tornaram-se prevalentes no campo. O objetivo deste trabalho foi determinar a estrutura genética de populações de begomovírus em tomateiro e plantas daninhas em regiões produtoras de tomate no sudeste brasileiro. Amostras foliares de tomateiro e plantas daninhas foram coletadas em seis locais nos estados do Rio de Janeiro e Minas Gerais, de maio de 2005 a maio de 2010. Um total de 126 DNAs-A e 58 DNAs-B foram obtidos por meio de amplificação por círculo rolante, clonados e sequenciados. Dois isolados da espécie tentativa Tomato mottle leaf curl virus (ToMoLCV) foram identificados em plantas de tomateiro coletadas em Jaíba, MG. Este vírus ainda não era reconhecido como uma espécie oficial, pois a sequência completa de seu DNA-A ainda não havia sido determinada. A caracterização molecular dos dois isolados de Jaíba

indica que o ToMoLCV é um típico geminivírus bissegmentado do Novo Mundo, com máxima identidade de sequência com outros begomovírus brasileiros. Análise filogenética confirmou o relacionamento do ToMoLCV com begomovírus do Brasil. Em conjunto, esses resultados apoiam a classificação do ToMoLCV como uma espécie do gênero *Begomovirus*. Além do ToMoLCV, outros oito begomovírus foram identificados nas amostras de tomateiro, e oito nas amostras de plantas daninhas. Quatro vírus foram identificados em tomateiros e plantas daninhas. Todos os vírus identificados já haviam sido previamente descritos e são de ocorrência restrita ao Brasil. Suas propriedades moleculares indicam que todos são begomovírus bissegmentados do Novo Mundo. Dois vírus (SiYLCV e ToCmMV) se agrupam com begomovírus de outros países das Américas em árvores filogenéticas. Análise de recombinação confirmou a natureza altamente recombinante dos begomovírus brasileiros. Vários eventos de recombinação envolvendo vírus de tomateiros tiveram vírus de plantas daninhas identificados como possíveis parentais. As populações virais apresentam subdivisões com base em região geográfica e são altamente variáveis. O BlYSV, um vírus encontrado apenas em plantas daninhas, apresenta uma variabilidade genética muito superior aos vírus de tomateiro (ToCmMV, ToCMoV, ToSRV e ToYVSV).

ABSTRACT

ROCHA, Carolina da Silva, D.Sc. Universidade Federal de Viçosa, July, 2011. **Variability and genetic structure of begomovirus populations in tomatoes and weeds in six localities in southeastern Brazil.** Adviser: Francisco Murilo Zerbini Júnior. Co-advisers: Claudine Marcia Carvalho and Poliane Alfenas-Zerbini.

The incidence of begomoviruses has sharply increased in Brazil since the mid 1990's, after the introduction of the B biotype of the whitefly *Bemisia tabaci*. It is believed that the insect vector transferred indigenous viruses infecting wild and weed hosts to tomato. After a rapid evolutionary process, novel species adapted to the new host became prevalent in the field. The objective of this work was to determine the genetic structure of begomovirus populations infecting tomatoes and weeds in major tomato growing regions of southeastern Brazil. Tomato and weed samples were collected at six locations in the states of Rio de Janeiro and Minas Gerais, from May 2005 to May 2010. A total of 126 DNA-A and 58 DNA-B full-length begomovirus components were amplified using rolling-cicle amplification, cloned and sequenced. Two isolates of the tentative species Tomato mottle leaf curl virus (ToMoLCV) were associated with tomato plants collected in Jaíba, MG. This virus had not yet been recognized as a distinct species because its DNA-A had not been completely sequenced. The complete DNA-A sequence and molecular characterization of the two isolates from Jaíba indicate that ToMoLCV is a typical New World, bipartite begomovirus with

greater sequence identity with begomoviruses from Brazil. Phylogenetic analysis confirmed the relationship of ToMoLCV with New World begomoviruses from Brazil. Together, these results support the classification of ToMoLCV as a new species in the genus *Begomovirus*. Besides ToMoLCV, eight begomoviruses were detected in tomatoes and eight begomoviruses in the weed samples, with four viruses present in both tomatoes and weeds. All of these viruses had been previously described and are restricted to Brazil. Their sequence features indicate that they are typical New World, bipartite begomoviruses. Two viruses (SiYLCV and ToCmMV) cluster with non-Brazilian viruses in phylogenetic trees. Recombination analysis confirmed the mosaic-like nature of Brazilian begomoviruses. Many of the recombination events involving tomato viruses had weed viruses as putative parents. Viral populations were structured with subdivisions based on location, and highly variable, with the weed-infecting BlYSV displaying higher genetic variability compared to the tomato-infecting ToCmMV, ToCMoV, ToSRV and ToYVSV.

INTRODUÇÃO GERAL

A família *Geminiviridae* engloba vírus com genoma composto por uma ou duas moléculas de DNA circular de fita simples, encapsidados em uma partícula icosaédrica geminada. A família é dividida em quatro gêneros (*Mastrevirus*, *Curtovirus*, *Begomovirus* e *Topocuvirus*), de acordo com o tipo de inseto vetor, gama de hospedeiros, organização do genoma e relacionamento filogenético (Stanley *et al.*, 2005).

Os begomovírus possuem um ou dois componentes genômicos, infectam plantas dicotiledôneas e são transmitidos naturalmente pela mosca-branca *Bemisia tabaci* (Homoptera:Aleyrodidae). Os begomovírus do "Velho Mundo" (Europa, Ásia e África) apresentam um ou dois componentes genômicos e frequentemente estão associados a DNAs satélites (Mansoor *et al.*, 2003). Por outro lado, begomovírus encontrados no "Novo Mundo" (Américas) apresentam dois componentes genômicos (denominados DNA-A e DNA-B), e até recentemente acreditava-se que não estavam associados a DNAs satélites. Entretanto, dois trabalhos recentes relataram a associação entre begomovírus e alfassatélites no Brasil e na Venezuela (Paprotka *et al.*, 2010c; Romay *et al.*, 2010).

Os begomovírus possuem grande importância econômica, principalmente em regiões tropicais e subtropicais, sendo uma das maiores ameaças à agricultura nestas regiões (Morales e Anderson, 2001; Monci *et al.*, 2002; Briddon, 2003; Were *et al.*, 2004). No Brasil, a incidência e os danos causados por *B. tabaci* aumentaram exponencialmente a partir da década de 70, em associação ao grande aumento da área plantada com soja. A soja é um excelente hospedeiro de *B. tabaci*, e sofre poucos danos com a presença da praga. A não adoção de medidas de controle permite que as populações de insetos atinjam níveis altíssimos, com a posterior migração para outras plantas após a colheita da soja. Esse contexto levou à disseminação do begomovírus *Bean golden mosaic virus* (BGMV), agente causal do mosaico dourado do feijoeiro, em plantios de feijoeiro próximos a cultivos de soja (Costa, 1975). O mosaico dourado continua causando grandes prejuízos à cultura do feijoeiro em pelo menos 12 países da América Latina (Faria *et al.*, 2000; Morales e Anderson, 2001).

Curiosamente, o aumento populacional de *B. tabaci* observado no Brasil durante as décadas de 1970 e 1980 não levou à disseminação de begomovírus na cultura do tomateiro, provavelmente porque o biótipo predominante naquela época (biótipo A) é pouco adaptado a esse hospedeiro. Na América Central e no Caribe predominava então o biótipo B (Brown e Bird, 1992). Ao contrário do biótipo A, o biótipo B possui maior gama de hospedeiros e é altamente adaptado para alimentação e oviposição em tomateiro, aumentando a probabilidade de transmissão de begomovírus para essa cultura (Schuster *et al.*, 1990). De fato, a partir do final da década de 1980 perdas consideráveis foram relatadas na cultura do tomateiro na Flórida, no México e em países da América Central e do Caribe devido à infecção por begomovírus (Brown e Bird, 1992).

O biótipo B de *B. tabaci* foi relatado pela primeira vez no Brasil no início da década de 1990 (Melo, 1992), e devido a suas características de maior adaptabilidade se disseminou rapidamente pelas regiões quentes e secas do país (Lourenção e Nagai,

1994). Concomitantemente, sintomas típicos de infecção por begomovírus em tomateiros foram relatados no Distrito Federal (Ribeiro *et al.*, 1994), Triângulo Mineiro (Rezende *et al.*, 1996; Zerbini, 1996), São Paulo (Faria *et al.*, 1997), Rio de Janeiro (Galvão *et al.*, 1998) e na região Nordeste, incluindo o estado da Bahia (Ribeiro *et al.*, 1996) e a região do Sub-Médio São Francisco, então a principal região produtora de tomate para processamento industrial no Brasil (Bezerra *et al.*, 1997). A explicação mais provável para o rápido surgimento e disseminação de begomovírus na cultura do tomateiro é a colonização de plantas silvestres e daninhas pelo biótipo B de *B. tabaci*, possibilitando que vírus nativos presentes nestas plantas sejam transferidos para o tomateiro.

A caracterização inicial das espécies de begomovírus associadas às epidemias em tomateiro nas regiões produtoras brasileiras revelou uma grande variabilidade genética (Ambrozevicius *et al.*, 2002; Ribeiro *et al.*, 2003), com a descrição de novas espécies como o *Tomato rugose mosaic virus* (ToRMV) (Fernandes *et al.*, 2006), *Tomato chlorotic mottle virus* (ToCMoV) (Ribeiro *et al.*, 2007) e *Tomato yellow spot virus* (ToYSV) (Calegario *et al.*, 2007), todas presentes no estado de Minas Gerais. Levantamentos realizados nos últimos cinco anos (Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007; Fernandes *et al.*, 2008) indicam que determinadas espécies tornaram-se prevalentes em diferentes regiões do país. Por outro lado, Castillo-Urquiza *et al.* (2008) relataram seis novas espécies em tomateiros e em plantas daninhas, indicando que novas espécies continuam emergindo.

O surgimento de novas espécies está relacionado com eventos de mutação, recombinação e pseudo-recombinação. Infecções por mais de um begomovírus são comuns no campo, favorecendo a recombinação e a pseudo-recombinação, o que pode levar ao surgimento de novas estirpes ou espécies mais adaptadas ao novo hospedeiro (Padidam *et al.*, 1999b; Pita *et al.*, 2001; Galvão *et al.*, 2003; Bull *et al.*, 2007;

Chakraborty *et al.*, 2008; Davino *et al.*, 2009; Patil e Fauquet, 2009). Um recombinante entre o *Tomato yellow leaf curl virus* (TYLCV) e *Tomato yellow leaf curl Sardinia virus* (TYLCSV) apresentou uma maior gama de hospedeiros quando comparado aos parentais e tornou-se prevalente na Espanha (Monci *et al.*, 2002). Em Uganda, um recombinante entre *African cassava mosaic virus* (ACMV) e *East African cassava mosaic virus* (EACMV) apresentou maior agressividade e virulência quando comparado aos parentais. Este recombinante foi responsável por uma epidemia severa na cultura da mandioca nesta região ao longo da década de 1990 (Harrison *et al.*, 1997; Zhou *et al.*, 1997).

No Brasil, o ToRMV e o *Tomato severe rugose virus* (ToSRV) são frequentemente encontrados no campo infectando plantações de tomate. A sequência completa dos DNAs-B do ToSRV e ToRMV apresenta identidade de 98%, e a elevada identidade de sequência da região comum (97,5%) sugere que os dois vírus compartilham o mesmo DNA-B (Silva *et al.*, 2010b). Ribeiro *et al.* (2007) sugerem que um evento de recombinação recente entre ToSRV e ToCMoV gerou o ToRMV, o qual teria capturado o DNA-B do ToSRV. Dessa forma, a origem do ToRMV envolveria tanto a recombinação quanto a pseudo-recombinação.

Além do ToRMV e do ToSRV, um outro begomovírus descrito em tomateiros em Minas Gerais e que possui propriedades biológicas e moleculares interessantes é o ToYSV. Apesar de ter sido isolado de tomateiro, suas características moleculares e filogenéticas são mais semelhantes às de begomovírus isolados de *Sida* sp., como o *Sida mottle virus* (SiMoV), *Sida yellow mosaic virus* (SiYMV) e *Sida micrantha mosaic virus* (SimMV) (Fernandes *et al.*, 1998; Jovel *et al.*, 2004; Andrade *et al.*, 2006a). Essa relação é especialmente evidente ao se compararem as sequências de aminoácidos das proteínas responsáveis pelo movimento viral na planta, NSP ("nuclear shuttle protein") e MP ("movement protein"), do ToYSV e do isolado B3 do SiMoV: o nível de

identidade é superior a 90%. Essas observações sugerem que o ToYSV pode ser originalmente um vírus que infectava *Sida*, transferido para o tomateiro pelo inseto vetor, reforçando a hipótese de que plantas silvestres ou daninhas são reservatórios naturais da diversidade genética de begomovírus no Brasil.

Estudos sobre a distribuição e prevalência de diferentes espécies de vírus fornecem informações úteis para programas de melhoramento na busca de fontes de resistência duráveis. A estrutura genética de uma população reflete a história evolutiva e o potencial dessa população para evoluir (Pinel *et al.*, 2003; Moreno *et al.*, 2004; Font *et al.*, 2007). O grau de variabilidade genética representa o potencial de um dado organismo em se adaptar ao ambiente. O entendimento da dinâmica da variabilidade de populações de vírus de plantas é necessário para entender como as populações evoluem, bem como as implicações para a durabilidade de medidas de manejo (Seal *et al.*, 2006a). Entretanto, no caso de begomovírus esses estudos eram até recentemente baseados na amplificação de fragmentos do genoma viral via PCR, o que limitava a análise a espécies virais já caracterizadas. O desenvolvimento da técnica de amplificação por círculo rolante ("rolling-circle amplification", RCA; Inoue-Nagata *et al.*, 2004), acoplado à grande redução nos custos do sequenciamento de DNA, veio permitir a clonagem e o sequenciamento de um grande número de genomas virais completos em um curto espaço de tempo, sem a necessidade de conhecimento prévio da sequência do genoma viral. Dessa forma, a análise genética de populações virais foi enormemente facilitada, não apenas em hospedeiros cultivados como o tomateiro, mas também em hospedeiros silvestres (Haible *et al.*, 2006). Embora diversos trabalhos nesse sentido já tenham sido relatados (Owor *et al.*, 2007b; Castillo-Urquiza, 2008; Varsani *et al.*, 2008; Harkins *et al.*, 2009; Varsani *et al.*, 2009), estudos em hospedeiros silvestres ainda são escassos, embora seja aceito que estes hospedeiros atuem como

fonte de inóculo (Idris *et al.*, 2003; Jovel *et al.*, 2004; Castillo-Urquiza *et al.*, 2008) podendo, portanto, contribuir para epidemias em hospedeiros cultivados.

Este trabalho teve como objetivo determinar a estrutura genética de populações de begomovírus infectando tomateiro e plantas daninhas associadas nos estados de Minas Gerais e Rio de Janeiro, Brasil.

REVISÃO DE LITERATURA

1. Família Geminiviridae

Os vírus pertencentes à família *Geminiviridae* apresentam genoma composto de DNA de fita simples (ssDNA) circular encapsidado em um capsídeo icosaédrico geminado. A família é dividida em quatro gêneros: *Mastrevirus*, *Curtovirus*, *Topocuvirus* e *Begomovirus*, com base no tipo de inseto vetor, gama de hospedeiros, organização genômica e relacionamento filogenético (Stanley *et al.*, 2005). O gênero *Mastrevirus* inclui os geminivírus com um componente genômico, transmitidos por diversas cigarrinhas (Homoptera: Cicadellidae) a plantas monocotiledôneas. A espécie-tipo é o *Maize streak virus* (MSV), um vírus economicamente importante para a cultura do milho (*Zea mays*). No gênero *Curtovirus* estão os geminivírus com um componente genômico, transmitidos por diversas cigarrinhas (Hemiptera: Cicadellidae) a plantas dicotiledôneas. O *Beet severe curly top virus* (BSCTV) é a espécie-tipo e mais importante economicamente. O gênero *Topocuvirus* possui uma única espécie, o *Tomato pseudo-curly top virus* (TPCTV), com um componente genômico, transmitido pela cigarrinha *Micrutalis malleifera* (Homoptera: Auchenorrhyncha) a plantas dicotiledôneas. O gênero *Begomovirus* engloba espécies com um ou dois componentes genômicos, transmitidas pela mosca-branca *Bemisia tabaci* (Homoptera: Aleyrodidae) a

plantas dicotiledôneas (Stanley *et al.*, 2005). A espécie-tipo é o *Bean golden yellow mosaic virus* (BGYMV) (Fauquet *et al.*, 2008).

Os begomovírus do "Velho Mundo" (Europa, Ásia e África) possuem em sua maioria um componente genômico (monossegmentados), e estão frequentemente associados a moléculas de ssDNA circular conhecidas como DNA-1 (alfassatélites) e DNA β (betassatélites) (Briddon, 2003; Briddon e Stanley, 2006). Os alfassatélites são semelhantes ao componente genômico denominado DNA-R dos nanovírus, os quais contêm uma ORF que codifica uma proteína associada à replicação (Rep), seguida de uma região rica em adenina e uma estrutura em forma de grampo que inclui a origem de replicação (Idris *et al.*, 2005). Os alfassatélites podem replicar autonomamente, mas requerem um vírus auxiliar para infecção sistêmica da planta e transmissão por inseto (Saunders e Stanley, 1999; Saunders *et al.*, 2000; Saunders *et al.*, 2002). Recentemente alfassatélites foram identificados no Brasil e na Venezuela associados aos begomovírus bissegmentados *Cleome leaf crumple virus* (ClLCrV), *Euphorbia mosaic virus 1* (EuMV) e *Melon chlorotic mosaic virus* (MeCMV), sendo esses os primeiros relato de alfassatélites associados a begomovírus ocorrendo naturalmente no "Novo Mundo" (Américas) (Paprotka *et al.*, 2010c; Romay *et al.*, 2010). Os betassatélites dependem do vírus auxiliar para replicação e movimento sistêmico na planta. Seu genoma contêm uma ORF, βC1, que codifica uma proteína responsável pela indução de sintomas e que atua como supressora do silenciamento gênico pós-transcricional (Cui *et al.*, 2004; Cui *et al.*, 2005; Briddon e Stanley, 2006).

Os begomovírus do Novo Mundo possuem dois componentes genômicos (bissegmentados), denominados DNA-A e DNA-B, cada um com aproximadamente 2600 nucleotídeos (Figura 1). Os dois componentes genômicos de uma mesma espécie viral não possuem identidade entre as suas sequências, exceto por uma região com

aproximadamente 200 nucleotídeos denominada região comum (RC), que inclui a origem de replicação (Hanley-Bowdoin *et al.*, 1999).

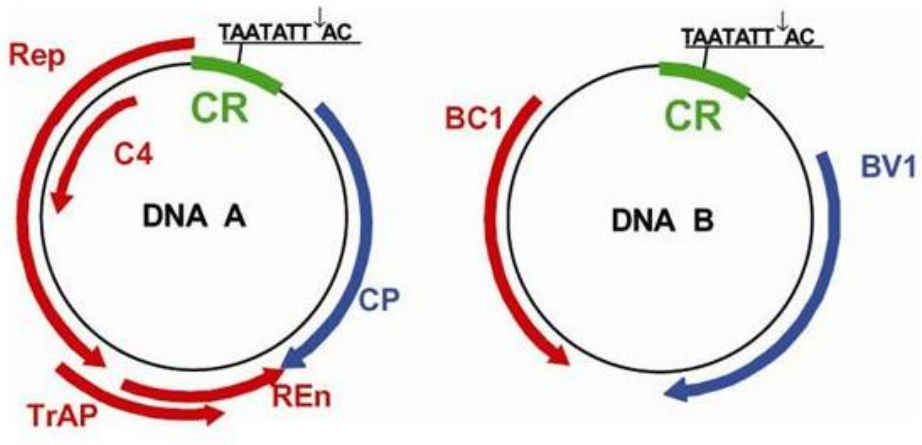

Figura 1. Representação esquemática do genoma do *Bean golden yellow mosaic virus* (BGYMV), espécie-tipo do gênero *Begomovirus.* Os círculos representam o genoma viral, com dois componentes (DNA-A e DNA-B) de aproximadamente 2.600 nucleotídeos cada. Uma sequência de aproximadamente 200 nucleotídeos, denominada região comum (CR), contém a origem de replicação viral, com uma estrutura em forma de grampo e uma sequência invariável de nove nucleotídeos (TAATATT↓AC), conservada em todos os membros da família *Geminiviridae*. A seta (↓) indica o sítio de início da replicação do DNA viral por círculo rolante. As setas azuis e vermelhas indicam os genes virais e a direção em que ocorre a transcrição (viral e complementar, respectivamente). Reproduzido de Gutierrez *et al.*(2004).

O DNA-A dos begomovírus bissegmentados pode codificar de quatro a seis proteínas: uma proteína associada à replicação, Rep ("replication-associated protein"), iniciadora do mecanismo de replicação por círculo rolante, com propriedade de ligação a ácidos nucléicos, endonuclease e ATPase (Fontes *et al.*, 1992; Orozco *et al.*, 1997); uma proteína transativadora, TrAP ("trans-activating protein"), fator transcricional dos genes *CP* e *NSP* e que também atua como supressora do silenciamento gênico (Sunter e

Bisaro, 1992; Voinnet *et al.*, 1999; Wang *et al.*, 2005); a proteína Ren ("replication-enhancer protein"), fator acessório da replicação viral (Sunter *et al.*, 1990; Pedersen e Hanley-Bowdoin, 1994); e a proteína capsidial ("coat protein", CP), que além de formar o capsídeo viral é essencial para a transmissão do vírus pelo inseto vetor (Briddon *et al.*, 1990; Hofer *et al.*, 1997a). O gene *AV2* ("pre-coat") está presente apenas nos begomovírus do Velho Mundo, e atua no movimento do vírus na planta (Padidam *et al.*, 1996). O gene *AC4* codifica uma proteína supressora de silenciamento gênico (Vanitharani *et al.*, 2004). O DNA B codifica as proteínas MP ("movement protein"), envolvida no movimento célula-a-célula do vírus por meio do aumento do limite de exclusão dos plasmodesmas (Noueiry *et al.*, 1994), e NSP ("nuclear shuttle protein"), responsável pelo transporte do DNA através do envelope nuclear (Noueiry *et al.*, 1994; Sanderfoot *et al.*, 1996).

2. Replicação viral

No processo de infecção dos geminivírus, as partículas virais são inoculadas na planta pelo inseto vetor e o genoma viral (ssDNA) se desassocia de forma espontânea do capsídeo (Lazarowitz, 1992; Palmer e Rybicki, 1998). No interior da célula o ssDNA viral é transportado para o núcleo, onde é convertido em um intermediário de fita dupla (dsDNA) denominado forma replicativa (RF). A maneira como esta conversão ocorre não é conhecida, no entanto evidências indiretas, como a necessidade de desestabilização local do dsDNA para o iniciação da replicação por círculo rolante em procariotos por "strand-nicking enzimes", indicam que é realizada por fatores do hospedeiro. A RF serve como molde para síntese dos novos componentes genômicos e também para a transcrição dos genes virais. O genoma viral é replicado via mecanismo de círculo rolante semelhante ao utilizado pelos bacteriófagos ϕX174 e M13, utilizando a RF como molde (Stenger *et al.*, 1991; Stanley, 1995).

A origem de replicação (*ori*) está localizada na região intergênica comum entre os dois componentes genômicos. A sequência da *ori* é conservada entre componentes de um mesmo vírus, porém variável entre espécies, com exceção de uma região de aproximadamente 30 nucleotídeos conservada entre todas as espécies (Davies *et al.*, 1987; Lazarowitz, 1992). Nesta região se localiza uma sequência repetida e invertida composta predominantemente por guanina e citosina, formando uma estrutura conservada em forma de grampo ("structurally-conserved element", SCE), com uma sequência invariável (5'-TAATATTAC-3') encontrada em todos os geminivírus, que constitui o domínio funcional da origem de replicação (Heyraud-Nitschke *et al.*, 1995; Orozco e Hanley-Bowdoin, 1998). É nesse nonanucleotídeo que ocorre a clivagem (TAATATT//AC) que inicia o processo de replicação por círculo rolante (Fontes *et al.*, 1994; Laufs *et al.*, 1995). Essa clivagem é realizada pela proteína Rep, que atua como endonuclease sítio-específica com requerimento de estrutura e sequência (Laufs *et al.*, 1995; Orozco e Hanley-Bowdoin, 1998). Na região comum encontram-se também sequências específicas para ligação da proteína Rep (Fontes *et al.*, 1992; Fontes *et al.*, 1994) e regiões promotoras da RNA polimerase tipo II de plantas, responsável pela transcrição dos genes virais (Hanley-Bowdoin *et al.*, 1999).

O sítio de ligação de Rep ao DNA viral está localizado entre a caixa TATA do gene *Rep* e a SCE (Orozco e Hanley-Bowdoin, 1998), sendo constituído por duas sequências em repetição direta e pelo menos uma repetição invertida denominadas "iterons" (Arguello-Astorga *et al.*, 1994). A ligação de Rep aos iterons é essencial para o início da replicação. Após a ligação de Rep ao DNA viral e estabilização do complexo formado por Rep, Ren e fatores do hospedeiro, a proteína Rep cliva o nonanucleotídeo localizado na SCE, dando início à replicação por círculo rolante (Gutierrez, 1999). O reconhecimento pela proteína Rep é considerado vírus-específico (Arguello-Astorga *et al.*, 1994; Harrison e Robinson, 1999; Ramos *et al.*, 2003), de modo que a proteína só

inicia a replicação de DNAs cognatos. O domínio funcional de Rep foi mapeado na sua região N-terminal e inclui o domínio de ligação a DNA, conservado em todas as proteínas Rep (Jupin, 1995; Gladfelter *et al.*, 1997; Chatterji *et al.*, 1999).

Uma vez que o reconhecimento e ligação aos iterons por Rep é específico, foi proposto que este depende da sequência de nucleotídeos dos iterons e da sequência de aminoácidos de um motivo conservado na proteína Rep denominado domínio relacionado aos iterons ("iteron-related domain", IRD) (Arguello-Astorga e Ruiz-Medrano, 2001). Porém, a replicação do DNA-B do *Tomato yellow spot virus* (ToYSV) pela Rep do *Tomato golden mosaic virus* (TGMV) indica que a interação entre os aminoácidos do IRD e os iterons não é a única forma de reconhecimento da origem de replicação, uma vez que tanto os iterons quanto os IRDs são diferentes entre esses dois vírus (Andrade *et al.*, 2006b). Além disso, a ausência de iterons nos DNAs satélites associados a begomovírus é uma evidência adicional de que outros fatores afetam o reconhecimento da origem de replicação pela proteína Rep (Lin *et al.*, 2003; Stanley, 2004).

3. Movimento do vírus na planta

O movimento do vírus no interior do hospedeiro pode ser dividido em dois processos: movimento célula-a-célula via plasmodesmas, e movimento a longa distância, no qual o vírus atinge o sistema vascular e é transportado sistemicamente para toda a planta. Para esse fim, a partir do DNA-B dos begomovírus bissegmentados são codificadas duas proteínas relacionadas ao movimento viral, NSP e MP. Como os begomovírus replicam no núcleo da célula hospedeira, necessitam de uma etapa adicional de transporte do núcleo para o citoplasma, a qual é realizada pela proteína NSP (Palmer e Rybicki, 1998). Já a proteína MP associa-se à membrana celular e altera o limite de exclusão dos plasmodesmas, viabilizando o transporte do genoma viral

(Noueiry *et al.*, 1994). Estas duas proteínas atuam de forma cooperativa para mediar o tráfego intra- e intercelular do DNA viral (Sanderfoot e Lazarowitz, 1995), permitindo ao vírus infectar sistemicamente o hospedeiro.

Os estudos sobre o movimento viral na planta tem como base a interação física entre as proteínas de movimento MP e NSP (Rojas *et al.*, 2005b). A interação direta das proteínas MP e NSP *in vitro* foi demonstrada para o TGMV utilizando-se o sistema duplo-híbrido de levedura (Mariano *et al.*, 2004). A interação *in vivo* entre NSP e MP do *Cabbage leaf curl virus* (CaLCuV) foi recentemente demonstrada, também utilizando-se o sistema duplo-híbrido de levedura. Nestes estudos foi identificada uma GTPase citoplasmática designada NIG (NSP-interacting GTPase), que interage com NSP de begomovírus *in vitro* e *in vivo* e promove o transporte da proteína viral do núcleo para o citoplasma, onde ela é redirecionada para a periferia da célula para interagir com MP (Carvalho *et al.*, 2008).

Dois modelos foram propostos para explicar o movimento intracelular de begomovírus (Levy e Tzfira, 2010). No primeiro modelo, denominado "couple-skating" (Kleinow *et al.*, 2008), NSP transporta ssDNA ou dsDNA do núcleo para a periferia da célula e, no citoplasma, MP atua nos plasmodesmas para facilitar o movimento célula-a-célula do complexo NSP-DNA (Sanderfoot e Lazarowitz, 1995; Frischmuth *et al.*, 2004; Frischmuth *et al.*, 2007; Kleinow *et al.*, 2008). No segundo modelo, denominado "relay-race", NSP inicialmente transporta o dsDNA do núcleo para o citoplasma. No citoplasma, o dsDNA se associa a MP, e o complexo MP-dsDNA se movimenta célula-a-célula através dos plasmodesmas (Noueiry *et al.*, 1994; Rojas *et al.*, 1998).

Seguindo o movimento célula-a-célula, o vírus atinge os plasmodesmas associados ao tecido vascular e então inicia-se o movimento a longa distância. O movimento viral a longa distância é passivo, acompanhando o fluxo de fotoassimilados dos tecidos fonte para os tecidos dreno através do sistema vascular. A grande maioria

dos vírus é transportada via floema na forma de partícula completa, atingindo, a partir do ponto de penetração, primeiramente as raízes, em seguida as folhas jovens e posteriormente a planta toda, estabelecendo uma infecção sistêmica (Jeffrey *et al.*, 1996).

Para mastrevírus, curtovírus e begomovírus monossegmentados, a proteína CP é necessária para os movimentos célula-a-célula e a longa distância (Rojas *et al.*, 2001; Gafni e Epel, 2002). Além da CP, as proteínas V2 e C4 também são necessárias para o movimento de begomovírus monossegmentados. No caso de *Tomato yellow leaf curl virus* (TYLCV), a CP é responsável pelo transporte do DNA do núcleo para o citoplasma, funcionando como uma proteína análoga a NSP dos begomovírus bissegmentados, e o movimento célula-a-célula através do plasmodesma é mediado pelas proteínas C4 e/ou V2 (Rojas *et al.*, 2001; Rojas *et al.*, 2005b). Recentemente, foi demonstrado que a proteína C4 do curtovírus *Beet severe curly top virus* (BSCTV) é capaz de se ligar de forma não específica a ssDNA e a dsDNA, é essencial para o desenvolvimento de sintomas e, quando expressa em plantas infectadas com mutantes deficientes para C4, pode complementar *in trans* o movimento sistêmico. Em conjunto, esses dados sugerem o envolvimento de C4 no movimento desse vírus (Chen *et al.*, 2010).

A proteína CP é dispensável para o estabelecimento da infecção sistêmica de begomovírus bissegmentados na maioria dos casos já estudados (Rojas *et al.*, 2005a). Tanto MP quanto NSP reconhecem o DNA viral de maneira específica com relação à forma e comprimento (Rojas *et al.*, 1998; Gilbertson *et al.*, 2003), o que elimina a necessidade da proteína capsidial para o movimento a longa distância. Raras exceções, como o begomovírus bissegmentado *Tomato chlorotic mottle virus* (ToCMoV), são capazes inclusive de infectar sistemicamente alguns hospedeiros na ausência do DNA-B cognato (Galvão *et al.*, 2003; Fontenelle *et al.*, 2007).

4. Evolução dos geminivírus

Os geminivírus podem ter evoluído a partir de um replicon primitivo de DNA extracromossomal, presente em procariotos ou em ancestrais primitivos das plantas (Rojas *et al.*, 2005b). Evidências indiretas, como características conservadas com as proteínas iniciadoras da replicação de replicons de procariotos e eucariotos contemporâneos (Ilyina e Koonin, 1992; Campos-Olivas *et al.*, 2002), presença de mRNAs policistrônicos, e a capacidade dos geminivírus de replicarem em *Agrobacterium tumefaciens* (Ridgen *et al.*, 1996; Selth *et al.*, 2002), apóiam esta hipótese. Durante a co-evolução com seus hospedeiros, estes replicons de DNA teriam adquirido novos genes por meio de recombinação com o DNA do hospedeiro ou com outros replicons (revisado por Rojas *et al.*, 2005b).

Estudos filogenéticos propõem que os geminivírus são derivados de um ancestral comum que possuía apenas um componente, infectava monocotiledôneas e era transmitido por cigarrinhas (Rybicki, 1994; Rojas *et al.*, 2005b). Comparações de sequências entre espécies dos gêneros *Mastrevirus*, *Curtovirus* e *Begomovirus* demonstraram que as primeiras são mais divergentes entre si, o que sugere que os mastrevírus divergiram por um período de tempo mais longo. O processo evolutivo levou à capacidade de infecção de plantas dicotiledôneas e em seguida à transmissão pela mosca-branca, uma vez que existem mastrevírus (transmitidos por cigarrinhas) que infectam dicotiledôneas, mas até o presente não foram encontrados geminivírus transmitidos por mosca-branca que infectem monocotiledôneas. Esse ancestral dos begomovírus modernos possuía apenas um componente. A aquisição do segundo componente teria ocorrido antes da separação dos continentes, uma vez que os begomovírus bissegmentados são encontrados tanto no Velho Mundo como no Novo Mundo.

Análises filogenéticas dos betassatélites e seus begomovírus associados sugerem que o satélite e o vírus auxiliar co-evoluíram como consequência do isolamento geográfico e adaptação ao hospedeiro (Zhou *et al.*, 2003; Rojas *et al.*, 2005b). Membros do gênero *Curtovirus* seriam derivados de antigas recombinações entre mastrevírus e begomovírus, resultando na aquisição da CP de um mastrevírus ancestral transmitido por uma cigarrinha primitiva, ao passo que um begomovírus teria contribuído com os genes associados à replicação (Rybicki, 1994; Padidam *et al.*, 1995). Outro evento de recombinação foi identificado para o TPCTV, o único membro do gênero *Topocuvirus,* que teria surgido após recombinação entre um curtovírus ancestral e um vírus que não possui semelhança com nenhum outro geminivírus, o que sugere que outros geminivírus, não relacionados com vírus pertencentes aos quatro gêneros atualmente reconhecidos, podem estar presentes no campo (Briddon *et al.*, 1996). De fato, tais vírus têm sido recentemente identificados e caracterizados (Yazdi *et al.*, 2008; Varsani *et al.*, 2009; Briddon *et al.*, 2010a).

Atualmente, com base em análises filogenéticas do DNA-A de 212 espécies, os begomovírus estão classificados em sete diferentes grupos de acordo com sua origem geográfica ou planta hospedeira (Padidam *et al.*, 1995; Fauquet *et al.*, 2008). Os begomovírus do Velho Mundo segregam em grupos originados na África, Índia, Japão e "resto da Ásia". Entretanto, um número crescente de vírus, os quais são referidos como "outsiders", não se encaixam nesses grupos baseados em região geográfica ou hospedeira. Esses vírus são originários da Indochina, Indonésia e Austrália. Begomovírus do Novo Mundo formam grupos de acordo com a origem geográfica (América Central ou do Sul). Duas espécies originárias do Vietnam isoladas de *Corchorus* sp. são relacionadas aos begomovírus do Novo Mundo, e formam um grupo referido como "corchovírus" (Ha *et al.*, 2006; 2008). Dois grupos de vírus, um infectando leguminosas originárias da Índia e Sudeste da Ásia ("legumovírus") e outro

composto de vírus isolados de *Ipomoea* spp., particularmente batata-doce (*I. batatas*) originários da América, Ásia e Europa ("sweepovírus"), são distintos e basais a todos os demais begomovírus. Esta posição anômala desses begomovírus reflete uma história evolutiva distinta. Para os legumovírus foi sugerido que isto seja devido ao isolamento genético de suas espécies hospedeiras (Qazi *et al.*, 2007).

Um cenário atual para a evolução da família *Geminiviridae* foi proposto por Nawaz-Ul-Rehman e Fauquet (2009). Nesse, plasmídeos que replicam em algas vermelhas e outras formas de vida mais primitivas conseguiram adquirir novos genes, tornando-se independentes de seu hospedeiro e assim capazes de infectar plantas, provavelmente em primeiro lugar monocotiledôneas, como um pré-mastrevírus. Esta evolução deve ter coincidido com a aquisição da transmissão por insetos. Em algum momento eles passaram a infectar dicotiledôneas, mas ainda tinham o mesmo tipo de vetor, as cigarrinhas. Com a aquisição de novos genes tornou-se um pré-monossegmentado transmitido por mosca-branca. Esse begomovírus monossegmentado teve a capacidade de capturar outras moléculas, adquirindo então um alfassatélite a partir de um pré-nanovírus ou um betassatélite de uma fonte desconhecida. Por recombinação entre um begomovírus monossegmentado que infecta dicotiledôneas e um mastrevírus foram formados híbridos que deram origem aos ancestrais dos curtovírus e topocuvírus. Em um período posterior, um monossegmentado conseguiu capturar um ancestral do que hoje é o componente B, e esta combinação de dois componentes foi extremamente bem sucedida ao ponto de begomovírus bissegmentados serem os únicos presentes no continente americano, seguindo a deriva dos continentes que aconteceu há cerca de 125 milhões de anos.

Briddon *et al.* (2010b) demonstraram por meio de análises filogenéticas e exaustivas comparações duas a duas do DNA-A e DNA-B de begomovírus que estas moléculas de fato tem histórias evolutivas diferentes. O DNA-B apresenta maior

variação genética quando comparado ao DNA-A. Esse fato pode ser atribuído à menor quantidade de funções codificadas pelo DNA-B, sendo assim mais permissivo à variação, evoluindo exclusivamente em resposta ao hospedeiro (o DNA-A deve manter também a interação com o vetor). Uma explicação alternativa é que o DNA-B teria uma origem distinta do DNA-A, surgido inicialmente como um satélite que foi capturado pelo seu progenitor monossegmentado e posteriormente evoluído para se tornar parte integral do genoma (Nawaz-Ul-Rehman e Fauquet, 2009; Briddon *et al.*, 2010b).

5. Variabilidade e estrutura genética de populações de geminivírus

Populações de geminivírus, incluindo os begomovírus, possuem um elevado grau de variabilidade genética. A alta taxa de mutação (Duffy e Holmes, 2008; Duffy e Holmes, 2009), a ocorrência de eventos frequentes de recombinação (Padidam *et al.*, 1999b) e a ocorrência de pseudo-recombinação entre vírus com genoma bissegmentado (Andrade *et al.*, 2006a) contribuem para esse elevado grau de variabilidade. Mutação, recombinação e pseudo-recombinação são as principais fontes de variabilidade genética de vírus em plantas (Garcia-Arenal *et al.*, 2003; Seal *et al.*, 2006b).

5.1. Mutação

Assim como para todos os vírus, a evolução dos geminivírus depende primariamente de mutações. Há evidências de que a rápida evolução dos geminivírus é, ao menos em parte, dirigida por processos mutacionais que agem especificamente sobre ssDNA (Harkins *et al.*, 2009).

O impacto das mutações pontuais tem sido estudado nesse grupo de vírus. Sob diferentes condições de seleção, como presença de um efeito gargalo (população inicial pequena do vírus, período curto de aquisição pelo vetor), transferências sucessivas entre hospedeiros sem emprego do vetor, e inoculação em plantas resistentes, isolados de

MSV apresentaram alta frequência de mutação, da ordem de 10^{-4} e 10^{-5} (Isnard *et al.*, 1998). Resultados similares foram obtidos num experimento controlado de análise da taxa de variabilidade genética do begomovírus *Tomato yellow leaf curl China virus* (TYLCCNV), onde foi encontrada uma frequência média de mutação de $3,5\times10^{-4}$ e $5,3\times10^{-4}$ após 60 dias de infecção em *N. benthamiana* e tomateiro, respectivamente (Ge *et al.*, 2007). Uma série de experimentos de evolução a longo prazo (de 6 a 32 anos) também revelaram alta frequência de mutação, entre 2 e 3×10^{-4}, para MSV e *Sugarcane streak Réunion virus* (SSRV), sugerindo que mastrevírus provavelmente não co-divergem com seus hospedeiros (Harkins *et al.*, 2009). Estes resultados discordam com a hipótese de aparente co-divergência entre alguns mastrevírus e seus hospedeiros, o que implicaria em taxas de substituições de apenas 10^{-8} subs/sítio/ano na natureza (Wu *et al.*, 2008).

Duffy e Holmes (2008, 2009) realizaram análises estruturadas no tempo de isolados de TYLCV e *East African cassava mosaic virus* (EACMV), para estimar a taxa de substituição de nucleotídeos desses begomovírus na natureza. Taxas de substituição para o TYLCV foram estimadas em $2,88\times10^{-4}$ subs/sítio/ano para o genoma completo (Duffy e Holmes, 2008). O gene *CP* apresentou uma taxa maior ($4,63\times10^{-4}$ subs/sítio/ano) e a região intergênica (não codificadora) apresentou uma taxa ainda maior ($1,75\times10^{-3}$ subs/sítio/ano). Entretanto, as substituições observadas foram na maioria sinônimas, sugerindo que as altas taxas observadas refletem mais uma rápida dinâmica mutacional do que uma frequência de evolução adaptativa (Duffy e Holmes, 2008). Para o EACMV as taxas foram estimadas em $1,6\times10^{-3}$ e $1,33\times10^{-4}$ subs/sítio/ano para o DNA-A e DNA-B, respectivamente (Duffy e Holmes, 2009). O gene *CP* apresentou $1,37\times10^{-3}$ subs/sítio/ano e o gene *Rep* mostrou $1,24\times10^{-3}$ subs/sítio/ano. Os genes presentes no DNA-B, *NSP* e *MP*, apresentaram $2,77\times10^{-4}$ e $3,45\times10^{-4}$, respectivamente. Contudo, os autores validaram esses altos níveis de heterogeneidade

apenas para o DNA-A e o gene *CP*. Foi observado então que as taxas de substituição indicadas para essas duas espécies de begomovírus, entre 10^{-3} e 10^{-5}, corroboram em geral aquelas determinadas experimentalmente para o MSV (Isnard *et al.*, 1998; Harkins *et al.*, 2009) e TYLCCNV (Ge *et al.*, 2007).

Erros de incorporação de nucleotídeos durante a replicação viral também contribuem para a variabilidade genética. Estudos de bactérias e sistemas animais indicaram que as taxas de substituição dos vírus de dsDNA e ssDNA diferem significativamente (Duffy *et al.*, 2008). Taxas de substituição para fagos bacterianos, poliomavírus e papilomavírus, com genoma composto de dsDNA, são da ordem de 10^{-7} a 10^{-8} subs/sítio/ano (Drake, 1991; Holmes, 2004; Raney *et al.*, 2004). Em contraste, altas taxas de substituição (10^{-4}) foram relatadas para parvovírus e circovírus (vírus de ssDNA) (Gallian *et al.*, 2002; Biagini, 2004). Semelhante aos geminivírus, os parvovírus e circovírus replicam seu genoma via mecanismo de círculo rolante, sugerindo que os altos níveis de heterogeneidade relatados para begomovírus e mastrevírus podem refletir erros de replicação (Arguello-Astorga *et al.*, 2004). Foi sugerido que os mecanismos de correção de erro associados à replicação de DNA em eucariotos não sejam eficientes na replicação por círculo rolante e, ou, na replicação de ssDNA (Van Der Walt *et al.*, 2008).

Mutantes para as proteínas Rep do TGMV e do CaLCuV que não permitem a interação com a proteína pRB, inoculados em protoplastos de fumo (*Nicotiana tabacum*) e em plantas *N. benthamiana*, apresentaram até 100% de frequência de reversão de mutações, evidenciando a capacidade de populações de geminivírus de evoluir rapidamente em respostas a mudanças deletérias em seu genoma (Arguello-Astorga *et al.*, 2007).

5.2. Recombinação

Recombinação é o processo pelo qual segmentos de uma fita de DNA ou RNA tornam-se incorporados na fita de um indivíduo diferente durante o processo de replicação (Padidam *et al.*, 1999b). A recombinação é um evento bastante comum em geminivírus (Padidam *et al.*, 1999b; Lefeuvre *et al.*, 2009), e parece contribuir grandemente para sua diversificação genética, aumentando seu potencial evolucionário e adaptação local (Harrison e Robinson, 1999; Padidam *et al.*, 1999b; Berrie *et al.*, 2001; Monci *et al.*, 2002). A elevada frequência de recombinação nesse grupo de vírus pode ser em parte explicada pela existência de uma possível estratégia de replicação dependente de recombinação (RDR) (Jeske *et al.*, 2001; Preiss e Jeske, 2003) em adição à replicação por círculo rolante (RCR) (Saunders *et al.*, 2001), e pela ocorrência frequente de infecções mistas (Torres-Pacheco *et al.*, 1996; Harrison *et al.*, 1997; Sanz *et al.*, 2000; Pita *et al.*, 2001; Ribeiro *et al.*, 2003; Garcia-Andres *et al.*, 2006; Davino *et al.*, 2009) com a evidência de infecção do mesmo núcleo da célula por mais de um begomovírus (Morilla *et al.*, 2004).

Eventos de recombinação têm sido diretamente implicados na emergência de novas doenças e epidemias em plantas cultivadas. Essas incluem a epidemia devastadora do mosaico da mandioca (*Manihot esculenta*), causada pelo recombinante EACMV na Uganda e países vizinhos (Zhou *et al.*, 1997; Pita *et al.*, 2001); as epidemias do complexo TYLCV na bacia ocidental do Mediterrâneo, com o surgimento dos recombinantes *Tomato yellow leaf curl Málaga virus* (TYLCMalV) e *Tomato yellow leaf curl Axarquía virus* (TYLCAxV) em campos de tomate na Espanha (Monci *et al.*, 2002; Garcia-Andres *et al.*, 2006; Garcia-Andres *et al.*, 2007a; Garcia-Andres *et al.*, 2007b); e as epidemias de *Cotton leaf curl virus* (CLCuV) no Paquistão causadas por um complexo de espécies incluindo diversos begomovírus recombinantes (Zhou *et al.*, 1998; Idris e Brown, 2002).

A emergência frequente de novas espécies de geminivírus devido a eventos de recombinação foi demonstrada por meio de análise de conversão gênica (Padidam *et al.*, 1999a). Embora na época o número de genomas completos sequenciados fosse pequeno, os autores analisaram todas as combinações dois-a-dois possíveis, e identificaram 420 fragmentos recombinantes tanto entre espécies como entre gêneros da família *Geminiviridae*.

Os mecanismos precisos que controlam a recombinação em begomovírus permanecem desconhecidos (Padidam *et al.*, 1999a). No entanto, é conhecido que sítios recombinantes não são uniformemente distribuídos ao longo do genoma, com a existência de sítios frequentes ("hot spots") e não-frequentes ("cold spots") (Stanley, 1995; Fauquet *et al.*, 2005; Garcia-Andres *et al.*, 2007b; Lefeuvre *et al.*, 2009). Análises bioinformáticas para detectar vírus recombinantes ocorrendo naturalmente revelaram que a origem de replicação viral é um sítio frequente de recombinação (Gutierrez, 1999; Hanley-Bowdoin *et al.*, 1999). A comparação de sequências de begomovírus mono- e bissegmentados depositadas no GenBank até maio de 2006 (123 e 116 sequências, respectivamente) indicou que a região do gene *Rep* que codifica a porção N-terminal da proteína Rep, assim como a região intergênica adjacente (RC), são frequentemente intercambiadas durante a replicação. Também foram identificados sítios frequentes de recombinação localizados na região intergênica entre os genes *CP* e *Ren* (Lefeuvre *et al.*, 2007).

A análise comparativa da distribuição de sítios de recombinação dentro do genoma de diversas famílias de vírus de ssDNA novamente sugeriu a distribuição não aleatória dos sítios e também uma tendência significativa para estes se localizarem tanto fora como na periferia dos genes. Além disso, foi observado que poucos sítios de recombinação foram encontrados dentro de genes que codificam proteínas estruturais (Lefeuvre *et al.*, 2009). Esses resultados sugerem que a seleção natural agindo contra

vírus que expressam proteínas recombinantes é a principal determinante na distribuição não aleatória dos sítios de recombinação na maioria das famílias de vírus de ssDNA (Lefeuvre *et al.*, 2009).

Eventos de recombinação também têm sido relatados entre begomovírus e DNA satélites, e entre diferentes moléculas de betassatélites (Briddon *et al.*, 2001; Saunders *et al.*, 2001; Briddon *et al.*, 2003; Nawaz-Ul-Rehman *et al.*, 2009).

5.3. Pseudo-recombinação

A existência de dois componentes genômicos na maioria dos begomovírus promove um mecanismo alternativo, conhecido como pseudo-recombinação, pelo qual a troca de material genético pode ocorrer sem necessidade de recombinação intermolecular, ocorrendo apenas a troca de componentes genômicos entre dois vírus distintos (Gilbertson *et al.*, 1993b; Sung e Coutts, 1995; Andrade *et al.*, 2006a; revisado por Rojas *et al.*, 2005b). A ocorrência natural de pseudo-recombinantes no campo foi verificada no México, em tomateiros infectados pelo *Chino del tomate virus* (CdTV) (Paplomatas *et al.*, 1994).

Experimentos com pseudo-recombinação são ferramentas úteis no estudo de funções de genes e podem revelar relações filogenéticas, como é o caso da mistura de componentes genômicos do BGYMV e do *Bean golden mosaic virus* (BGMV), que possuem identidade inferior a 75% em suas sequências de nucleotídeos e não formam pseudo-recombinantes infecciosos (Gilbertson *et al.*, 1993a). Por outro lado, pseudo-recombinantes formados a partir da mistura de componentes genômicos de dois isolados de BGYMV mostraram-se infecciosos. Quando inoculada, a mistura formada a partir de DNA-A do isolado da Guatemala (BGYMV-GA) e DNA-B do isolado da República Dominicana (BGYMV-DR) foi capaz de induzir os mesmos sintomas apresentados pelos parentais, enquanto o pseudo-recombinante recíproco induziu sintomas atenuados e tardios. Esses resultados demonstram que geminivírus com regiões comuns

suficientemente similares podem formar pseudo-recombinantes infecciosos, mas ressaltam que frequentemente os pseudo-recombinantes recíprocos apresentam diferenças na eficiência de replicação e infecção sistêmica (Faria *et al.*, 1994). Esse fato foi também observado para o *African cassava mosaic virus* (ACMV) (Stanley *et al.*, 2005) e TGMV (Von Arnim e Stanley, 1992).

A especificidade da ligação da proteína Rep aos iterons é considerada a principal determinante da formação de pseudo-recombinantes viáveis entre diferentes espécies/estirpes de begomovírus (Arguello-Astorga *et al.*, 1994; Eagle *et al.*, 1994; Fontes *et al.*, 1994; Chatterji *et al.*, 1999; Andrade *et al.*, 2006a; Bull *et al.*, 2007). Outro fator importante é a conservação da sequência de aminoácidos da proteína Rep, especialmente os três aminoácidos do IRD que estariam envolvidos diretamente na ligação aos iterons (Arguello-Astorga e Ruiz-Medrano, 2001; Ruiz-Medrano *et al.*, 2001). A viabilidade de pseudo-recombinantes indica que fatores envolvidos na replicação e movimento são intercambiáveis entre espécies altamente relacionadas, ou entre estirpes de uma mesma espécie. Por outro lado, a assimetria entre pseudo-recombinantes recíprocos indica que a pseudo-recombinação entre begomovírus é um fenômeno complexo que envolve interações entre fatores do vírus e do hospedeiro (Hill *et al.*, 1998).

Embora a pseudo-recombinação seja comum entre estirpes de uma mesma espécie de begomovírus, a formação de pseudo-recombinantes viáveis entre espécies distintas é mais difícil. Um pseudo-recombinante foi obtido entre o DNA-A do *Abutilon mosaic virus* (AbMV) e o DNA-B do *Sida golden mosaic Costa Rica virus* (SiGMCRV), porém o pseudo-recombinante recíproco não foi infeccioso (Hofer *et al.*, 1997b). Similarmente, um pseudo-recombinante viável foi formado pelo DNA-A de um isolado de *Sida golden mosaic virus* (SiGMV) de Honduras (SiGMV-[Ho_{yv}]) e o DNA-B do SiGMCRV (Unseld *et al.*, 2000). Entretanto, dentre os pseudo-recombinantes

recíprocos formados pelo DNA-A do SiGMCRV combinado ao DNA-B de três isolados de SiGMV-[Ho_{yv}] que possuíam pequenas diferenças na composição de nucleotídeos, apenas um mostrou-se viável, porém pouco eficiente, e não foi capaz de infectar a planta a partir da qual foi originalmente isolado (Unseld *et al.*, 2000). Pseudo-recombinantes infecciosos entre o DNA-A do CdTV e o DNA-B do BGYMV foram formados apesar da baixa identidade da região comum (68%), porém o pseudo-recombinante recíproco não foi infeccioso quando inoculado em feijoeiro (*Phaseolus vulgaris*) (Garrido-Ramirez *et al.*, 2000).

Um pseudo-recombinante produzido entre o DNA-A do *Tomato mottle virus* (ToMoV) e o DNA-B do *Bean dwarf mosaic virus* (BDMV), embora infeccioso, apresentou acúmulo reduzido do DNA-B e induziu sintomas atenuados em *N. benthamiana* (Gilbertson *et al.*, 1993b; Hou e Gilbertson, 1996). Entretanto, após três passagens mecânicas sucessivas nesse hospedeiro, os sintomas tornaram-se idênticos aos produzidos pelo ToMoV e o nível do DNA-B tornou-se igual ao do DNA-A. A análise das regiões comuns dos DNAs-A e -B do pseudo-recombinante comprovou a ocorrência de recombinação intermolecular na região comum do BDMV, que foi substituída quase que totalmente pela região comum do DNA-A do ToMoV (Hou e Gilbertson, 1996). Assim, o DNA-B passou a ser reconhecido com 100% de eficiência pela proteína Rep do ToMoV. Esse resultado evidencia a importância da pseudo-recombinação e da recombinação na evolução de geminivírus e em sua adaptação a novos hospedeiros.

Pseudo-recombinantes infecciosos foram formados entre o DNA-A do TGMV e o DNA-B do ToYSV, que possuem iterons similares. A não formação do pseudo-recombinante recíproco sugere que a proteína Rep do TGMV tem maior versatilidade em termos de reconhecimento de componentes de DNA heterólogos comparada à do ToYSV (Andrade *et al.*, 2006a).

O *Passion fruit severe leaf distortion virus* (PSLDV) e o ToCMoV, os quais apresentam os mesmos iterons, formam um pseudo-recombinante viável, pois todas as plantas de *Nicotiana benthamiana* inoculadas com a combinação PSLDV-A e ToCMoV-B foram infectadas sistemicamente. Entretanto, o pseudo-recombinante recíproco (PSLDV-B e ToCMoV-A) não foi viável. Os mesmos resultados foram observados com PSLDV e ToYSV, os quais possuem iterons distintos. O pseudo-recombinante PSLDV-A e ToYSV-B foi viável em 20% das plantas de *N. benthamiana* inoculadas (Ferreira *et al.*, 2010). Estes resultados reforçam a hipótese de que outros fatores além da identidade dos iterons estão associados à viabilidade de pseudo-recombinantes.

5.4. Estrutura genética de populações de geminivírus

A estrutura genética de populações de vírus de plantas refere-se à quantidade de variabilidade genética e a sua distribuição dentro e entre subpopulações (Garcia-Arenal *et al.*, 2001). Definir a estrutura genética é o primeiro passo para se estudar as populações virais, pois a estrutura genética reflete a história evolutiva e o potencial da população para evoluir (Pinel *et al.*, 2003; Moreno *et al.*, 2004; Font *et al.*, 2007). Para a maior parte dos objetivos, a genética de populações fornece a ferramenta mais conveniente para estimar a variabilidade genética de populações de patógenos. Os principais mecanismos evolutivos que afetam a variabilidade das populações são seleção, deriva genética, migração, mutação e recombinação (Hartl e Clark, 2007). Quantificar a contribuição de cada mecanismo é importante e constitui o objetivo de vários estudos de biologia de populações de vírus de plantas (Bull *et al.*, 2006; Wang *et al.*, 2006; Garcia-Andres *et al.*, 2007a).

Diversos estudos já foram realizados com o objetivo de investigar a estrutura genética de populações de geminivírus em diversos hospedeiros e em diferentes regiões

geográficas. Trabalhos realizados ao longo das décadas de 1990 e 2000 avaliaram a estrutura populacional de begomovírus infectando mandioca na África Sub-Sahariana e no Sub-Continente Indiano. Nos países dessas regiões, a mandioca pode ser infectada por diversas espécies de begomovírus (Fauquet e Fargette, 1990; Legg e Raya, 1993; Fargette *et al.*, 1994) (curiosamente, não existem relatos de begomovírus que infectam mandioca no Brasil, o centro de origem e diversidade genética desta cultura). Os estudos realizados demonstraram um elevado grau de variabilidade genética da população viral em diversos países. A ocorrência frequente de infecções mistas facilita a ocorrência de pseudo-recombinação e recombinação, e em pelo menos dois casos foi demonstrada a emergência de novas espécies como consequência direta desses mecanismos (Zhou *et al.*, 1997; Fondong *et al.*, 2000).

Na Tanzânia, sete espécies de begomovírus descritas que infectam mandioca foram relatadas (Ndunguru *et al.*, 2005). Diversos eventos de recombinação foram detectados entre as estirpes TZ1 e TZ7 do *East African cassava mosaic Cameron virus* (EACMCV). A análise das sequências indicou que as duas estirpes têm a mesma origem e não foram introduzidas recentemente. A variabilidade genética da população viral foi analisada também com base no DNA-B, o que indicou a existência de diversos eventos de recombinação. Os resultados indicam que a região central do continente africano é um centro de diversidade genética de begomovírus (Ndunguru *et al.*, 2005).

Além dos begomovírus que infectam a mandioca, a África também é o centro de origem dos mastrevírus que infectam gramíneas (Palmer e Rybicki, 1998). Um estudo recente utilizando RCA analisou a estrutura genética da população viral em Uganda, um dos países mais afetados pelo estriado do milho causado pelo MSV (Owor *et al.*, 2007a). Amostras foram coletadas em 155 locais cobrindo todo o país. Inicialmente, fragmentos do genoma viral foram amplificados via PCR e a variabilidade foi analisada por meio de PCR-RFLP. Um total de 49 variantes foram identificadas a partir de 391

isolados virais. A partir dessas 49 variantes, um total de 62 genomas completos foram sequenciados, e uma origem recombinante foi demonstrada para 52 desses genomas. Entretanto, um único recombinante, denominado MSV-A(1)UgIII, estava presente em infecção simples em mais de 60% das amostras infectadas em todo o país. Os autores concluíram que, embora a ocorrência de recombinação entre mastrevírus seja tão ou mais frequente em comparação com os begomovírus, o MSV deve estar sujeito a gargalos que limitam a variabilidade genética das populações naturais (Owor *et al.*, 2007a).

Font *et al.* (2007) determinaram a estrutura e variabilidade genética de populações de *Tomato yellow leaf curl Sardinia virus* (TYLCSV) e TYLCV em plantas de tomateiro em seis regiões da Espanha (Andaluzia, Ilhas Canárias, Lanzarote, Levante, Majorca e Murcia) entre os anos de 1997 e 2001. A análise de PCR-RFLP do gene *CP* e da RC de 358 isolados revelou a presença de 14 haplótipos, e eventos de recombinação foram identificados na RC. Em todas as regiões geográficas, exceto Murcia, as populações eram compostas de um haplótipo predominante com uma baixa diversidade genética (<0,0180), ou estavam evoluindo para esta condição. Em Murcia houve mudanças na predominância de haplótipos. O haplótipo I (TYLCSV) era predominante em 1997, mas sua frequência decresceu em 1998, com o aumento correspondente do haplótipo III (TYCLV), de modo que ambos os haplótipos apresentaram frequências semelhantes. Em 1999, o haplótipo II surgiu e rapidamente tornou-se predominante na população. Esses resultados sugerem que a seleção negativa ocorreu de forma acentuada nessas populações. No entanto, o surgimento de haplótipos altamente adaptados se dispersando na população indica que seleção positiva também estava ocorrendo.

No Brasil, Castillo-Urquiza (2008), estudando duas populações de begomovírus que infectam tomateiro, *Tomato yellow vein streak virus* (ToYVSV) e *Tomato common*

mosaic virus (ToCmMV) na região Sudeste do Brasil (municípios de Coimbra, MG e Paty do Alferes, RJ), observou maior variabilidade genética na população de ToCmMV. Demonstrou ainda que entre subpopulações de ToCmMV em Coimbra e Paty de Alferes havia maior variabilidade na subpopulação localizada em Coimbra.

6. Diversidade de begomovírus infectando plantas cultivadas e invasoras no Brasil

Durante as duas últimas décadas, begomovírus têm emergido como um dos principais patógenos de plantas, particularmente nas regiões tropicais e subtropicais no mundo, causando severas perdas econômicas (Morales, 2006). No Brasil, as culturas mais severamente afetadas são o feijoeiro e tomateiro (Faria e Maxwell, 1999; Zerbini *et al.*, 2005). Embora existam relatos de infecção por begomovírus em outras culturas importantes como a soja (*Glycine max*) (Mello *et al.*, 2000; Mello *et al.*, 2002) e o pimentão (*Capsicum annum*) (Nozaki *et al.*, 2005), esses ocorrem esporadicamente nas áreas de cultivo, não sendo considerados fatores limitantes à produção.

Begomovírus que infectam feijoeiro (*Phaseolus* spp.) são distribuídos através das Américas, sendo sua incidência um fator limitante para a produtividade dessa cultura. Quatro espécies já foram descritas: *Bean calico mosaic virus* (BCaMV), *Bean dwarf mosaic virus* (BDMV), BGMV e BGYMV (Fauquet *et al.*, 2008). Foi demonstrado que isolados brasileiros de BGMV apresentam um baixo grau de variabilidade genética, o que não é comum para begomovírus (Faria e Maxwell, 1999). No entanto, estudos mais recentes realizados em populações de BGMV infectando fava (*P. lunatus*) baseados na análise de genomas completos indicaram alta variabilidade genética (Silva, 2006; Ramos-Sobrinho *et al.*, 2010).

Apesar da ocorrência frequente de BGMV em feijoeiro, infecções de begomovírus em soja não são comuns no Brasil. Ocorrências esporádicas têm sido relatadas desde 1980, com a detecção de BGMV, *Sida mottle virus* (SiMoV) e duas

possíveis novas espécies em amostras coletadas na região Sudeste (Mello *et al.*, 2002); e BGMV, *Sida micrantha mosaic virus* (SiMMV) e *Okra mottle virus* (OMoV) na região Centro-Oeste do país (Fernandes *et al.*, 2009). Este cenário está em contraste com a Argentina, onde a infecção de soja por três begomovírus distintos, incluindo o SiMoV, é frequente na região Noroeste, causando perdas moderadas a severas na produção (Rodríguez-Pardina *et al.*, 2010).

Uma situação oposta é observada para begomovírus que infectam solanáceas, a exemplo do tomateiro e do pimentão, onde um grande número de espécies tem sido descritas, e a variabilidade genética entre os isolados de uma determinada espécie é normalmente muito alta (Ribeiro *et al.*, 2003; Castillo-Urquiza *et al.*, 2008; Fernandes *et al.*, 2008).

O primeiro relato de begomovírus em tomateiro no Brasil foi feito na década de 1970 (Costa *et al.*, 1975). O vírus foi caracterizado e denominado TGMV. Além do TGMV, cinco outros vírus transmitidos por mosca-branca foram identificados, porém sem causar danos de importância econômica (Matyis *et al.*, 1975). Isso provavelmente ocorria porque o biótipo A de *B. tabaci*, o único presente no país naquela época, coloniza o tomateiro com baixa eficiência (Bedford *et al.*, 1994). No entanto, no início da década de 1990 um complexo de begomovírus surgiu em tomateiro no Brasil, coincidindo com a introdução e disseminação do biótipo B de *B. tabaci* (Ambrozevicius *et al.*, 2002; Ribeiro *et al.*, 2003). Desde então, oito espécies de begomovírus já foram descritas: ToCMoV, ToYSV, ToRMV, ToSRV, ToCmMV, ToYVSV, *Tomato leaf distortion virus* (ToLDV) e *Tomato mild mosaic virus* (ToMlMV) (Faria e Maxwell, 1999; Fernandes *et al.*, 2006; Calegario *et al.*, 2007; Ribeiro *et al.*, 2007; Castillo-Urquiza *et al.*, 2008), e seis outras foram descritas a partir de sequências parciais (Ribeiro *et al.*, 2003; Fernandes *et al.*, 2008). Algumas dessas espécies encontram-se amplamente distribuídas pelo país, enquanto outras estão restritas a certas regiões. Por

exemplo, o ToSRV já foi relatado nos estados de Goiás, Minas Gerais, Pernambuco, Rio de Janeiro, Santa Catarina e São Paulo (Rezende *et al.*, 1997; Lima *et al.*, 2006; Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007; Fernandes *et al.*, 2008). Por outro lado, o ToYSV foi relatado apenas em Minas Gerais (Calegario *et al.*, 2007).

Levantamentos realizados para acessar a diversidade de begomovírus em tomateiro indicam que determinadas espécies tornaram-se prevalentes em diferentes regiões do país (Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007; Castillo-Urquiza, 2008; Fernandes *et al.*, 2008). O sequenciamento direto de fragmentos de PCR de amostras de tomateiro coletadas na região central do estado de São Paulo nos anos de 2003 e 2004 revelou como espécie predominante o ToSRV, presente em 50% das amostras analisadas. O ToYVSV e o SiMoV também estavam presentes (Cotrim *et al.*, 2007). A mesma estratégia foi utilizada para identificar begomovírus em amostras de tomateiro coletadas entre 2002 e 2004 no Distrito Federal e nos estados da Bahia, Goiás, Minas Gerais, Pernambuco e São Paulo. Verificou-se a presença do ToSRV em 61% das amostras, além do ToYVSV, Tomato mottle leaf curl virus (ToMoLCV) e duas possíveis novas espécies (Fernandes *et al.*, 2008).

Nos anos de 2005 e 2007 foi realizado um estudo sobre a diversidade de begomovírus em duas importantes regiões produtoras de tomate no Sudeste do Brasil, Paty do Alferes (RJ) e Coimbra (MG). A análise de sequências do genoma completo do DNA-A revelou que em Paty do Alferes o ToYVSV era o vírus predominante, encontrado em 56% das amostras analisadas, seguido pelo ToCmMV. Já em Coimbra o ToCmMV foi o único vírus encontrado infectando tomateiro (Castillo-Urquiza, 2008).

Acredita-se que a emergência dos begomovírus que infectam tomateiro no Brasil seja resultado da transferência horizontal de vírus nativos que infectam plantas silvestres ou invasoras pelo biótipo B da mosca-branca. Uma vez presentes no novo hospedeiro, esses vírus evoluiram rapidamente via recombinação e pseudo-

recombinação, dando origem às espécies atualmente detectadas no campo. A predominância de algumas espécies poderia ser devido a diferenças na adaptação ao tomateiro ou diferenças na eficiência de transmissão pelo vetor (Castillo-Urquiza *et al.*, 2008). Três observações corroboram essa hipótese. Em primeiro lugar, todas as espécies de begomovírus detectadas até o presente em tomateiro no Brasil são de ocorrência restrita ao país. Em segundo lugar, a caracterização biológica de algumas espécies (ToRMV, ToCMoV e ToYSV) confirmou que plantas daninhas como *Nicandra physaloides*, *Solanum nigrum* e *Datura stramonium* são hospedeiras (Fernandes *et al.*, 2006; Calegario *et al.*, 2007; Ribeiro *et al.*, 2007). Por fim, begomovírus originalmente descritos em plantas silvestres/daninhas, como o SiMoV (Fernandes *et al.*, 1999) e o SimMV (Jovel *et al.*, 2004), já foram encontrados infectando naturalmente o tomateiro (Calegario, 2004; Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007).

A presença de diversas espécies no campo, todas transmitidas pelo mesmo inseto vetor, torna comum a ocorrência de infecções mistas, com dois ou mais vírus presentes simultaneamente na mesma planta, aumentando a probabilidade da ocorrência de eventos de recombinação e pseudo-recombinação, o que pode levar ao surgimento de espécies melhor adaptadas ao hospedeiro (Andrade *et al.*, 2006a; Inoue-Nagata *et al.*, 2006; Ribeiro *et al.*, 2007). Evidências de recombinação e pseudo-recombinação já foram encontradas em associação ao complexo de begomovírus infectando o tomateiro no Brasil. Galvão *et al.* (2003) e Ribeiro *et al.* (2007) sugeriram que os isolados MG-Bt1 e BA-Se1 do ToCMoV possuem origem recombinante. A formação de pseudo-recombinantes viáveis entre clones infecciosos do TGMV (DNA-A) e ToYSV (DNA-B), e entre o ToYSV (DNA-A) e o Tomato crinkle leaf yellow virus (ToCrLYV) já foi demonstrada (Andrade *et al.*, 2006a).

Paprotka *et al.* (2010a) estudaram a diversidade genética de begomovírus presentes em acesso de batata-doce naturalmente infectados em um banco de

germoplasma brasileiro. Nesse estudo foram identificadas duas novas espécies, Sweet potato golden vein-associated virus (SPGVaV) e Sweet potato mosaic-associated virus (SPMaV), além de três novos isolados e vários variantes do *Sweet potato leaf curl virus* (SPLCV). A comparação de sequências dos begomovírus encontrados nesses acessos revelou a presença de sinais de recombinação em seus genomas, ressaltando o risco do surgimento de novos begomovírus no material propagado vegetativamente no banco de germoplasma.

Além das plantas cultivadas, muitas espécies silvestres e/ou invasoras têm sido relatadas como hospedeiras de begomovírus em vários países, incluindo o Brasil (Idris *et al.*, 2003; Jovel *et al.*, 2004; Varsani *et al.*, 2009; Fiallo-Olive *et al.*, 2010; Mubin *et al.*, 2010). As espécies mais comumente relatadas como hospedeiras pertencem às famílias Malvaceae, Euphorbiaceae e Fabaceae (Morales e Anderson, 2001). Alguns estudos demonstraram que begomovírus provenientes de plantas invasoras podem ser transmitidos para espécies cultivadas pelo inseto vetor ou mediante inoculação via extrato vegetal tamponado (Frischmuth *et al.*, 1997; Faria *et al.*, 2000; Morales e Anderson, 2001; Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007).

No Brasil já se realizaram alguns estudos com o objetivo de caracterizar molecularmente isolados de begomovírus que infectam plantas silvestres e daninhas, sobretudo em associação às culturas do feijoeiro e do tomateiro (Ribeiro *et al.*, 1998; Faria e Maxwell, 1999; Castillo-Urquiza *et al.*, 2008). Os resultados desses estudos revelaram que, a exemplo do que ocorre com plantas cultivadas, a diversidade genética é alta entre os isolados de begomovírus que infectam plantas invasoras (Ambrozevicius *et al.*, 2002; Calegario, 2004; Castillo-Urquiza, 2008).

Na Serra do Ibiapaba, CE, amostras assintomáticas de plantas invasoras de sete famílias botânicas e 18 espécies vegetais foram avaliadas por ELISA e PCR para infecção por begomovírus. Espécies de plantas daninhas pertencentes às famílias

Amaranthaceae (*Amaranthus deflexus, A. spinosus*, *A. viridis*), Asteraceae (*Acanthospermum hispidum*, *Ageratum conyzoides*, *Bidens pilosa*), Euphorbiaceae (*Euphorbia heterophylla*) e Rubiaceae (*Borreria capitata*) foram identificadas como hospedeiras naturais de begomovírus (Santos *et al.*, 2003; Arnaud *et al.*, 2007). Silva *et al.* (2010a) realizaram ensaios de inoculação por mosca-branca e enxertia com o objetivo de observar a transmissão de begomovírus a partir de tomateiros infectados para quatro espécies de plantas invasoras (*Amaranthus spinosus*, *A. viridis*, *Ageratum conizoydes* e *B. pilosa*) e verificação de seu retorno para o tomateiro. Os resultados indicaram que o vetor transmitiu eficientemente o vírus para as quatro espécies. Por enxertia, apenas *B. pilosa* foi infectada. Esses resultados demonstram que as espécies invasoras são hospedeiras alternativas dos begomovírus de tomateiro presentes na região da Serra de Ibiapaba e, em condições de campo, na presença do vetor, podem constituir importantes fontes de inóculo para essa cultura. No entanto, as espécies de begomovírus infectando estas plantas não foram identificadas.

Plantas daninhas coletadas em municípios dos estados de Alagoas, Bahia e Pernambuco, com sintomas de mosaico amarelo, deformação do limbo foliar e redução do crescimento, foram avaliadas para a presença de begomovírus via PCR (Assunção *et al.*, 2006). A infecção viral foi confirmada em *Cleome affinis* (Capparaceae), *Cnidoscolus urens* (Euphorbiaceae), *Desmodium* sp., *Macroptilium lathyroides* (Fabaceae), *Herissantia crispa*, *Sidastrum micranthum, S. rhombifolia*, *Sida spinosa* (Malvaceae), *Triumfetta semitriloba* e *Waltheria indica* (Sterculiaceae). Padrões distintos de clivagem obtidos em análise de PCR-RFLP sugeriram a existência de um alto grau de variabilidade genética (Assunção *et al.*, 2006). Entretanto, as espécies de begomovírus infectando estas plantas não foram identificadas.

Castillo-Urquiza *et al.* (2008) analisaram a presença de begomovírus em tomateiro e plantas invasoras associadas à cultura. Foram encontradas seis novas

espécies, três provenientes do tomateiro e três provenientes das invasoras *Blainvillea rhomboidea* (*Blainvillea yellow spot virus*, BlYSV), *Sida rhombifolia* (*Sida yellow mosaic virus*, SiYMV) e *Sida micrantha* (*Sida common mosaic virus*, SiCmMV).

A partir de material foliar de plantas sintomáticas pertencentes às famílias Malvaceae, Euphorbiaceae e Capparaceae, coletadas no município de Miranda (Mato Grosso do Sul), foram identificadas duas novas espécies de begomovírus, *Cleome leaf crumple virus* (ClLCrV), obtido de *Cleome affinis*, e *Sida mosaic Brazil virus* (SiMBV). Além disso, foram encontrados dois alfassatélites associados ao *Euphorbia mosaic virus* (Euphorbia mosaic virus Mato Grosso do Sul-associated DNA1) e ao ClLCrV (Cleome leaf crumple virus-associated DNA1) (Paprotka *et al.*, 2010c).

Um novo begomovírus, *Abutilon Brazil virus* (AbBV), foi identificado infectando *Abutilon* sp. no estado da Bahia. Análises filogenéticas demonstraram que ambos os componentes genômicos são distintos da espécie clássica, *Abutilon mosaic virus* (AbMV), originária do oeste da Índia. Além disso, inoculação via biobalística comprovou sua transmissão para *Malva parviflora*, a qual desenvolveu sintomas característicos de clareamento de nervuras e mosaico (Paprotka *et al.*, 2010b).

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CAPÍTULO 1

MOLECULAR CHARACTERIZATION OF THE BEGOMOVIRUS *Tomato mottle leaf curl virus* (ToMoLCV)

Rocha, C.S., Xavier, C.A.D., Lima, A.T.M., Silva, F.N., & Zerbini, F.M. Molecular characterization of the begomovirus *Tomato mottle leaf curl virus* (ToMoLCV). Archives of Virology, *in preparation*.

Molecular characterization of the begomovirus *Tomato mottle leaf curl virus* (ToMoLCV)

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Running title: Characterization of the begomovirus ToMoLCV

Abstract

Begomoviruses cause economic losses in many crops, mainly in tropical and subtropical regions. Begomoviruses have one or two components and are transmitted by the whitefly *Bemisia tabaci* to dicotyledonous plants. In Brazil, a viral complex comprised of at least eight species is responsible for severe losses in tomato. Tomato and weed samples were collected in tomato growing regions of Minas Gerais state in southeastern Brazil in 2008 and 2010. Previously described viruses were prevalent in the samples. Two isolates of the partially sequenced Tomato mottle leaf curl virus (ToMoLCV) were associated with tomato plants collected in Jaíba. Here, we describe its complete DNA-A sequence and molecular characterization. Genome analysis indicates that ToMoLCV is a typical New World, bipartite begomovirus with greater sequence identity with begomoviruses from Brazil and Central America. Phylogenetic analysis confirms that ToMoLCV clusters with New World begomoviruses from Brazil and Central America. Together, these results support the classification of ToMoLCV as a distinct species in the genus *Begomovirus*.

Key words: geminivirus; begomovirus; diversity; tomato.

Begomovirus diseases are a major limiting factor to crop yields in tropical and subtropical regions [18, 23, 29, 30]. The genus *Begomovirus* belongs to the *Geminiviridae* family, and includes viruses with one or two genomic components which infect dicotyledonous plants and are transmitted by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) [31]. The B biotype of *B. tabaci* was first reported in Brazil in the early 1990s [20], and due to its characteristics of greater adaptability it has spread rapidly throughout the hot and dry regions of the country [17]. Following the dissemination of the B biotype of *B. tabaci*, begomovirus epidemics have greatly increased in Brazil. It is believed that the insect vector transferred indigenous viruses infecting wild and weed hosts to tomato.

The initial characterization of begomoviruses associated with epidemics in tomato in Brazil indicated a high genetic diversity [1, 26], with the description of eight new species including *Tomato rugose mosaic virus* (ToRMV) [12], *Tomato chlorotic mottle virus* (ToCMoV) [27] and *Tomato yellow spot virus* (ToYSV) [4], all present in the state of Minas Gerais. Surveys conducted over the past five years [5, 8, 11] indicate that certain species have become prevalent in different regions of the country. However, Castillo-Urquiza *et al.* [6] recently reported six new begomoviruses species in tomato and weeds, indicating that new species continue to emerge.

The emergence of new viruses is dependent on mutation, recombination and, in viruses with divided genomes such as most begomoviruses, pseudorecombination events. Mixed infections with more than one begomovirus are common in the field, favoring recombination and pseudorecombination and facilitating the emergence of new strains or species better adapted to new hosts [3, 7, 9, 14, 22-24].

As part of an ongoing study of the genetic diversity of tomato-infecting begomoviruses in Brazil, 117 tomato and 23 weed samples were collected in July 2008

in tomato fields located around the city of Jaíba, in northern Minas Gerais state (15°11'01"S; 43°49'07"W). Total DNA was extracted as described by Doyle and Doyle [10] and full-length viral genomes were amplified by rolling-circle amplification [15]. After monomerization with the restriction enzymes *Apa* I, *Bam*H I, *Cla* I, *Hin*d III, *Kpn* I, *Pst* I, *Ssp* I or *Sac* I, samples that displayed a restriction pattern including a 2,600 bp band were selected for cloning of full-length viral genome components in pBLUESCRIPT KS+ (Stratagene). Recombinant plasmids with inserts corresponding to full-length begomovirus components were identified by restriction analysis, and the viral inserts were completely sequenced at Macrogen, Inc. (Seoul, South Korea). Pairwise *p*-distance comparisons of the nucleotides sequences of the complete DNA-A and of the five genes in the DNA-A (Replication-associated protein, *Rep*; Transactivating protein, *Trap*; Replication enhancer protein, *Ren*; *AC4*; and Coat protein, *CP*) were performed using MEGA 5 with exclusion of alignment gaps [32]. The deduced amino acid sequences of the five proteins were compared using EMBOSS (http://www.ebi.ac.uk/Tools/psa/) with default settings. Maximum likelihood phylogenetic trees were inferred using PAUP 4.0 [33], using a full-length genome dataset. Phylogenetic trees based on recombinant and non-recombinant regions were constructed using Bayesian inference and Markov chain Monte Carlo (MCMC) simulation implemented in MrBayes 3.0 [28] with the evolution models selected by MrModeltest2.2 [21] using the Akaike Information Criterion (AIC). The program ModelTest 3.7 [25] was used to predict the best-fit model GTR+I+G. The MCMC simulation was run for 10 million generations, and sampled at every 1000 steps. Convergence was assessed on the basis of the effective sampling size after a 25% burn-in using Tracer version 1.5. The tree was viewed using FigTree version 1.3.1 and edited using Corel Draw X3.

A total of seven DNA-A and three DNA-B clones were obtained. Sequence comparisons indicated that the DNA-A components from two different samples corresponded to the previously described Tomato mottle leaf curl virus (ToMoLCV), sharing 97% identity with the partial sequence reported by Ribeiro *et al.* [26] (a detailed analysis of the remaining clones will be reported elsewhere). The two isolates (BR:Jai13:08 and BR:Jai56:08) have 99% nucleotide sequence identity with each other. The highest nucleotide sequence identity with other begomoviruses is 80% with *Passionfruit severe leaf distortion virus* (PSLDV) and *Tomato chlorotic mottle virus* (ToCMoV) (Figure 1A). Although, the DNA-B has not been cloned, the two ToMoLCV isolates have a DNA-A organization typical of New Worlds bipartite begomoviruses, with five genes (CP, Ren, Trap, Rep and AC4). The *CP* gene is the most conserved, with 92% amino acid sequence identity with *Potato yellow mosaic Panama virus* (PYMPV) (Figure 1B). The *Rep* gene is the least conserved in terms of nucleotide sequence, showing the greatest identity with PSLDV (Figure 1C).

Phylogenetic analysis based on the DNA-A and including all begomoviruses from the Americas placed ToMoLCV in a monophyletic cluster which includes *Cleome leaf crumple virus* (ClLCrV), which so far has been found only in the weed *Cleome affinis*, the passionfruit-infecting PSLDV, two tomato-infecting begomoviruses from Brazil [*Tomato golden mosaic virus* (TGMV) and *Tomato yellow vein streak virus* (ToYVSV)], two tomato-infecting begomoviruses from Mexico [*Tomato golden mottle virus* (ToGMoV) and *Tomato chino La Paz virus* (ToChLPV)], and one soybean-infecting begomovirus from Argentina (*Soybean blistering mosaic virus*, SoBlMV) (Figure 2).

Recombination analysis using RDP3 [19] indicated that BR:Jai13:08 and BR:Jai56:08 are recombinants. The single recombination event was detected by all

methods of the RDP3 package using a data set including only Brazilian viruses known to infect tomato or weeds, with ToCMoV identified as one of the parents (Table 1A). However, using a data set including all American begomoviruses, PSLDV was identified as the recombinant parent (Table 1B). Phylogenetic analysis based only on the putative recombinant region (Table 1A) placed ToMoLCV together with PSLDV and ToCMoV (Figure 3A), but when the analysis was based only on the non-recombinant region ToMoLCV was placed together with PSLDV (Figure 3B). Together, these results indicate that ToMoLCV has a common ancestor with PSLDV and is a recombinant with ToCMoV.

The tomato samples from which BR:Jai13:08 and BR:Jai56:08 were cloned were also infected with ToYSV (data not shown). Detailed analysis of the common regions (CR) of isolates BR:Jai13:08 and BR:Jai56:08 indicated that they share similar iterons with PSLDV, ToCMoV and ToYSV. Interestingly, one of the direct repeats (GGGG) is identical to the one from PSLDV and ToCMoV, whereas the other (GGTG) is identical to the one from ToYSV (Figure 4). The inverted repeat (CCAC) is the same for all four viruses (Figure 4). These features indicate that the formation of viable pseudorecombinants among these viruses may occur. In fact, PSLDV (DNA-A) and ToCMoV (DNA-B), which have identical iterons but share only 70% nucleotide sequence identity in their CRs, form viable pseudorecombinants in *Nicotiana benthamiana* [13]. Also, a PSLDV-A and ToYSV-B pseudorecombinant was viable in 20% of the inoculated *N. benthamiana* plants [13]. These results confirm the close relationship among these viruses, and are an additional line of evidence pointing at a common origin. Evidently, in the case of ToMoLCV this must by confirmed with the production of infectious clones and plant inoculations with the mixtures of its genomic components with those from PSLDV, ToCMoV and ToYSV.

We have carried out the molecular characterization of the begomovirus Tomato mottle leaf curl virus (ToMoLCV), detected in two tomato samples collected in northern Minas Gerais state, Brazil. This virus had not yet been recognized as a distinct species, because its DNA-A had not been completely sequenced [26]. Our results support the classification of ToMoLCV as a species in the genus *Begomovirus*.

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Table 1. Recombination events detected between ToMoLCV isolates BR:Jai13:08 and BR:Jai56:08 and begomoviruses infecting tomato and weeds in Brazil and in the Americas. **(A)** Results based on a data set comprising only Brazilian begomoviruses. **(B)** Results based on data set comprising all begomoviruses from the Americas.

	Parents		Breakpoints[1]		P-value						
	Major	**Minor**	**Initial**	**Final**	**R[2]**	**G**	**B**	**M**	**C**	**S**	**3S**
A											
BR:Jai13:08	Unknown	ToCMoV	1819	2614	8.1×10^{-16}	3.2×10^{-15}	1.6×10^{-14}	4.1×10^{-14}	4.2×10^{-11}	7.5×10^{-24}	2.2×10^{-09}
BR:Jai56:08	Unknown	ToCMoV	1819	2614	8.1×10^{-15}	3.2×10^{-15}	1.6×10^{-14}	4.1×10^{-14}	4.2×10^{-11}	7.5×10^{-24}	2.2×10^{-09}
B											
BR:Jai13:08	Unknown	PSLDV	1932	2145	7.1×10^{-5}	6.2×10^{-14}	2.1×10^{-05}	1.1×10^{-05}	4.7×10^{-03}	1.1×10^{-13}	-
BR:Jai56:08	Unknown	PSLDV	1932	2145	7.1×10^{-5}	6.2×10^{-14}	2.1×10^{-05}	1.1×10^{-05}	4.7×10^{-03}	1.1×10^{-13}	-

[1]Numbering starts at the first nucleotide after the cleavage site at the origin of replication and increases clockwise.

[2] R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, CHIMAERA; S, SisScan; 3, 3SEQ.

Figure legends

Figure 1. Percent nucleotide sequence identities between the full-length DNA-A **(A)**, and percent nucleotide (below the diagonal) and deduced amino acid (above the diagonal) sequence identities of the **(B)** *CP*, **(C)** *Rep*, **(D)** *Trap*, **(E)** *Ren* and **(F)** *AC4* genes of the BR:Jai13:08 and BR:Jai56:08 isolates and the most closely related begomoviruses.

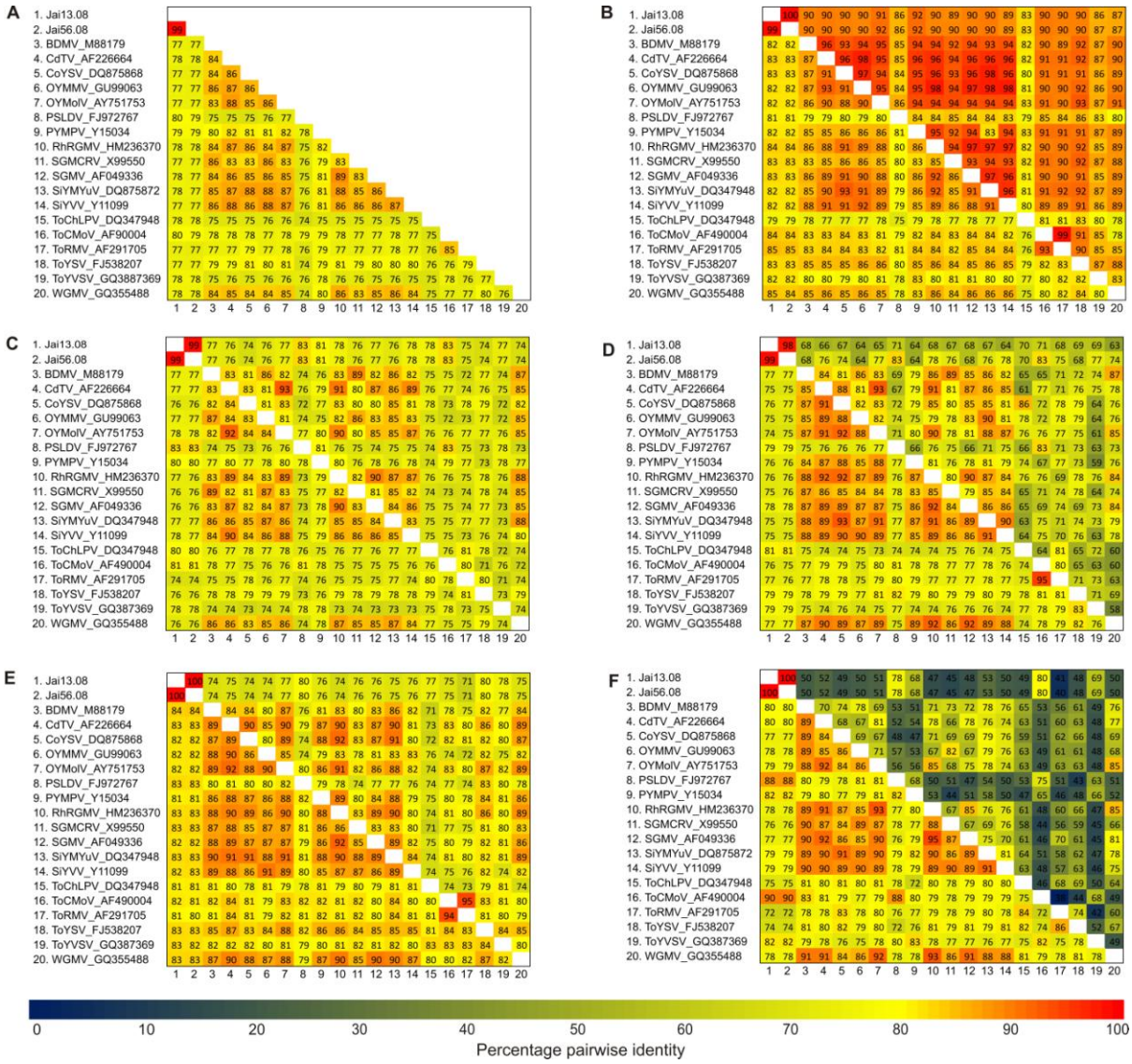
Figure 2. Maximum likelihood tree obtained from the alignment of the full-length DNA-A sequences of begomoviruses from the Americas, including the ToMoLCV isolates BR:Jai13:08 and BR:Jai56:08. Numbers on branches indicate bootstrap values (1000 replications).

Figure 3. Phylogenetic reconstruction, using the Bayesian method, based on the alignment of the **(A)** recombinant and **(B)** non-recombinant regions detected in the genomes of ToMoLCV isolates BR:Jai13:08 and BR:Jai56:08. Numbers at the nodes indicate Bayesian posterior probabilities.

Figure 4. (**A)** Alignment of the common regions of the ToMoLCV isolates BR:Jai13:08 and BR:Jai56:08 with those from ToYSV, ToCMoV and PSLDV. The TATA box and the conserved nonanucleotide are highlighted in grey. Iterated direct and inverted repeats (iterons) are boxed. The arrows indicate the direction of the repeats. Asterisks indicate nucleotide positions which are conserved among all four aligned sequences. Nucleotide differences in the ToMoLCV iterons are highlighted in yellow. (**B)** Partial alignment of the amino acid sequence of the Rep proteins of ToMoLCV, ToYSV,

ToCMoV and PSLDV. The domain associated with sequence-specific recognition of iterons (iteron-related domain, IRD) is boxed. Red asterisks indicate nucleotide positions which are conserved among all six aligned sequences. Motif 1, motif 2 and the specificity determinants (SPDs), which according to Arguello-Astorga and Ruiz-Medrano [2] and Londono *et al.* [16] are conserved in rolling-circle replication initiator proteins, including geminivirus Rep proteins, are highlighted in grey.

Figure 1


A
B
C
D
E
F
1. Jai13.08
2. Jai56.08
3. BDMV_M88179
4. CdTV_AF226664
5. CoYSV_DQ875868
6. OYMMV_GU99063
7. OYMoIV_AY751753
8. PSLDV_FJ972767
9. PYMPV_Y15034
10. RhRGMV_HM236370
11. SGMCRV_X99550
12. SGMV_AF049336
13. SiYMYuV_DQ347948
14. SiYVV_Y11099
15. ToChLPV_DQ347948
16. ToCMoV_AF490004
17. ToRMV_AF291705
18. ToYSV_FJ538207
19. ToYVSV_GQ387369
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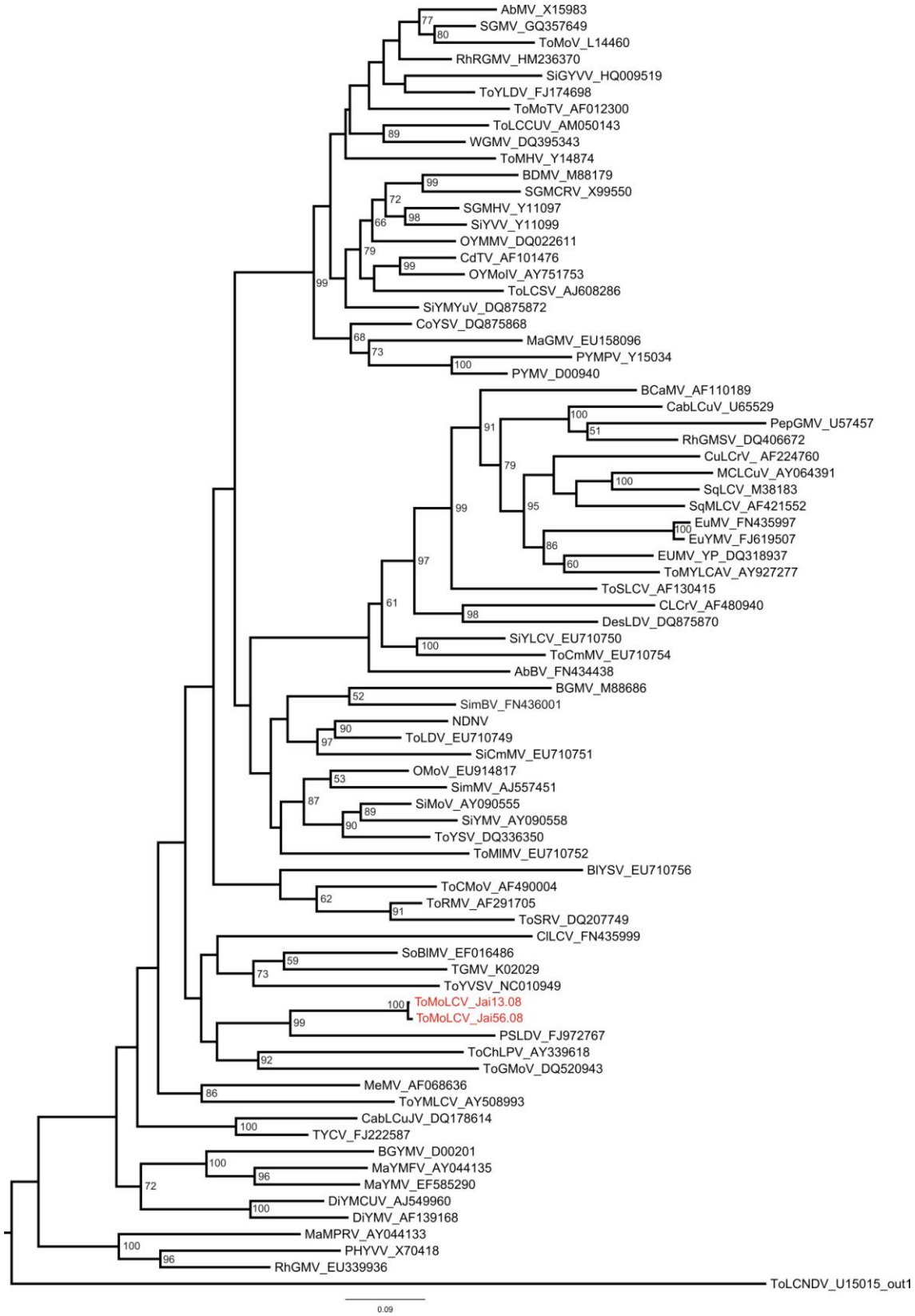
10
20
30
40
50
60
70
80
90
100
Percentage pairwise identity

Figure 2


AbMV_X15983
SGMV_GQ357649
ToMoV_L14460
RhRGMV_HM236370
SiGYVV_HQ009519
ToYLDV_FJ174698
ToMoTV_AF012300
ToLCCUV_AM050143
WGMV_DQ395343
ToMHV_Y14874
BDMV_M88179
SGMCRV_X99550
SGMHV_Y11097
SiYVV_Y11099
OYMMV_DQ022611
CdTV_AF101476
OYMoIV_AY751753
ToLCSV_AJ608286
SiYMYuV_DQ875872
CoYSV_DQ875868
MaGMV_EU158096
PYMPV_Y15034
PYMV_D00940
BCaMV_AF110189
CabLCuV_U65529
PepGMV_U57457
RhGMSV_DQ406672
CuLCrV_ AF224760
MCLCuV_AY064391
SqLCV_M38183
SqMLCV_AF421552
EuMV_FN435997
EuYMV_FJ619507
EUMV_YP_DQ318937
ToMYLCAV_AY927277
ToSLCV_AF130415
CLCrV_AF480940
DesLDV_DQ875870
SiYLCV_EU710750
ToCmMV_EU710754
AbBV_FN434438
BGMV_M88686
SimBV_FN436001
NDNV
ToLDV_EU710749
SiCmMV_EU710751
OMoV_EU914817
SimMV_AJ557451
SiMoV_AY090555
SiYMV_AY090558
ToYSV_DQ336350
ToMIMV_EU710752
BlYSV_EU710756
ToCMoV_AF490004
ToRMV_AF291705
ToSRV_DQ207749
CILCV_FN435999
SoBIMV_EF016486
TGMV_K02029
ToYVSV_NC010949
ToMoLCV_Jai13.08
ToMoLCV_Jai56.08
PSLDV_FJ972767
ToChLPV_AY339618
ToGMoV_DQ520943
MeMV_AF068636
ToYMLCV_AY508993
CabLCuJV_DQ178614
TYCV_FJ222587
BGYMV_D00201
MaYMFV_AY044135
MaYMV_EF585290
DiYMCUV_AJ549960
DiYMV_AF139168
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PHYVV_X70418
RhGMV_EU339936
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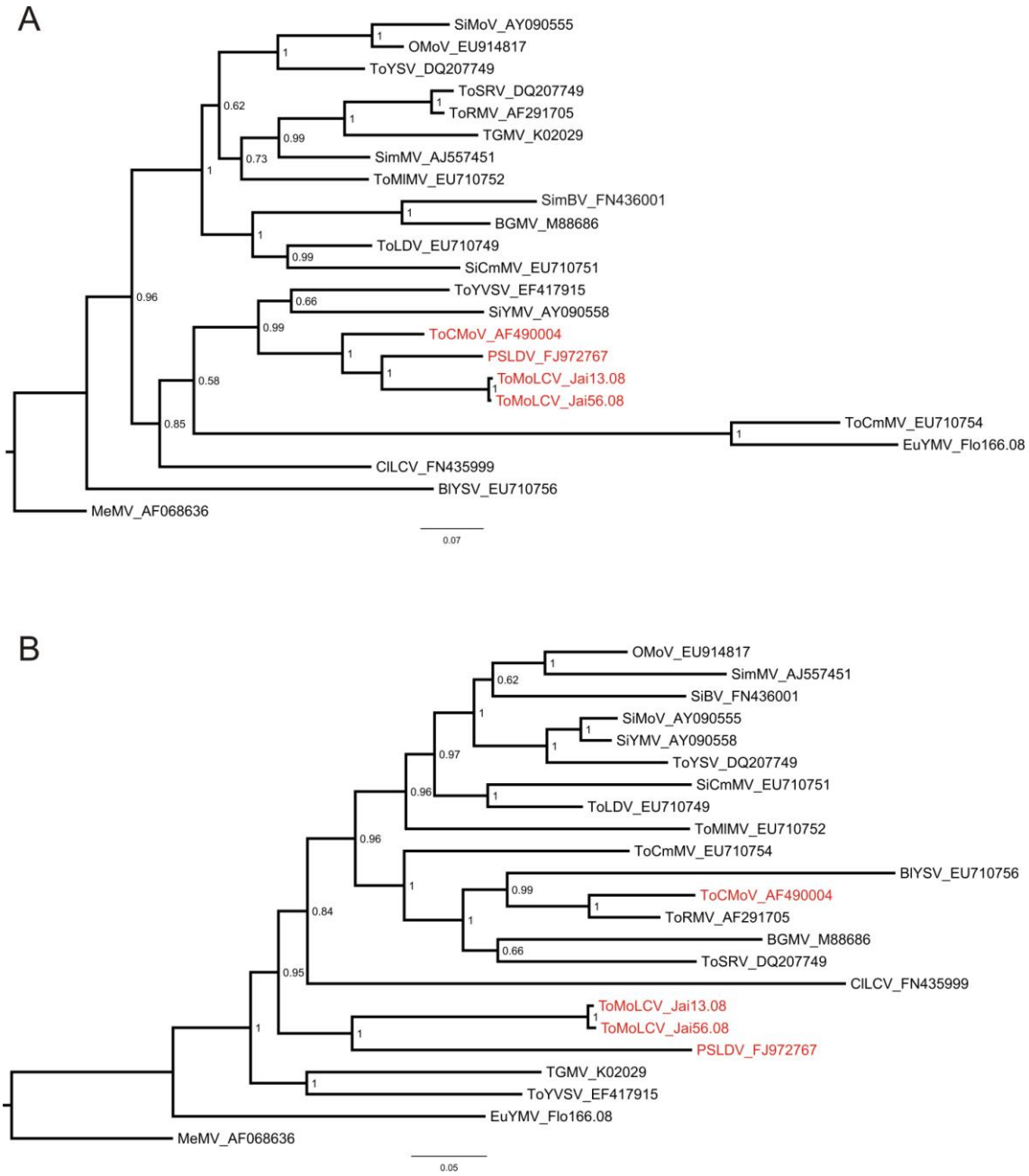
0.09

Figure 3


A
SiMoV_AY090555
OMoV_EU914817
ToYSV_DQ207749
ToSRV_DQ207749
ToRMV_AF291705
TGMV_K02029
SimMV_AJ557451
ToMlMV_EU710752
SimBV_FN436001
BGMV_M88686
ToLDV_EU710749
SiCmMV_EU710751
ToYVSV_EF417915
SiYMV_AY090558
ToCMoV_AF490004
PSLDV_FJ972767
ToMoLCV_Jai13.08
ToMoLCV_Jai56.08
ToCmMV_EU710754
EuYMV_Flo166.08
ClLCV_FN435999
BlYSV_EU710756
MeMV_AF068636
0.07
B
OMoV_EU914817
SimMV_AJ557451
SiBV_FN436001
SiMoV_AY090555
SiYMV_AY090558
ToYSV_DQ207749
SiCmMV_EU710751
ToLDV_EU710749
ToMlMV_EU710752
ToCmMV_EU710754
BlYSV_EU710756
ToCMoV_AF490004
ToRMV_AF291705
BGMV_M88686
ToSRV_DQ207749
ClLCV_FN435999
ToMoLCV_Jai13.08
ToMoLCV_Jai56.08
PSLDV_FJ972767
TGMV_K02029
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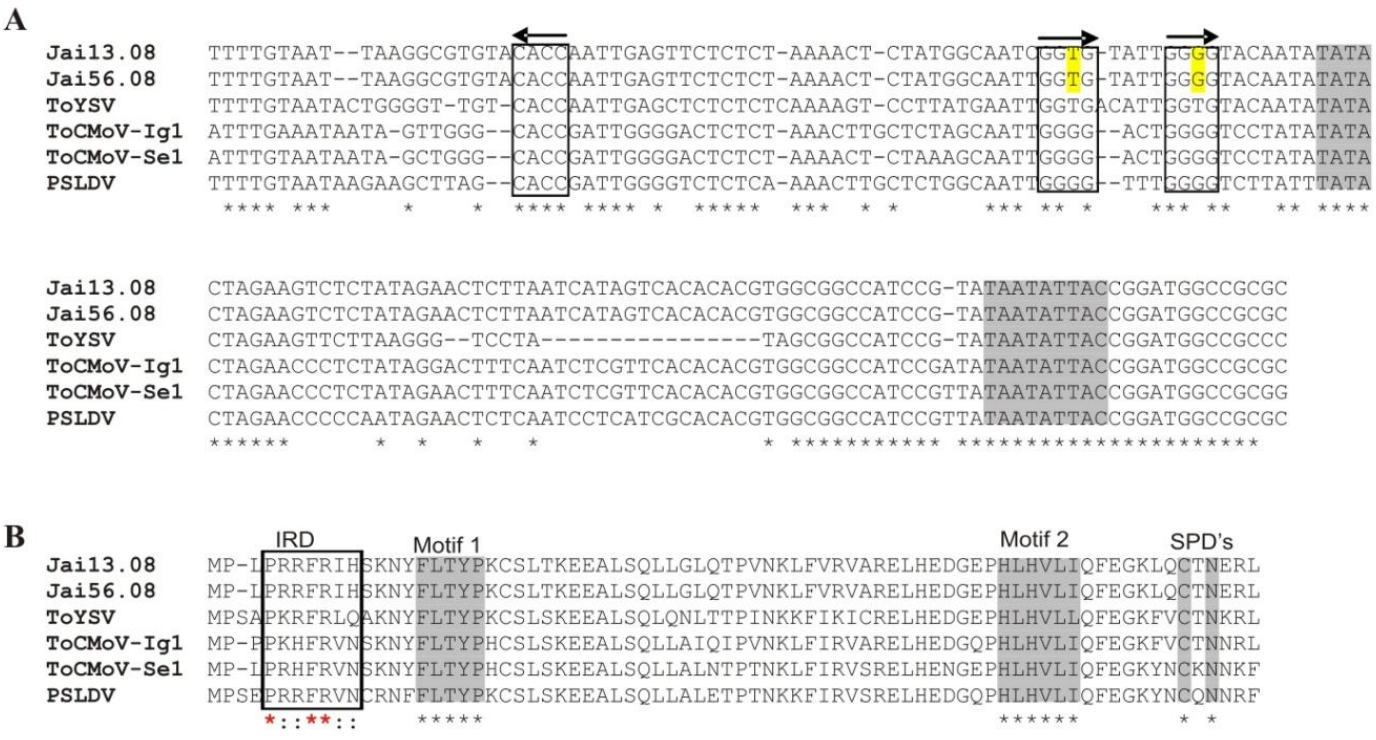
EuYMV_Flo166.08
MeMV_AF068636
0.05

Figure 4


A
Jai13.08
Jai56.08
ToYSV
ToCMoV-Ig1
ToCMoV-Se1
PSLDV
B
IRD
Motif 1
Motif 2
SPD's
Jai13.08
Jai56.08
ToYSV
ToCMoV-Ig1
ToCMoV-Se1
PSLDV

CAPÍTULO 2

BRAZILIAN BEGOMOVIRUS POPULATIONS ARE HIGHLY RECOMBINANT, RAPIDLY EVOLVING, AND STRUCTURED BASED ON GEOGRAPHICAL LOCATION

Rocha, C.S., Castillo-Urquiza, G.P., Lima, A.T.M., Silva, F.N., Xavier, C.A.D., Barros, D.R., Hora-Júnior, B.T., Beserra-Júnior, J.E.A., Malta, A.W.O., Martin, D.P., Varsani, A., Alfenas-Zerbini, P., Mizubuti, E.S.G., Zerbini, F.M. Brazilian begomovirus populations are highly recombinant, rapidly evolving, and structured based on geographical location. PloS Pathogens, *submitted*.

Brazilian begomovirus populations are highly recombinant, rapidly evolving, and structured based on geographical location

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Abstract

The incidence of begomoviruses has sharply increased in Brazil since the mid 1990's, after the introduction of the B biotype of the whitefly *Bemisia tabaci*. It is believed that the insect vector transferred indigenous viruses infecting wild and weed hosts to tomato. After a rapid evolutionary process, novel species adapted to the new host became prevalent in the field. The objective of this work was to determine the genetic structure of begomovirus populations infecting tomatoes and weeds in major tomato growing regions of southeastern Brazil. Tomato and weed samples were collected at six locations in the states of Rio de Janeiro and Minas Gerais, from May 2005 to May 2010. A total of 126 DNA-A and 58 DNA-B full-length begomovirus components were amplified using rolling-cicle amplification, cloned and sequenced. We detected the presence of nine begomoviruses in tomatoes and eight begomoviruses in the weed samples, with four viruses present in both tomatoes and weeds. All thirteen viruses had been previously described and are restricted to Brazil. Their sequence features indicate that they are typical New World, bipartite begomoviruses. Two viruses (SiYLCV and ToCmMV) cluster with non-Brazilian viruses in phylogenetic trees. Recombination analysis confirmed the mosaic-like nature of Brazilian begomoviruses. Many of the recombination events involving tomato viruses had weed viruses as putative parents. Viral populations were geographically structured and highly variable, with the weed-infecting BlYSV displaying higher genetic variability compared to the tomato-infecting ToCmMV, ToCMoV, ToSRV and ToYVSV.

Key words: geminivirus, genetic variability, phylogeny, recombination, evolution

Introduction

The *Geminiviridae* family is comprised of viruses with circular, single-stranded DNA genomes and particles structured as twinned imperfect icosahedra [1]. The family is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus*) based on the type of insect vector, host range, genome organization and phylogenetic relationships [2]. The genus *Begomovirus* includes viruses with one or two genomic components which infect dicotyledonous plants and are transmitted by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) [2]. Begomovirus diseases are a major limiting factor to crop yields in tropical and subtropical regions [3,4,5,6]. Tomatoes (*Solanum lycopersicum* L.) are seriously affected by begomoviruses on a worldwide scale [7,8,9,10]. In the Americas, diseases caused by begomoviruses have been causing significant losses in tomato production since the 1980's [11,12,13]. With the exception of *Tomato yellow leaf curl virus* (TYLCV), introduced into the Dominican Republic from tomato seedlings imported from Israel [14] and now spread from the USA to Venezuela [15], all begomoviruses isolated and characterized from tomato plants in countries from the American continent are native to this continent, and have never been found elsewhere (for example, [16,17,18,19]). In fact, the phylogeny of the *Geminiviridae* family (and not only of the tomato-infecting geminiviruses) is highly related to the geographical distribution of individual viruses [2,20]. Based on genome organization, genetic diversity and geographical distribution, begomoviruses have been divided into two groups: Old World (OW; Europe, Africa, Asia and Australia) and New World (NW; the Americas) [21].

In Brazil, eight begomoviruses, all restricted to the country, are currently recognized infecting tomatoes: *Tomato golden mosaic virus* (TGMV) [22], *Tomato rugose mosaic virus* (ToRMV) [23], *Tomato chlorotic mottle virus* (ToCMoV) [24],

Tomato yellow spot virus (ToYSV) [25], *Tomato yellow vein streak virus* (ToYVSV) [26], *Tomato severe rugose virus* (ToSRV) [27], *Tomato common mosaic virus* (ToCmMV) and *Tomato mild mosaic virus* (ToMlMV) [19]. At least nine additional viruses, not yet completely characterized, have also been described [13,28,29]. Some of these viruses are widely distributed throughout the country, while others are restricted to certain regions. For example, ToSRV has been reported in six different states covering more than 1,300,000 sq km [27,30,31,32,33]. On the other hand, ToYSV has been reported only in the state of Minas Gerais [25].

The most accepted hypothesis to explain the sudden emergence of tomato-infecting begomoviruses in Brazil assumes that indigenous viruses were transferred from wild hosts to tomatoes after the introduction and dissemination of the B biotype of *B. tabaci* in the early 1990's. The new vector allowed these indigenous viruses to reach the tomato, and a rapid evolutionary process gave rise to novel viruses with greater fitness to the new host [13,24,34]. The biological characterization of some of the tomato viruses (ToRMV, ToCMoV and ToYSV) confirmed that ubiquitous weeds such as *Nicandra physaloides*, *Solanum nigrum* and *Datura stramonium* are hosts [23,24,25]. Moreover, begomoviruses originally found in common weeds/wild plants, such as *Sida mottle virus* (SiMoV) and *Sida micrantha mosaic virus* (SimMV), have been found in tomatoes in natural infections [31,32].

As for all viruses, the evolution of geminiviruses is driven mainly by mutation and recombination [1,35]. Mutation frequencies or nucleotide substitution rates have been estimated for the begomoviruses TYLCV, *Tomato yellow leaf curl China virus* (TYLCCNV) and *East African cassava mosaic virus* (EACMV) and for the mastrevirus *Maize streak virus* (MSV), and were shown to be similar as those estimated for RNA viruses (~10^{-4} substitutions per site per year) [36,37,38,39]. The presence of several

begomoviruses in the field, all transmitted by the same vector, allows for the frequent occurrence of mixed infections in which two or more viruses are present simultaneously in the same plant. This increases the probability of recombination and/or pseudo-recombination (reassortment of genomic components) among the viral genomic components, which may give rise to better adapted viruses [24,29,40,41,42,43,44].

Management strategies of plant viral diseases are based on preventive measures, and are therefore more efficient when established on an epidemiological, population-based framework [45,46,47,48]. Although much has been done to characterize Brazilian tomato-infecting begomoviruses and also to prospect sources of natural resistance [49,50], population studies which might provide valuable clues on the potential of these viruses to evolve are still lacking.

We have carried out a large-scale study to determine the genetic structure of begomovirus populations associated with tomato crops and weeds in five important tomato-producing regions of southeastern Brazil. Our results confirm the presence of several begomoviruses in the field and demonstrate that viruses originally detected in tomatoes can also be found (eventually) in weeds, and vice-versa. The weed-infecting viruses are more genetically variable than the tomato-infecting viruses, and in either case the DNA-B component is more variable than the DNA-A. Phylogenetic analysis showed local division between the populations. Recombination analysis confirmed the previously suggested mosaic-like pattern of recombination among Brazilian begomoviruses, with weed viruses often identified as recombinant parents of tomato viruses but not vice-versa. Together, these results point to the rapidly evolving nature of tomato-infecting begomoviruses in Brazil, and stress the need for a management strategy that must include, but also go beyond, the deployment of resistant cultivars.

Methods

Sample collection and storage

Foliar samples with typical symptoms of begomovirus infection were collected in tomato fields located in the cities of Paty do Alferes, Rio de Janeiro (RJ) state (119 tomato samples collected in May 2005), Coimbra, Minas Gerais (MG) state (17 tomato and 43 weed samples collected in July 2007), Florestal, MG (50 tomato and 10 weed samples collected in July 2008), Jaíba, MG (117 tomato and 23 weed samples collected in July 2008), Carandaí, MG (23 tomato and 4 weed samples collected in July 2008), and Viçosa, MG (26 weed samples collected in May 2010). For each sample the following information was recorded: plant species (weed samples) or cultivar/hybrid (tomato samples), date of collection, GPS coordinates of the sampling location, and symptoms (description and digital image of the sample at the time of collection). Samples were either stored in an ultrafreezer (-80^{o}C) as desiccated foliar material, or press-dried and stored at room temperature as herbarium-like samples until analyzed.

DNA amplification and cloning

Total DNA was extracted as described by Doyle & Doyle [51], and full-length viral genomes were amplification by rolling-circle amplification [52]. RCA products were cleaved with *Apa* I, *Bam*H I, *Cla* I, *Eco*R I, *Hin*d III, *Kpn* I, *Pst* I, *Sac* I or *Spe* I, and ligated to the pBLUESCRIPT KS+ (Stratagene) plasmid vector, previously cleaved with the same enzyme. RCA products were also cleaved with *Hae* III to check for the presence of satellite-like DNA molecules. Viral inserts were completely sequenced at Macrogen, Inc. (Seoul, South Korea) by primer walking. All genome sequences were organized to begin at the nicking site in the invariant nonanucleotide at the origin of replication (5'TAATATT//AC3').

Sequence comparisons and phylogenetic analysis

Sequences were initially analysed with the BLAST*n* algorithm [53] to determine viral species with greatest identity. Specific sets of sequences were then prepared for each analysis that was performed. Besides the sequences determined in this study, reference sequences for each begomovirus from Brazil and selected begomoviruses from the Americas were retrieved from GenBank (Table 1).

Multiple nucleotide sequence alignments used for the recombination and phylogenetic analyses were prepared using the MUSCLE program [54]. Phylogenies for each data set were reconstructed using maximum likelyhood and Bayesian analyses.

Maximum likelihood (ML) trees were inferred using PAUP v. 4.0 [55]. The program ModelTest v. 3.7 [56] was used to provide the nucleotide substitution model with the best fit for each data set. A heuristic search was initiated with a neighbor-joining tree using the tree-bissection-reconnection (TBR) algorithm to optimize the ML tree. The robustness of each internal branch was estimated using a nonparametric test [57] with 1,000 bootstrap replications. The Nearest Neighbor Interchange (NNI) algorithm was used to optimize the bootstrap replications of the ML tree.

Additional phylogenetic trees were constructed using Bayesian inference performed with MrBayes v. 3.0b4 [58], with the model selected by MrModeltest v. 2.2 [59] in the Akaike Information Criterion (AIC). The analyses were carried out running 10,000,000 generations and excluding the 2,000,000 first generations as burn in. Trees were visualized using the TreeView program [60] and edited using CorelDraw X3.

Recombination analysis

Evidence for recombination was initially assessed using the Neighbor-Net method implemented in the program SplitsTree4 v. 4.10 [61]. Parental sequences and

recombinations breakpoints were then determined using the Recombination Detection Program (RDP) v. 3.44 [62]. The analyses were performed with default settings and a Bonferroni-corrected *p*-value cutoff of 0.05. Only the recombination events detected by more than four out of the seven methods implemented in RDP were considered to be reliable.

General descriptors of the genetic structure of viral populations

The partition of genetic variability and inferences about population structure were based on Wright's F fixation index [63]. Analysis of molecular variance (AMOVA) was performed to estimate the Φ_{ST} parameter, using the program Arlequin v. 3.11 [64] with the Kimura 2-parameter distance and estimating statistical significance by permutation analysis with 1,000 replications.

The program Structure v. 2.3.1 was used to examine the genetic structure among subpopulations and to identify individuals that were admixed or had migrated. One run of one to 10 subpopulations (K = 1 to 10) was performed using 1,000,000 Markov chain steps after a burn-in period of 100,000 steps, to select the number of clusters that best represents the population structure. We compared the likelihood estimate of each of the K values, based on the maximum log probability of data [ln $P(D)$], assayed to determine the number of K values present in the populations.

The main descriptors of molecular variability were estimated for each population/subpopulation, including the total number of segregating sites (s), total number of mutations (Eta), average number of nucleotide differences between sequences (k), nucleotide diversity (π), mutation frequencies, number of haplotypes (h), haplotype diversity (Hd), Watterson's estimate of the population mutation rate based on

the total number of segregating sites (θ-w) and on the total number of mutations (θ-Eta). Diversity indices were calculated using the DnaSP software v. 5.10 [65].

Parameterization of evolutionary mechanisms

Four types of neutrality tests were used to test the hypothesis of occurrence of selection in populations: Tajima's *D*, Fu and Li's *D** and *F**, and the test based on the number of non-synonymous (dN) and synonymous (dS) substitutions with the Pamilo-Bianchi-Li (PBL) model. These analyses were performed using DnaSP v. 5.10, with different sets of data considering the unique populations or subpopulations separated on the basis of geographical location.

Results

Viral detection and sequence comparisons

A total of 432 samples (326 tomato, 106 weeds) were analyzed, from which 219 were tentatively positive for the presence of a begomovirus based on the detection of a ~2,600 bp band after digestion of the RCA products with restriction enzymes (data not shown). From these samples, 126 full length DNA-A and 58 DNA-B components were cloned (Table 2; this table lists only the 132 samples from which full-length DNA componentes were cloned, not all 219 begomovirus-positive samples). BLAST analysis and pairwise sequence comparisons indicated the presence of thirteen begomoviruses, all previously described and, with one exception (ToYSV, detected in Argentina [66]), so far reported only in Brazil. An exhaustive analysis of the RCA amplification products after digestion with *Hae* III failed to identify bands which could correspond to satellite DNAs.

From the tomato samples, ToYVSV and ToCmMV were the most frequently cloned viruses in Paty do Alferes (23 and 19 out of 49 clones, respectively) (Table 3). ToCmMV was also found in Coimbra (12 out of 12 clones, the only virus cloned from tomato samples at this location) and Jaíba (one out of six clones) (Table 3). ToSRV was the predominantly cloned virus in Carandaí (18 out of 24 clones), followed by ToCMoV (6 out of 24 clones) (Table 3). Interestingly, these same two viruses were found in Florestal, but in opposite proportions: out of 24 clones, 19 were identified as ToCMoV and 5 as ToSRV (Table 3). Together, these four viruses (ToCmMV, ToCMoV, ToSRV and ToYVSV) accounted for 103 out of 115 (89.5%) clones obtained from tomato samples. Five other viruses were cloned at a much lower frequency (Table 3). ToMlMV and ToLDV were each found in two samples from Paty do Alferes. SimMV was found in three samples from Paty do Alferes and one sample from Jaíba. ToYSV was detected in two samples from Jaíba, which were also infected with *Tomato mottle leaf curl virus* (ToMoLCV).

Four tomato samples in Paty do Alferes had mixed infections: two samples with ToCmMV and ToYVSV, one sample with SimMV and ToCmMV and one sample with ToLDV and ToYVSV. One tomato sample collected in Carandaí had a mixed infection with ToCMoV and ToSRV. ToMoLCV was found in mixed infection with *Tomato yellow spot virus* (ToYSV) in Jaíba (Table 2).

The weed samples were infected with *Blainvillea yellow spot virus* (BlYSV, cloned from one *Blainvillea rhomboidea* sample from Coimbra, and nine *B. rhomboidea* and one *Physalis* sp. sample from Viçosa), *Euphorbia yellow mosaic virus* (EuYMV, one *Euphorbia* sp. sample from Florestal), *Sida common mosaic virus* (SiCmMV, two *Sida micrantha* samples from Coimbra), *Sida micrantha mosaic virus* (SimMV, one *Sida* sp. sample from Viçosa) and *Sida yellow leaf curl virus* (SiYLCV, cloned from

two *Sida rhombifolia* samples from Coimbra) (Table 2). Also, ToCMoV was cloned from one *Sida* sp. sample from Florestal, ToMlMV was cloned from three *Sida urens* samples from Viçosa, and ToSRV was cloned from two *Sida* sp. samples, one from Carandaí and one from Viçosa (Table 2).

Phylogenetic analysis

Phylogenetic relationships were analyzed based on complete DNA-A nucleotide sequences. A ML tree was constructed including sequences of one isolate from each begomovirus obtained in this study, plus reference sequences from all Brazilian begomoviruses available in GenBank, plus representative begomoviruses from the Americas (Figure 1). This analysis indicated that the Brazilian begomoviruses form seven clusters (Figure 1, clusters I to VII). Clusters I to III contain mostly viruses from cultivated hosts, and clusters IV to VII contain mostly viruses from wild hosts.

Clusters I to III contain nine viruses, and isolates of four of them (ToCMoV, ToMoLCV, ToSRV and ToYVSV) were obtained in this work (Figure 1; Table 2). The viruses in this clusters seem to be well adapted to cultivated plants, as ToCMoV, ToSRV and ToYVSV were three of the most frequently detected here (Table 3) and in other studies [27], and *Soybean blistering mosaic virus* (SoBlMV) is widespread in soybean fields in northwestern Argentina [66]. ToMoLCV, although detected in a small number of samples, seems to be widespread in tomato fields in the Brazilian northeast [27].

The opposite is observed in cluster IV, which contains mostly viruses from wild hosts. The fact that ToLDV, ToMlMV and ToYSV are included in this cluster (Figure 1), coupled with the fact that these three viruses are detected in tomatoes at a low frequency, suggests that they are actually "wild" viruses which are poorly adapted to

infect tomatoes. ToMlMV was detected in three *Sida urens* plants (Table 2), and the analysis of a larger number of samples of this host could indicate whether it is the "natural" host of this virus. All isolates of ToLDV and ToYSV obtained so far were recovered from tomato plants, and therefore their "natural" hosts remain unknown.

Cluster V has a more diverse composition, including viruses which so far have been detected only in wild hosts (such as BlYSV) and viruses which are widespread in cultivated hosts (such as *Bean golden mosaic virus*, BGMV).

Clusters VI and VII, which contain *Abutilon Brazil virus* (AbMV), EuYMV, SiYLCV and ToCmMV, are part of a monophyletic branch containing viruses from several countries in Central and North America (Figure 1; Table 1). Thus, these four viruses are members of a lineage of New World begomoviruses which is distinct from all other Brazilian begomoviruses. Interestingly, ToCmMV is one of the viruses which were most frequently found in our tomato samples (Table 3).

Bayesian inference was employed to reconstruct the phylogenies of three viral populations for which location-based subdivision was suspected to occur: ToCmMV (including isolates from Coimbra, Jaíba and Paty do Alferes), ToCMoV (including isolates from Carandaí and Florestal, plus three previously sequenced isolates available in GenBank) and ToSRV (isolates from Carandaí, Florestal, Jaíba and Viçosa, plus five previously sequenced isolates available in GenBank) (Figure 2). The results are consistent with the subdivision hypothesis for all three viruses, as the isolates were clearly split according to geographical location (Figure 2).

Recombination analysis

The occurrence of recombination within the populations of ToCmMV, ToCMoV, ToSRV and ToYVSV was initially tested by neighbor-net/reticulate network

analysis. The results did not indicate any significant evidence of recombination within the ToCmMV and ToYVSV populations (data not shown). However, the populations of ToCMoV and ToSRV were found to be potentially recombinant (Figure 3).

Strong evidence of recombination was also obtained using neighbor net analysis with a data set including all Brazilian begomoviruses plus one isolate from each begomovirus obtained in this study (Figure 4). The strongest signals were obtained for ToCMoV, ToRMV and ToSRV, which have previously been suggested to be recombinants [24], and for AbBV, EuYMV, SiYLCV and ToCmMV.

To further investigate these putative recombination signals, data sets including either all Brazilian begomoviruses or all begomoviruses from the Americas, both including one isolate from each begomovirus obtained in this study, were analyzed using the RDP3 package (Tables 4 and 5). This analysis identified several unique recombination events. Most recombination events have breakpoints located within the Rep gene and the CR, consistent with previous studies which identified these regions as recombination hot spots [67,68,69]. Interestingly, analysis based on both data sets indicated that a large number of recombination events involving tomato viruses have viruses from wild/weed hosts as parents, but not vice-versa (the vast majority of the recombination events involving viruses from wild/weed hosts have other viruses from wild/weed hosts as parents). Although parent identification is not always reliable, these results are an additional line of evidence indicating that tomato viruses have evolved/emerged from viruses infecting wild hosts.

Recombination analysis with the data set including all viruses from the Americas identified *Cabbage leaf curl virus* (CabLCuV) as the minor parent of a recombination event involving AbBV, SiYLCV and ToCmMV, and *Tomato yellow margin leaf curl virus* (ToYMLCV) as the major parent in a recombination event involving EuYMV

(Table 5). To determine whether these two recombination events were associated with major changes in tree topology, Bayesian inference was used to reconstruct the phylogenies based on sequence alignments corresponding to either side of the putative recombination break-point. This analysis supported the recombination event for AbBV, ToCmMV and SiYLCV: in the "recombinant" tree (Figure 5A), the three viruses clustered with Central/North American viruses, while in the "non-recombinant" tree (Figure 5B) they clustered with Brazilian viruses. However, the same analysis did not support the recombination event for EuYMV (data not shown).

Genetic structure of BlYSV, ToCmMV, ToCMoV, ToSRV and ToYVSV populations

The 39 ToYVSV sequences (26 DNA-A and 13 DNA-B), 38 ToCmMV sequences (22 DNA-A and 16 DNA-B), 28 ToCMoV sequences (22 DNA-A and six DNA-B), 34 ToSRV sequences (27 DNA-A and seven DNA-B) and 14 BlYSV sequences (seven DNA-A and seven DNA-B) were used to characterize these populations (Table 6).

DNA-B sequences were more diverse than DNA-A sequences for all five populations. For example, the average number of nucleotide differences between the seven BlYSV DNA-A sequences was 65.619, while for the seven DNA-B sequences it was 121.905 (Table 6). Comparing the populations of each virus, BlYSV has a much higher degree of genetic variability compared to the tomato viruses. For example, values for nucleotide diversity (DNA-A) are 0.02466 for BlYSV, 0.0143 for ToCmMV, 0.0071 for ToCMoV, 0.0102 for ToSRV and 0.0021 for ToYVSV (Table 6). ToYVSV is the least diverse virus, with lower values for every descriptor (Table 6).

Mutation frequencies were determined for the five populations (Table 6) and, except for the populations from Paty do Alferes, were found to be higher than those

determined for other begomoviruses. The ToYVSV population, comprised entirely of isolates from Paty do Alferes, had a mutation frequency in the order of 10^{-4} for both the DNA-A and the DNA-B (Table 6). Similar values were found for the ToCmMV subpopulation from Paty do Alferes. These values are quite similar to those determined for other ssDNA viruses [38,70,71]. However, all other populations had mutation frequencies in the order of 10^{-3} (Table 6), which are equivalent to the frequencies determined for RNA viruses [72]. Strikingly, the population of the weed-infecting BlYSV has an even higher frequency, in the order of 10^{-2} (Table 6). In order to verify if mutations are evenly distributed throughout the genome, we determined the mutation frequencies for each coding region (CP, Rep, Trap and Ren) as well as the non-coding sequences (IR). In most cases the highest mutation frequencies were observed in intergenic region (Figure 6). For the ToYVSV population, mutation frequencies were similar throughout the genome (Figure 6).

Two of the five populations analyzed (ToCmMV and ToSRV) included enough DNA-A and DNA-B sequences from isolates collected at two locations to allow for a segregated analysis. For ToCmMV, both the DNA-A and DNA-B sequences could be divided into Coimbra and Paty do Alferes groups. The analysis indicated that the DNA-A and DNA-B sequences from Coimbra have a much greater genetic variability than the Paty do Alferes sequences (Table 6). In fact, the values obtained for the Paty do Alferes group are equivalent to, and in many cases even lower than, those obtained for the ToYVSV population (which is comprised entirely of isolates from this same location). For ToSRV, DNA-A sequences could be divided into Carandaí and Florestal groups. The Florestal sequences are more diverse than the Carandaí ones, although in this case the result is not as clear due to the discrepancy in the size of each group (19 sequences from Carandaí and only five from Florestal) (Table 6).

Differences in genetic variability between these groups of sequences indicate the existence of two subpopulations of ToCmMV (Coimbra and Paty do Alferes) and ToSRV (Carandaí and Florestal). To verify the subdivision of these populations and estimate the variability within and among subpopulations, AMOVA, Fst and Nst tests were performed. Analyses of population differentiation using the Fst and Nst statistics for nucleotide diversity confirmed population subdivision for ToCmMV and ToSRV, and also for ToCMoV (Table 7). The AMOVA results indicated that 43.1% of the genetic variability is found among subpopulations and 56.9% within subpopulations for ToCMoV. For ToCmMV, 72.4% of the variability is found between, and 27.6% within, subpopulations. For ToSRV, 72.2% of the variability is found between, and 27.8% within, subpopulations.

A cluster-based method (Structure) was used to identify individuals that were admixed or had migrated in tomato-infecting begomovirus populations, as well as to infer the cluster number (*K*) for each population. ToCMoV, ToCMoV, ToSRV and ToYVSV populations shown K = 3, K = 2, K = 2 and K = 1, respectively (Figure 7), results which are consistent with Bayesian tree topology (Figure 2). Two ToCmMV isolates from Coimbra (Coi21 and Coi22) appear as immigrants in Paty do Alferes (Figure 7B). ToSRV isolates from Viçosa and Jaíba group together with the Carandaí subpopulation (Figure 7C).

Neutrality tests were used to assess what kind of selection is acting on the coding sequences of the BlYSV, ToCmMV, ToCMoV, ToSRV and ToYVSV populations. Statistically significant values were obtained for different ORFs depending on the population being analyzed (Table 8). The absolute majority of these statistically significant values were negative, indicating the occurrence of negative (purifying) selection or a recently population expansion. For example, statistically supported

negative values were obtained for Tajima's *D*, Fu and Li's *D** and Fu and Li's *F** tests for the Rep and CP ORFs, and for Tajima's *D* test for the MP ORF in the ToYVSV population (Table 8). The ToSRV subpopulation from Carandaí presented statistically supported, negatives values for Tajima's *D*, Fu and Li's *D** and Fu and Li's *F** tests for the CP, Rep, Trap and Ren ORFs (Table 8). The only exception was the Ren ORF in the ToCmMV subpopulation from Coimbra, which displayed a statistically supported positive value in Fu and Li's *D** test (Table 8), indicative of positive (diversifying) selection.

Most of the ORFs in all populations presented dN/dS values <1, indicative of negative (purifying) selection. Only ToCMoV Trap, ToSRV (Carandaí) NSP and MP and ToYVSV Trap and Ren displayed values >1, indicative of positive (diversifying) selection (Table 8).

Discussion

Begomoviruses became established in tomato crops in Brazil after the introduction of the B biotype of the whitefly vector in the mid 1990's, and a large number of viral species have since been described and characterized [13,19,23,24,25,44]. These viral species have never been detected anywhere else, which strongly indicates that they have evolved from indigenous populations. Begomoviruses are notoriously recombination-prone [42,67,69,73], and have mutation frequencies which are comparable to those of RNA viruses [38,74]. The results described here, based on sequences of more than 200 viral genomes cloned from samples collected over a five-year period, support the hypothesis that tomato-infecting begomoviruses from Brazil evolved from indigenous populations in a process driven by high mutation frequencies and widespread recombination. This process culminated with the emergence

of a number of viruses which are highly adapted to infect tomatoes (*eg*, ToCMoV, ToCmMV, ToSRV), plus a number of viruses which are less well adapted (*eg*, ToYSV, ToLDV, ToMlMV).

The first step of our analysis was to investigate which begomovirus species were present and prevalent in each region analyzed. Minas Gerais state is over 500,000 sq km large, and some of the sampling locations within the state, such as Coimbra and Jaíba, are as far away from each other as 900 km (Paty do Alferes and Jaíba are over 1,200 km away from each other). Four viruses (ToCmMV, ToCMoV, ToSRV and ToYVSV) were present in more than 95% of the samples. Interestingly, the prevalence of each one of these viruses at each location varied widely. For example, ToCmMV was the only virus detected in Coimbra and was one of the two prevalent viruses in Paty do Alferes, but was not found at the other locations. ToYVSV was the other prevalent virus in Paty do Alferes, but was not found anywhere else. ToCMoV and ToSRV were the prevalent viruses in Carandaí and Florestal, but at opposite proportions at each location. Thus, it is evident that different viruses predominate at distinct geographical locations. This could reflect the presence of distinct natural reservoirs, of distinct vector populations with differential transmission efficiencies for each virus, different types of tomato cultivars/hybrids, or could be the result of introductions. This latter possibility seems particular likely to explain the results at Paty do Alferes, considering the extremely low degree of genetic variability of the two populations analyzed at that location (ToCmMV and ToYVSV). The combined observations that the ToCmMV population from Coimbra has a higher degree of variability, that two isolates from Coimbra were identified as phylogenetically closer to the Paty do Alferes population, and that no begomovirus-positive samples were found in a survey conducted at Paty do Alferes in 1999 [28], all provide additional support for the hypothesis of a recent introduction of

begomoviruses at Paty do Alferes. Obviously, once a given virus emerges and becomes established in a particular location, there is no particular reason why it should stay restricted to that location. Tomatoes are often transported over long distances in Brazil, with little or no interstate sanitary inspections (and of course no "within-state" inspections). It will thus be interesting to continue monitoring the prevalence of these viruses at each location over the years. In case the location-based segregation of virus species reported here is maintained, factors such as natural reservoirs or local vector populations must be contributing and should be investigated.

Recombination is a common event for begomoviruses [67,68], and appears to contribute greatly to their genetic diversification, increasing their evolutionary potential and local adaptation [43,67,75,76]. As reported for other Brazilian begomoviruses [24,29], recombination was found to be widespread among the viruses detected in this work. Most recombination events occurred at the N-terminal region of the *Rep* gene, the common region and the intergenic region between the *CP* and *Ren* genes (Tables 4 and 5), all of which have been reported to be recombination hot spots [69]. Interestingly, most recombination events detected in tomato-infecting begomoviruses had viruses from wild or weed hosts identified as putative parents, while the opposite was not true. Another interesting observation was that three of the four Brazilian viruses which were found to cluster with non-Brazilian viruses in a phylogenetic tree (ToCmMV and SiYLCV, detected in this work, plus AbBV) were identified as recombinants having CabLCuV as a minor parent (Table 5). This was reinforced by phylogenetic analysis based on the putative recombinant and non-recombinant regions (Figure 5). SiYLCV and AbBV are "wild" viruses, but ToCmMV is one of the four prevalent tomato viruses. Therefore, a recombination event apparently involving viruses from distinct

phylogenetic lineages and which do not have tomato as a host, gave rise to a novel virus which is well adapted to tomatoes.

The relevance of recombination notwithstanding, virus evolution (and the consequent emergence of novel viruses) is primarily dependent on mutations. On this regard, the ssDNA begomoviruses display a high frequency of mutation [38] or substitution rates [36,37], equivalent to those found for RNA viruses. The mutation frequencies determined for the tomato-infecting viruses ToCMoV, ToCmMV, ToSRV and ToYVSV are consistent with those previously determined for TLCCNV. However, the weed-infecting BlYSV has a mutation frequency which is one order of magnitude higher. When mutation frequencies were determined for different regions of the viral genome, the intergenic (common) region was identified as the most rapidly evolving region (Figure 6). Although this could apparently be explained by the fact that this is a non-coding region, it is also the region which includes the viral origin of replication, the high affinity Rep binding sites, and the promoters for the CP and Rep genes. Thus, our preferred explanation is that it reflects the genetic variability of the host. We have recently observed similar mutation frequencies in a population of *Macroptilium yellow spot virus* (MaYSV), a novel species described infecting the ubiquitous weed *Macroptilium lathyroides* in northeastern Brazil (S.J.C. Silva and F.M. Zerbini, *unpublished results*). Further studies analyzing mutation frequencies of begomovirus populations (ideally of the same virus) infecting "domestic" and "wild" hosts must be carried out to verify this hypothesis. A good candidate for such study is BGMV, which infects both the cultivated ("domestic") host *Phaseolus vulgaris* and the "wild" host *M. lathyroides*, as well as hosts with an intermediate degree of domestication such as *Phaseolus lunatus*.

New World begomoviruses have a bipartite genome, with a clear "division of labour" between the two DNA components: the DNA-A encodes all replication-related functions as well as the coat protein (which is also the only viral protein necessary for whitefly transmission), while the DNA-B encodes the movement-related functions. The DNA-B of all the viruses analyzed in our work was more variable than the DNA-A. For example, BlYSV seven DNA-A sequences have a total number of mutations equal to 211, while the corresponding value for the same number of DNA-B sequences is 326 (Table 6). This fact could be attributed to the inespecific nature of the movement functions carried out by the DNA-B encoded proteins, which would thus be more permissive to changes. An alternative explanation would be that the DNA-B had a separate origin from the DNA-A, possibly as a satellite that was captured by a parent monopartite virus and later evolved to become an integral part of the genome, as suggested by Nawaz-Ul-Rehman and Fauquet [21] and Briddon et al. [77].

ToCmMV, ToCMoV and ToSRV populations were split according to geographical location, confirming the existence of population subdivision already hinted by phylogenetic analysis. Neutrality tests were performed to assess what kind of selection is acting on the coding sequences of each begomovirus populations. With the exception of ToCmMV in Coimbra, all ORFs with significant values for at least one neutrality test displayed negative values, indicating either purifying selection or a recently population expansion. However, in protein-coding sequences, selection pressures can be more accurately identified by the ratio of nonsynonymous (dN; amino acid-replacing) and synonymous (dS; silent) substitution rates. The dN/dS is expected to exceed unity when natural selection promotes changes in the protein sequence (diversifying selection), whereas a ratio lower than unity is expected if natural selection suppress protein changes (purifying selection) [78]. Only the MP and NSP ORFs of

ToSRV and the Trap and Ren ORFs of ToYVSV displayed dN/dS values greater than 1, indicating diversifying selection (Table 8). All the other ORFs of the five populations analyzed displayed dN/dS values lower than 1, indicating purifying selection. Purifying selection and population expansion were concluded to be the major evolutionary forces acting on TLCV in *Eupatorium makinoi* [79] and on *Tomato spotted wilt virus* (TSWV) in peanut [48].

Consistent with our hypothesis that the insect vector transferred indigenous viruses infecting wild and weed hosts to tomato, viruses of weed hosts were found in tomato plants (*eg*, SimMV isolates BR:Pda8:05, BR:Pda37:05 and BR:Pda43:05) and vice-versa (*eg*, ToSRV isolates BR:Car228:08 and BR:Vic25:10, both found in *Sida* sp.) indicating that at least some of the "wild" viruses can infect tomatoes, even if at a low frequency, and that the "domestic" begomoviruses which are well adapted to tomato crops can reinfect weeds under field conditions. Interestingly, ToMlMV, a virus which clusters with weed viruses in both the ML and the species tree (Figure 1) was found in both tomato (at a very low frequency) and weeds (Table 1). Based on these results, we suggest that this begomovirus is actually a "wild" virus which can eventually infect tomatoes. The same is probably true for ToYSV.

Understanding the variability dynamics of virus populations in plants is necessary in order to understand how these populations evolve, as well as the implications for the durability of control measures. We have assessed the genetic structure and variability of Brazilian begomovirus populations, and found that the viruses comprising these populations are recombinant, rapidly evolving agents which are well adapted to tomatoes but can also reinfect weeds. It is thus evident that, as much as resistance-based approaches must be actively sought in order to allow the economically feasible and environmentally friendly control of these viruses, this

strategy by itself will most likely fail in the long term. Complementary control measures that do not place additional selection pressure upon viral populations must be concurrently employed.

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Table 1. Begomoviruses used in pairwise sequence comparisons, phylogenetic and recombination analyses.

Virus	Acronym	GenBank access # (DNA-A)
From Brazil		
Abutilon Brazil virus	AbBV	FN434438
Bean golden mosaic virus	BGMV	M88686
Cleome leaf crumple virus	ClLCrV	FN35999
Euphorbia yellow mosaic virus	EuYMV	FJ619507
Nicandra deforming necrosis virus	NDNV	n.a.
Okra mottle virus	OMoV	EU914817
Passionfruit severe leaf distortion virus	PSLDV	FJ972767
Sida mosaic Brazil virus	SiBV	FN436001
Sida micrantha mosaic virus	SimMV	AJ557451
Sida mottle virus	SiMoV	AY090555
Sida yellow mosaic virus	SiYMV	AY090558
Soybean blistering mosaic virus	SoBlMV	EF016486
Tomato chlorotic mottle virus	ToCMoV	AF490004; AY090557; DQ336353
Tomato golden mosaic virus	TGMV	K02029
Tomato rugose mosaic virus	ToRMV	AF291705
Tomato severe rugose virus	ToSRV	DQ207749; EU086569; HQ606467; FJ824808; AY029750
Tomato yellow spot virus	ToYSV	DQ336350
Tomato yellow vein streak virus	ToYVSV	EF417915
From other countries in the Americas		
Abutilon mosaic virus	AbMV	X15983
Bean calico mosaic virus	BCaMV	AF110189
Bean dwarf mosaic virus	BDMV	M88179
Bean golden yellow mosaic virus	BGYMV	D00201
Cabbage leaf curl virus	CabLCuV	U65529
Chino del tomate virus	CdTV	AF101476
Cotton leaf curl virus	CLCrV	AF480940
Corchorus yellow spot virus	CoYSV	DQ875868
Curcubit leaf crumple virus	CuLCrV	AF224760
Desmodium leaf distortion virus	DesLDV	DQ875870
Dicliptera yellow mosaic virus	DiYMV	AF139168
Dicliptera yellow mosaic Cuba virus	DiYMCUV	AJ549960
Euphobia yellow mosaic virus	EuYMV	FN435997
Euphobia mosaic virus	EuMV	DQ318937
Macroptillium golden mosaic virus	MaGMV	EU158096
Macroptillium mosaic Puerto Rico virus	MaMPRV	AY044133
Macroptilium yellow mosaic Florida virus	MaYMFV	AY044135
Macroptilium yellow mosaic virus	MaYMV	EF585290
Melon chlorotic leaf curl virus	MCLCuV	AY064391

Merremia mosaic virus	MeMV	AF068636
Okra yellow mosaic Mexico virus	OYMMV	DQ022611
Okra yellow mottle Iguala virus	OYMoIV	AY751753
Pepper golden mosaic virus	PepGMV	U57457
Pepper huasteco yellow vein virus	PHYVV	X70418
Potato yellow mosaic Panama virus	PYMPV	Y15034
Potato yellow mosaic virus	PYMV	D00940
Rhyncosia golden mosaic Sinaloa virus	RhGMSV	DQ406672
Rhyncosia golden mosaic virus	RhGMV	EU339936
Rhyncosia rugose golden mosaic virus	RhRGMV	HM236370
Sida golden mosaic Costa Rica virus	SGMCRV	X99550
Sida golden mosaic Honduras virus	SGMHV	Y11097
Sida golden mosaic virus	SGMV	GQ357649
Sida golden yellow vein virus	SiGYVV	HQ009519
Sida yellow mosaic Yucatan virus	SiYMYuV	DQ875872
Sida yellow vein virus	SiYVV	Y11099
Squash leaf curl virus	SqLCV	M38183
Squash mild leaf curl virus	SqMLCV	AF421552
Tomato Chino La Paz virus	ToChLPV	AY339618
Tomato golden motlle virus	ToGMoV	DQ520943
Tobacco leaf curl Cuba virus	TLCCUV	AM050143
Tomato leaf curl Sinaloa virus	ToLCSV	AJ608286
Tomato mosaic Havana virus	ToMHV	Y14874
Tomato mottle Taino virus	ToMoTV	AF012300
Tomato mottle virus	ToMoV	L14460
Tomato mild yellow leaf curl Aragua virus	ToMYLCAV	AY927277
Tomato yellow leaf distortion virus	ToYLDV	FJ174698
Tomato yellow margin leaf curl virus	ToYMLCV	AY508998
Tomato severe leaf curl virus	ToSLCV	AF130415
Tobacco yellow crinkle virus	TYCV	FJ222587
Wissadula golden mosaic virus	WGMV	DQ395343
Outgroup		
Tomato leaf curl New Delhi virus	ToLCNDV	U15015

Table 2. Begomovirus clones and corresponding isolates obtained from tomato and weed samples collected in Minas Gerais and Rio de Janeiro states, Brazil, from May 2005 to July 2010.

Sample code	Location	Date	Host	Enzyme[1]		Isolate	GenBank access #	
				DNA-A	DNA-B		DNA-A	DNA-B
Blainvillea yellow spot virus (BlYSV)[2]								
S57	Coimbra	July, 2007	*Blainvillea rhomboidea*	*Hin*d III	*Hin*d III	BR:Coi25:07	EU710756	
HV4	Viçosa	May, 2010	*Blainvillea rhomboidea*	*Apa* I		BR:Vic04.1:10		
				Apa I		BR:Vic04.2:10		
HV7	Viçosa	May, 2010	*Blainvillea rhomboidea*		*Sac* I	BR:Vic07:10		
HV8	Viçosa	May, 2010	*Blainvillea rhomboidea*	*Apa* I	*Sac* I	BR:Vic08:10		
HV9	Viçosa	May, 2010	*Blainvillea rhomboidea*	*Apa* I		BR:Vic09:10		
HV11	Viçosa	May, 2010	*Blainvillea rhomboidea*	*Apa* I	*Sac* I	BR:Vic11:10		
HV13	Viçosa	May, 2010	*Blainvillea rhomboidea*	*Apa* I		BR:Vic13:10		
HV18	Viçosa	May, 2010	*Blainvillea rhomboidea*		*Sac* I	BR:Vic18:10		
HV20	Viçosa	May, 2010	*Physalis* sp.	*Apa* I		BR:Vic20:10		
HV21	Viçosa	May, 2010	*Blainvillea rhomboidea*		*Sac* I	BR:Vic21:10		
HV26	Viçosa	May, 2010	*Blainvillea rhomboidea*		*Sac* I	BR:Vic26s:10		
					Cla I	BR:Vic26c:10		
Euphorbia yellow mosaic virus (EuYMV)								
DV166	Florestal	July, 2008	*Euphorbia* sp.	*Apa* I		BR:Flo166:08		
Sida common mosaic virus (SiCmMV)								
M7	Coimbra	July, 2007	*Sida micrantha*	*Bam*H I		BR:Coi4:07		
M8	Coimbra	July, 2007	*Sida micrantha*	*Bam*H I		BR:Coi5:07		
Sida micrantha mosaic virus (SimMV)								
B1d	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Pst* I		BR:Pda8:05		
C18a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda37:05		
D2a[3]	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda43:05		
DV43	Jaíba	July, 2008	*Solanum lycopersicum*		*Bam*H I	BR:Jai43:08		

DV175	Jaíba	July, 2008	*Sida* sp.		*Bam*H I	BR:Jai175:08
HV24	Viçosa	May, 2010	*Sida* sp.		*Bam*H I	BR:Vic24:10
Sida yellow leaf curl virus (SiYLCV)						
S2	Coimbra	July, 2007	*Sida rhombifolia*	*Bam*H I		BR:Coi1:07
				*Bam*H I		BR:Coi2:07
S4	Coimbra	July, 2007	*Sida rhombifolia*	*Bam*H I		BR:Coi3:07
Tomato chlorotic mottle virus (ToCMoV)						
DV153	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo153:08
DV154	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo154:08
DV170	Florestal	July, 2008	*Sida* sp.	*Apa* I		BR:Flo170a:08
				Sac I		BR:Flo170s:08
DV171	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo171:08
DV180	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo180:08
DV181	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo181:08
DV182	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo182:08
DV184	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo184:08
DV186	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo186:08
DV187	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo187:08
DV188	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo188:08
DV191	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo191:08
DV192	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo192:08
DV193	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo193:08
DV194	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I	*Bam*H I	BR:Flo194:08
DV195	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo195:08
DV197	Florestal	July, 2008	*Solanum lycopersicum*		*Apa* I	BR:Flo197:08
DV209	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo209:08
DV210	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo210:08
DV211	Florestal	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Flo211:08

DV217	Carandaí	July, 2008	*Solanum lycopersicum*		*Kpn* I	BR:Car217.1:08
DV219	Carandaí	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Car219.3:08
DV231	Carandaí	July, 2008	*Solanum lycopersicum*		*Bam*H I	BR:Car231:08
DV234	Carandaí	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Car234.3:08
DV236	Carandaí	July, 2008	*Solanum lycopersicum*		*Bam*H I	BR:Car236.2:08
DV238	Carandaí	July, 2008	*Solanum lycopersicum*		*Bam*H I	BR:Car238.1:08
Tomato common mosaic virus (ToCmMV)						
B1d	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Hind* III	BR:Pda8:05
B1f	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda9:05
B2a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda10:05
B2g	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Hind* III	BR:Pda20:05
B4f	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda19:05
C9a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda25:05
C10a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda27:05
C13a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda31:05
C15a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda32:05
C19a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda39:05
C25a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda40:05
D1a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Hin*d III	BR:Pda42:05
D2a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda44:05
E2a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda48:05
E3b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda51:05
E4a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Hin*d III	BR:Pda53:05
E6a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda55:05
E8b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Kpn* I		BR:Pda56:05
E11b	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda57:05
T25	Coimbra	July, 2007	*Solanum lycopersicum*		*Cla* I	BR:Coi6:07
T27	Coimbra	July, 2007	*Solanum lycopersicum*	*Pst* I		BR:Coi7:07

T28	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I		BR:Coi8:07
				Apa I		BR:Coi10:07
T30	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I	*Cla* I	BR:Coi11:07
				Apa I		BR:Coi12:07
T31	Coimbra	July, 2007	*Solanum lycopersicum*		*Cla* I	BR:Coi13:07
T40	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I	*Apa* I	BR:Coi15:07
T42	Coimbra	July, 2007	*Solanum lycopersicum*		*Hin*d III	BR:Coi17:07
T43	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I	*Apa* I	BR:Coi18:07
T44	Coimbra	July, 2007	*Solanum lycopersicum*	*Kpn* I		BR:Coi20:07
				Kpn I		BR:Coi21:07
T48	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I	*Apa* I	BR:Coi22:07
T52	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I		BR:Coi23:07
T53	Coimbra	July, 2007	*Solanum lycopersicum*	*Apa* I		BR:Coi24:07
DV131	Jaíba	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Jai131:08
Tomato leaf distortion virus (ToLDV)						
A12a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda4:05
D6a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda47:05
Tomato mild mosaic virus (ToMlMV)						
A1b	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Eco*R I	BR:Pda1:05
PD	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Apa* I	*Cla* I	BR:Pda58:05
				Apa I	*Cla* I	BR:Pda59:05
				Apa I	*Bam*H I	BR:Pda60:05
HV14	Viçosa	May, 2010	*Sida urens*		*Bam*H I	BR:Vic14:10
HV17	Viçosa	May, 2010	*Sida urens*	*Apa* I		BR:Vic17.1:10
				Apa I		BR:Vic17.2:10
HV19	Viçosa	May, 2010	*Sida urens*	*Sac* I		BR:Vic19:10
Tomato mottle leaf curl virus (ToMoLCV)						
DV13	Jaíba	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Jai13:08

DV56	Jaíba	July, 2008	*Solanum lycopersicum*	*Apa* I		BR:Jai56:08
Tomato severe rugose virus (ToSRV)						
DV125	Jaíba	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Jai125:08
DV127	Jaíba	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Jai127:08
DV165	Florestal	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Flo165:08
DV202	Florestal	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Flo202:08
DV203	Florestal	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Flo203:08
DV206	Florestal	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Flo206:08
DV208	Florestal	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Flo208:08
DV214	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car214:08
DV217	Carandaí	July, 2008	*Solanum lycopersicum*		*Apa* I	BR:Car217.6:08
DV218	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car218.1:08
				*Bam*H I		BR:Car218.3:08
DV219	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car219.10:08
DV220	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car220:08
DV221	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car221:08
DV223	Carandaí	July, 2008	*Solanum lycopersicum*		*Apa* I	BR:Car223:08
DV224	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car224:08
DV226	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car226.3:08
				*Bam*H I		BR:Car226.5:08
DV227	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car227:08
DV228	Carandaí	July, 2008	*Sida* sp.	*Bam*H I		BR:Car228:08
DV230	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car230:08
DV232	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car232:08
DV233	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car233:08
DV234	Carandaí	July, 2008	*Solanum lycopersicum*		*Apa* I	BR:Car234.5:08
DV235	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I	*Apa* I	BR:Car235:08
DV236	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I		BR:Car236.1:08

DV237	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I	*Kpn* I	BR:Car237:08
				*Bam*H I	*Kpn* I	BR:Car237.6:08
DV238	Carandaí	July, 2008	*Solanum lycopersicum*	*Bam*H I	*Apa* I	BR:Car238:08
HV25	Viçosa	May, 2010	*Sida* sp.	*Sac* I		BR:Vic25:10
Tomato yellow spot virus (ToYSV)						
DV13	Jaíba	July, 2008	*Solanum lycopersicum*		*Sac* I	BR:Jai13.1:08
DV56	Jaíba	July, 2008	*Solanum lycopersicum*	*Sac* I		BR:Jai56.1:08
Tomato yellow vein streak virus (ToYVSV)						
A2a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda2:05
				*Bam*H I	*Bam*H I	BR:Pda3:05
A15a	Paty do Alferes	May, 2005	*Solanum lycopersicum*		*Bam*H I	BR:Pda5:05
B1b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda6:05
B1c	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda7:05
B2g	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda21:05
B3b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda12:05
B4a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda13:05
				*Bam*H I	*Bam*H I	BR:Pda14:05
B4b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda17:05
C7a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda22:05
C8b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda23:05
C9a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda24:05
C10a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda26:05
C11a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda28:05
				*Bam*H I		BR:Pda29:05
C13a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda30:05
C16a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda33:05
C17a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda35:05
C19a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda38:05

D1a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda41:05
D3a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda45:05
D6a	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda46:05
E2b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda50:05
				*Bam*H I		BR:Pda49:05
E3b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I	*Bam*H I	BR:Pda52:05
E4b	Paty do Alferes	May, 2005	*Solanum lycopersicum*	*Bam*H I		BR:Pda54:05

[1]Restriction enzyme used for cloning of the respective DNA component.
[2]Species assignment based on the ICTV-established criteria of 89% nucleotide sequence identity for the full-length DNA-A [80].
[3]Underlined samples had a mixed infection.

Table 3. Viruses cloned from the tomato samples collected at five different locations in the states of Rio de Janeiro and Minas Gerais, Brazil.

	Number of samples infected with each virus									
	ToCmMV	**ToCMoV**	**ToSRV**	**ToYVSV**	**ToLDV**	**ToMlMV**	**ToMoLCV**	**ToYSV**	**SimMV**	**Total**
Carandaí	-	6	18	-	-	-	-	-	-	**24**[a]
Coimbra	12	-	-	-	-	-	-	-	-	**12**
Florestal	-	19	5	-	-	-	-	-	-	**24**
Jaíba	1	-	2	-	-	-	2	2	2	**9**[b]
Paty do Alferes	19	-	-	23	2	2	-	-	3	**49**[c]
Total	**32**	**25**	**25**	**23**	**2**	**2**	**2**	**2**	**5**	**118**

[a] One sample with a mixed infection with ToCMoV and ToSRV

[b] Two samples with a mixed infection with ToMoLCV and ToYSV

[c] Two samples with a mixed infection with ToCmMV and ToYVSV; one sample with a mixed infection with SimMV and ToCmMV; one sample with a mixed infection with ToLDV and ToYVSV

Table 4. Putative recombination events detected among the tomato- and weed-infecting begomoviruses from Rio de Janeiro and Minas Gerais states, Brazil, based on a data set including only begomoviruses from Brazil.

Recombinant[1]	Recombinantion breakpoints[2]		Parents		Methods[3]	*p*-value
	Initial	Final	Major	Minor		
EuYMV (Flo166:08)	1547	2537	ClLCV	Unknown	RGBMC**S**	9.3×10^{-18}
SiCmMV (Coi4:07)	1333	2285	OMoV	Unknown	RGBMC**S**3	8.2×10^{-15}
SiCmMV(Coi4:07)	1929	(?)	Unknown	NDNV	RGBMC**S**3	3.3×10^{-07}
SimMV (Pda37:05)	1619	2606	OMoV	Unknown	RBMC**S**3	3.2×10^{-21}
SimMV (Pda37:05)	1915	(?)	Unknown	NDNV	R**G**BMCS	3.3×10^{-07}
SiYLCV (Coi3:07)	66	1997	EuYMV	SiMoV	RGBMCS**3**	4.5×10^{-21}
ToCmMV (Coi22:07)	35	1856	EuYMV	SiMoV	RGBMCS**3**	4.5×10^{-21}
ToCmMV (Coi22:07)	1917	2516	Unknown	ToMlMV	RGBMC**S**	9.3×10^{-18}
ToLDV (Pda4:05)	(?)	921	AbBV	SimMV	RGBMC**3**	1.7×10^{-07}
ToLDV (Pda4:05)	1582	2231	OMoV	Unknown	RGBMC**S**3	8.2×10^{-15}
ToMlMV (Pda58:05)	1935	2635	ToCMoV	Unknown	RGBMC**S**	5.2×10^{-05}
ToMoLCV (Jai13:08)	(?)	1159	SiMoV	ToSRV	RB**M**CS	6.2×10^{-04}
ToMoLCV (Jai13:08)	1945	2330	Unknown	ToCMoV	**R**GBMCS	5.2×10^{-15}
ToSRV (Flo165:08)	1860	2179	ToCMoV	SimMV	R**G**BMCS3	1.7×10^{-14}
ToYSV (Jai56.1:08)	1898	2422	ToCMoV	Unknown	RGBMC**S**	5.2×10^{-05}
ToYVSV (Pda3:05)	831	1046	Unknown	ToSRV	RBMC**S**	1.3×10^{-06}

[1] For simplicity, only one isolate of each species is listed for each recombination event.
[2] Numbering starts at the first nucleotide after the cleavage site at the origin of replication and increases clockwise. (?) Indicates that the breakpoint could not be precisely pinpointed.
[3] R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, CHIMAERA; S, SisScan; 3, 3SEQ. The reported *p*-value is for the program in bold, underlined type and is the lowest *p*-value calculated for the region in question.

Table 5. Putative recombination events detected among the tomato- and weed-infecting begomoviruses from Rio de Janeiro and Minas Gerais states, Brazil, based on a data set including all begomoviruses from the Americas.

Recombinant[1]	Recombination breakpoints[2]		Parental[3]		Methods[4]	*p*-value
	Initial	Final	Major	Minor		
BlYSV (Vic04.1:09)	2099	2283	*RhGMV*	Unknown	RGBMC**S**	4.6×10^{-12}
EuYMV (Flo166:08)	1753	2519	*ToYMLCV*	Unknown	RGBMC**S**	1.1×10^{-20}
SiCmMV (Coi4:07)	203	614	AbBV	SiMoV	RBMC**S**3	7.2×10^{-05}
SiCmMV (Coi4:07)	70	1445	*MaGMV*	SiMoV	RBMC**S**3	2.3×10^{-10}
SimMV (Pda37:05)	181	1619	OmoV	Unknown	RGBMCS**3**	2.7×10^{-19}
SimMV (Pda37:05)	1976	2234	*OYMolV*	ToRMV	R**G**BMC3	1.5×10^{-13}
SiYLCV (Coi3:07)	28	1999	SiMoV	*CabLCuV*	RGBMCS**3**	5.6×10^{-21}
ToCMoV (Flo182:08)	1790	2551	BGMV	PSLDV	RGBMC**S**3	6.7×10^{-20}
ToCmMV (Coi22:07)	1783	2501	SiMoV	*CabLCuV*	RGBMCS**3**	5.6×10^{-21}
ToLDV (Pda4:05)	270	929	AbBV	SimMV	RGBMCS**3**	3.9×10^{-07}
ToLDV (Pda4:05)	592	1845	*MaGMV*	SiMoV	RBMC**S**3	2.3×10^{-10}
ToMlMV (Pda58:05)	502	2050	*RhGMV*	*TSLCV*	RGBMCS**3**	1.2×10^{-12}
ToMoLCV (Jai13:08)	1608	2353	Unknown	PSLDV	RGBMC**S**	2.7×10^{-11}
ToSRV (Car224:08)	968	1907	*RhGMV*	*TSLCV*	RGBMCS**3**	1.2×10^{-12}
ToYSV (Jai56.1:08)	2561	(?)	SoBlMV	SiBV	RGM**C**3	4.8×10^{-08}
ToYVSV (Pda3:05)	(?)	2200	TGMV	Unknown	RGBMC**S**	7.8×10^{-05}

[1] For simplicity, only one isolate of each species is listed for each recombination event.
[2] Numbering starts at the first nucleotide after the cleavage site at the origin of replication and increases clockwise. (?) Indicates that the breakpoint could not be precisely pinpointed.
[3] Brazilian viruses underlined; viruses from other countries in the Americas in italics.
[4] R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, CHIMAERA; S, SisScan; 3, 3SEQ. The reported *p*-value is for the program in bold, underlined type and is the lowest *p*-value calculated for the region in question.

Table 6. Genetic structure of *Blainvilea yellow spot virus* (BlYSV), *Tomato chlorotic mottle virus* (ToCMoV), *Tomato common mosaic virus* (ToCmMV), *Tomato severe rugose virus* (ToSRV) and *Tomato yellow vein streak virus* (ToYVSV) populations from Rio de Janeiro and Minas Gerais states, Brazil.

Population	Number of sequences	Genome size	s^{*}	Eta[†]	$k^{‡}$	$\pi^{§}$	Mutation frequency	h[‖]	Hd[¶]	θ-w[#]	θ-Eta[*]
DNA-A											
BlYSV (Viçosa)	7	2661	200	211	65.619	0.02466	1.2×10^{-2}	7	1.000	0.0307	0.0323
ToCmMV (Total)	22	2560	103	104	36.645	0.0143	1.9×10^{-3}	20	0.987	0.0110	0.0111
Paty do Alferes	10	2560	11	11	2.200	0.0009	4.8×10^{-4}	8	0.933	0.0015	0.0015
Coimbra	12	2560	91	92	26.258	0.01026	3.1×10^{-3}	11	0.985	0.0312	0.0321
ToCMoV (Total)[a]	22	2619	135	138	18.351	0.00701	2.4×10^{-3}	22	1.000	0.0141	0.0144
Florestal	20	2619	120	122	16.589	0.00633	2.3×10^{-3}	20	1.000	0.0129	0.0131
ToSRV (Total)[b]	27	2588	148	159	26.530	0.0102	2.7×10^{-3}	26	0.997	0.0148	0.0159
Carandaí	19	2589	73	74	10.474	0.0040	1.8×10^{-3}	18	0.994	0.0080	0.0081
Florestal	5	2592	37	37	19.000	0.0073	3.5×10^{-3}	5	1.000	0.0068	0.0068
ToYVSV (Paty do Alferes)	26	2562	49	49	5.381	0.0021	7.4×10^{-4}	25	0.997	0.0050	0.0050
DNA-B											
BlYSV (Viçosa)	7	2625	326	346	121.905	0.04644	$\mathbf{1.8\times10^{-2}}$	7	1.000	0.0506	0.0538
ToCmMV (Total)	16	2500	205	214	60.475	0.0242	5.1×10^{-3}	14	0.975	0.0248	0.0258
Paty do Alferes	9	2500	14	14	3.111	0.0012	6.2×10^{-4}	7	0.917	0.0021	0.0021
Coimbra	7	2500	191	196	99.762	0.0400	1.1×10^{-2}	7	1.000	0.0312	0.0321
ToCMoV (Total)[c]	6	2554	146	146	73.467	0.02877	$\mathbf{8.8\times10^{-3}}$	5	0.933	0.0250	0.0250
Carandaí	4	2557	22	22	11.000	0.00430	$\mathbf{1.8\times10^{-3}}$	4	1.000	0.0046	0.0046
ToSRV (Carandaí)	7	2568	50	50	15.905	0.00619	$\mathbf{4.5\times10^{-3}}$	7	1.000	0.0079	0.0079
ToYVSV (Paty do Alferes)	13	2507	51	51	10.615	0.0042	1.6×10^{-3}	12	0.987	0.0066	0.0066

[*] Total number of segregating sites.
[†] Total number of mutations.
[‡] Average number of nucleotide differences between sequences (Tajima's estimate of the population mutation rate, θ).
[§] Nucleotide diversity.
[‖] Haplotype number.

[¶] Haplotype diversity.
[#] Watterson's estimate of the population mutation rate based on the total number of segregating sites.
[*] Watterson's estimate of the population mutation rate based on the total number of mutations.
[a] Including two sequences from Carandaí.
[b] Including two sequences from Jaíba and one sequence from Viçosa.
[c] Including two sequences from Florestal.

Table 7. Results of subdivision tests performed on the populations of *Tomato common mosaic virus* (ToCmMV), *Tomato chlorotic mottle virus* (ToCMoV) and *Tomato severe rugose virus* (ToSRV) from Rio de Janeiro and Minas Gerais states, Brazil.

Population	**Nst**[1]	**Fst**[1]
ToCmMV (DNA-A)		
Paty do Alferes/Coimbra	0.742	0.741
ToCmMV (DNA-B)		
Paty do Alferes/Coimbra	0.358	0.358
ToCMoV (DNA-A)		
Carandaí/Florestal	0.638	0.640
ToCMoV (DNA-B)		
Carandaí/Florestal	0.958	0.958
ToSRV (DNA-A)	0.738	0.735
Carandaí/Jaíba	0.502	0.502
Carandaí/Florestal	0.743	0.740
Jaíba/Florestal	0.793	0.791

[1] Values from 0 to 0.05 indicate little genetic differentiation; from 0.05 to 0.15, moderate differentiation; from 0.15 to 0.25, great differentiation; and >0.25, high differentiation.

Table 8. Results of the five neutrality tests for each open reading frame (ORF) in the DNA-A and DNA-B of viral isolates comprising populations of *Blainvilea yellow spot virus* (BlYSV), *Tomato chlorotic mottle virus* (ToCMoV), *Tomato common mosaic virus* (ToCmMV), *Tomato severe rugose virus* (ToSRV) and *Tomato yellow vein streak virus* (ToYVSV) from Rio de Janeiro and Minas Gerais states, Brazil.

Population	ORF	Tajima's *D*	Fu and Li's *D**	Fu and Li's *F**	dN/dS
BlYSV	CP[1]	**-1.517***	-1.458	-1.608	0.040
(Viçosa)	Rep	-1.296	-1.306	-1.429	0.124
	Trap	-0.799	-0.825	-0.889	0.036
	Ren	-1.160	-1.161	-1.253	0.195
	NSP	-1.197	-1.131	-1.270	0.175
	MP	-0.505	-0.298	-0.382	0.050
ToCmMV	CP	-1.562	-1.784	-1.934	0.145
(Paty do Alferes)	Rep	-1.562	-1.784	-1.934	0.605
	Trap	-1.667	-1.916	-2.076	0.304
	Ren	-1.401	-1.587	-1.719	0.287
	NSP	-1.677	-1.881	-2.039	0.461
	MP	**-1.728***	**-1.943***	-2.107	0.293
ToCmMV	CP	-0.604	0.993	0.654	0.090
(Coimbra)	Rep	-0.772	0.259	-0.013	0.278
	Trap	0.027	1.036	0.881	0.320
	Ren	0.065	**1.433***	1.226	0.425
	NSP	1.816	1.287	1.556	0.162
	MP	1.533	1.241	1.445	0.135
ToCMoV	CP	**-1.973***	-2.2557	-2.529	0.090
(Florestal)	Rep	**-2.182** ‡	**-2.836***	**-3.078** †	0.014
	Trap	-1.608	-2.012	-2.191	1.705
	Ren	-1.612	-0.687	-1.101	0.314
ToSRV	CP	**-2.282**‡	**-3.168**†	**-3.376**†	0.389
(Carandaí)	Rep	**-2.302**‡	**-2.970**†	**-3.223**†	0.068
	Trap	**-2.010***	-2.326	**-2.587***	0.364
	Ren	**-2.059***	**-2.456***	**-2.712***	0.647
	NSP	-1.314	-1.310	-1.443	2.165
	MP	-0.999	-0.904	-1.016	1.225
ToSRV	Rep	0.812	0.812	0.865	0.313
(Florestal)	Trap	0.243	0.243	0.239	0
	Ren	1.459	1.459	1.431	Ind.
	CP	0.708	0.708	0.749	0.211
ToYVSV	CP	**-2.135***	**-2.723***	**-2.969***	0.027
(Paty do Alferes)	Rep	**-2.156**†	**-3.341**†	**-3.483**†	0.152
	Trap	-1.369	-2.042	2.143	1.600
	Ren	-1.706	-2.400	-2.560	1.630
	NSP	-1.495	-1.917	-2.057	0.760
	MP	**-1.830***	-2.060	-2.272	0.033

[1] CP, Coat protein; Rep, Replication-associated protein; Trap, Trans-activating protein; Ren, Replication enhancer protein; NSP, Nuclear shuttle protein; MP, Movement protein.
*Significant values that reject the null hypothesis of selective neutrality; $p<0.05$
†Significant values that reject the null hypothesis of selective neutrality; $p<0.02$
‡Significant values that reject the null hypothesis of selective neutrality; $p<0.01$
Significant values that reject the null hypothesis of selective neutrality; $p<0.001$

Figure legends

Figure 1. Maximum likelihood tree based on the complete DNA-A nucleotide sequences of one isolate of each begomovirus obtained in this study (indicated in red), plus reference sequences of all Brazilian begomoviruses and selected begomoviruses from the Americas. *Tomato leaf curl New Delhi virus* (ToLCNDV), an Old World bipartite begomovirus, was used as outgroup. Clusters including Brazilian begomoviruses are indicated at the right.

Figure 2. Bayesian 50% majority rule consensus tree based on the complete DNA-A nucleotide sequences of **(A)** *Tomato chlorotic mottle virus* (ToCMoV), **(B)** *Tomato severe rugose virus* (ToSRV), and **(C)** *Tomato common mosaic virus* (ToCmMV) isolates. Numbers at the nodes indicate Bayesian posterior probabilities. The color-coded bars indicated the host and geographical (state) origin of each isolate.

Figure 3. Phylogenetic evidence for recombination within populations of the begomoviruses **(A)** *Tomato chlorotic mottle virus* (ToCMoV) and **(B)** *Tomato severe rugose virus* (ToSRV). Neighbor Net network analysis was performed using SplitsTree4. Formation of a reticular network rather than a single bifurcated tree is suggestive of recombination.

Figure 4. Phylogenetic evidence for recombination among begomoviruses from the Americas, including some of the isolates described in this study. Neighbor Net network analysis was performed using SplitsTree4. Formation of a reticular network rather than

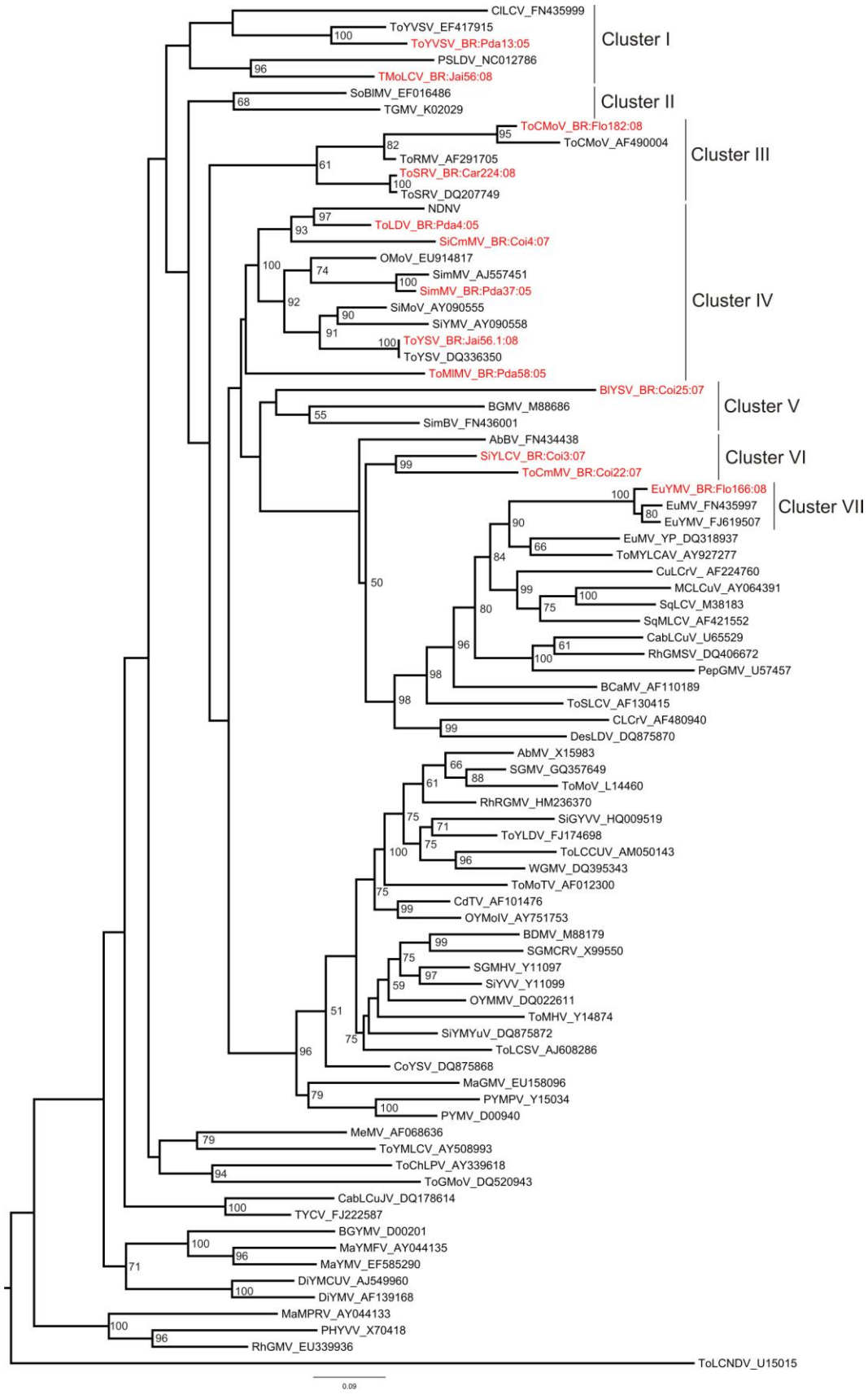
a single bifurcated tree is suggestive of recombination. The branches that include isolates obtained in this study are indicated in red.

Figure 5. Bayesian 50% majority rule consensus trees based on partial DNA-A nucleotide sequences of *Abutilon Brazil virus* (AbBV), *Sida yellow leaf curl virus* (SiYLCV), *Tomato common mosaic virus* (ToCmMV) (indicated in red), *Cabbage leaf curl virus* (CabLCuV) and additional begomoviruses from the Americas. **(A)** Tree based on the putative recombinant region between AbBV/SiYLCV/ToCmMV and CabLCuV detected by RDP3. **(B)** Tree based on the non-recombination region. Numbers at the nodes indicate Bayesian posterior probabilities.

Figure 6. Mutation frequencies determined for each coding sequence in the populations of *Blaivillea yellow spot virus* (BlYSV), *Tomato common mosaic virus* (ToCmMV), *Tomato chlorotic mottle virus* (ToCMoV), *Tomato severe rugose virus* (ToSRV) and *Tomato yellow vein streak virus* (ToYVSV). BlYSV, ToCmMV, ToCMoV, ToSRV and ToYVSV ORFs are indicated in amber, orange, blue, red and purple, respectively. "ToCMoV Total" refers to the ToCMoV population from Florestal plus two isolates from Carandaí; "ToSRV Total" refers to the ToSRV populations from Carandaí and Florestal; "ToCmMV Total" refers to the ToCmMV populations from Coimbra and Paty do Alferes. The genome organization of a typical New World begomovirus is displayed below the graph, showing the precise location of each ORF (CP, coat protein; Rep, replication-associated protein; Trap, transcriptional activator protein; Ren, Replication enhancer protein) and the IR (intergenic or common region).

Figure 7. Cluster analysis of population subdivision using Structure. Each individual is represented by a vertical black line divided into *K* colors, where *K* is the number of clusters assumed. Individuals are sorted according to *Q*. **(A)** ToCMoV population, *K* = 3; **(B)** ToCmMV population, *K* = 2; **(C)** ToSRV population, *K* = 2; **(D)** ToYVSV population, K = 1. Car, Coi, Flo, Pda, and Vic correspond to isolates from Carandaí, Coimbra, Florestal, Paty do Alferes and Viçosa, respectively.

Figure 1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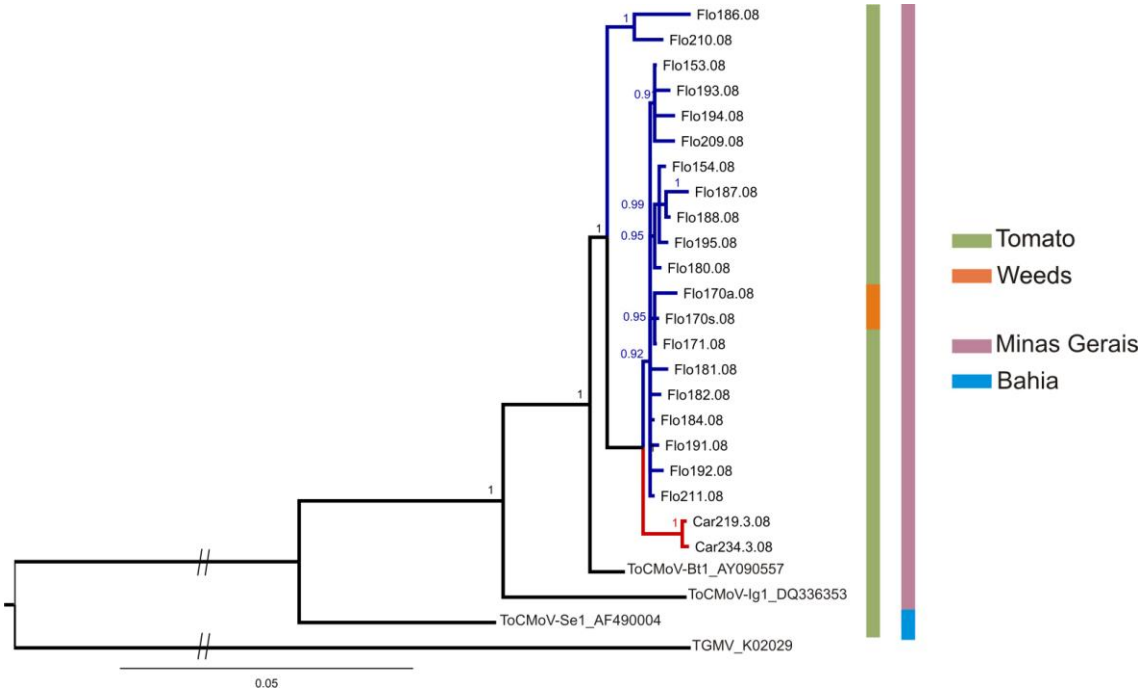
Cluster III
Cluster IV
Cluster V
Cluster VI
Cluster VII

Figure 2A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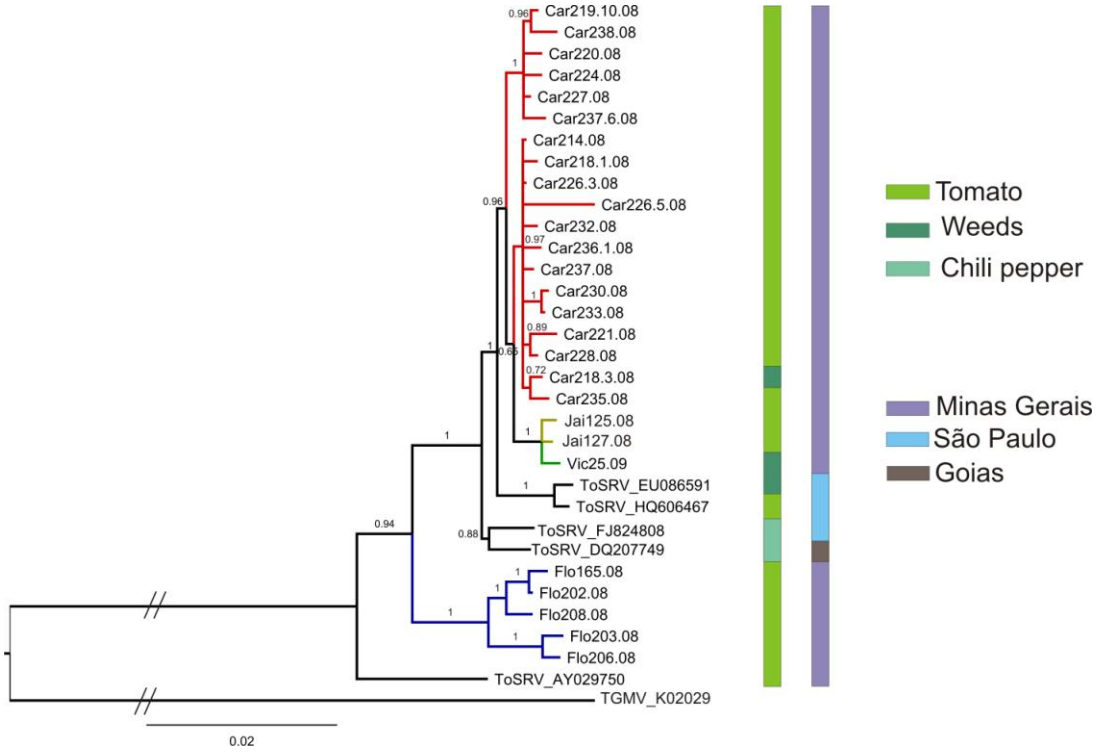
Weeds
Minas Gerais
Bahia

Figure 2B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São Paulo
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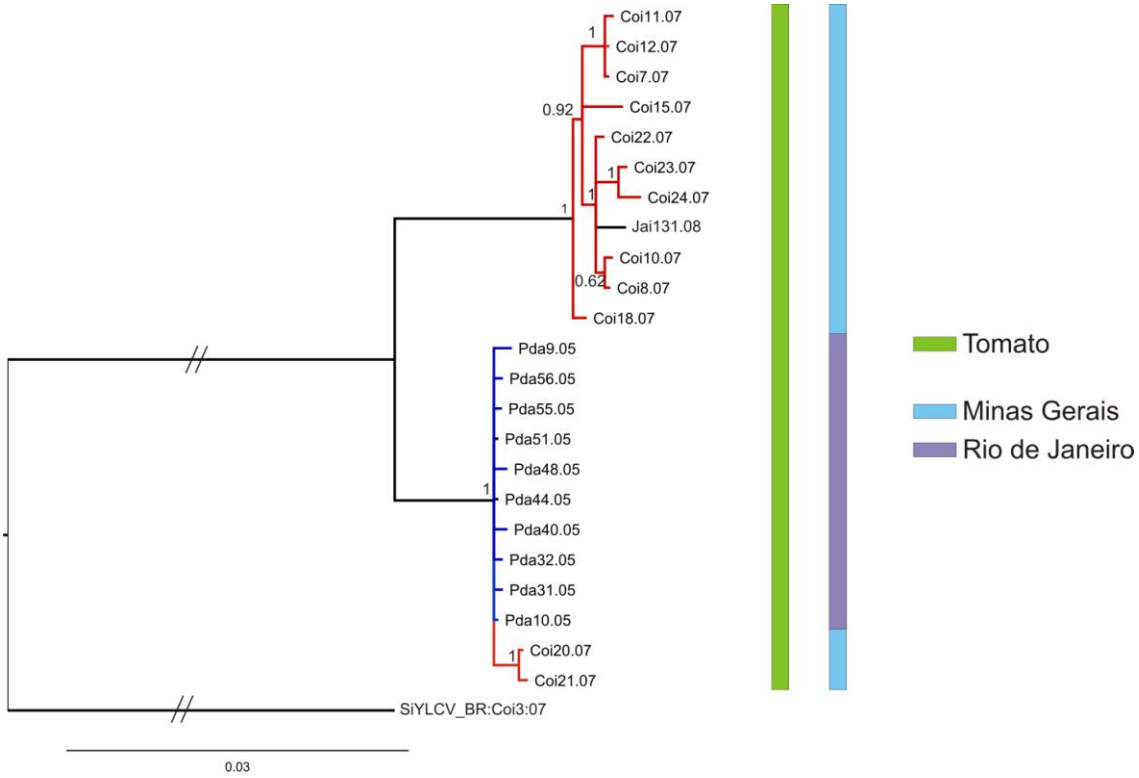
Figure 2C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Tomato
Minas Gerais
Rio de Janeiro

Figure 3

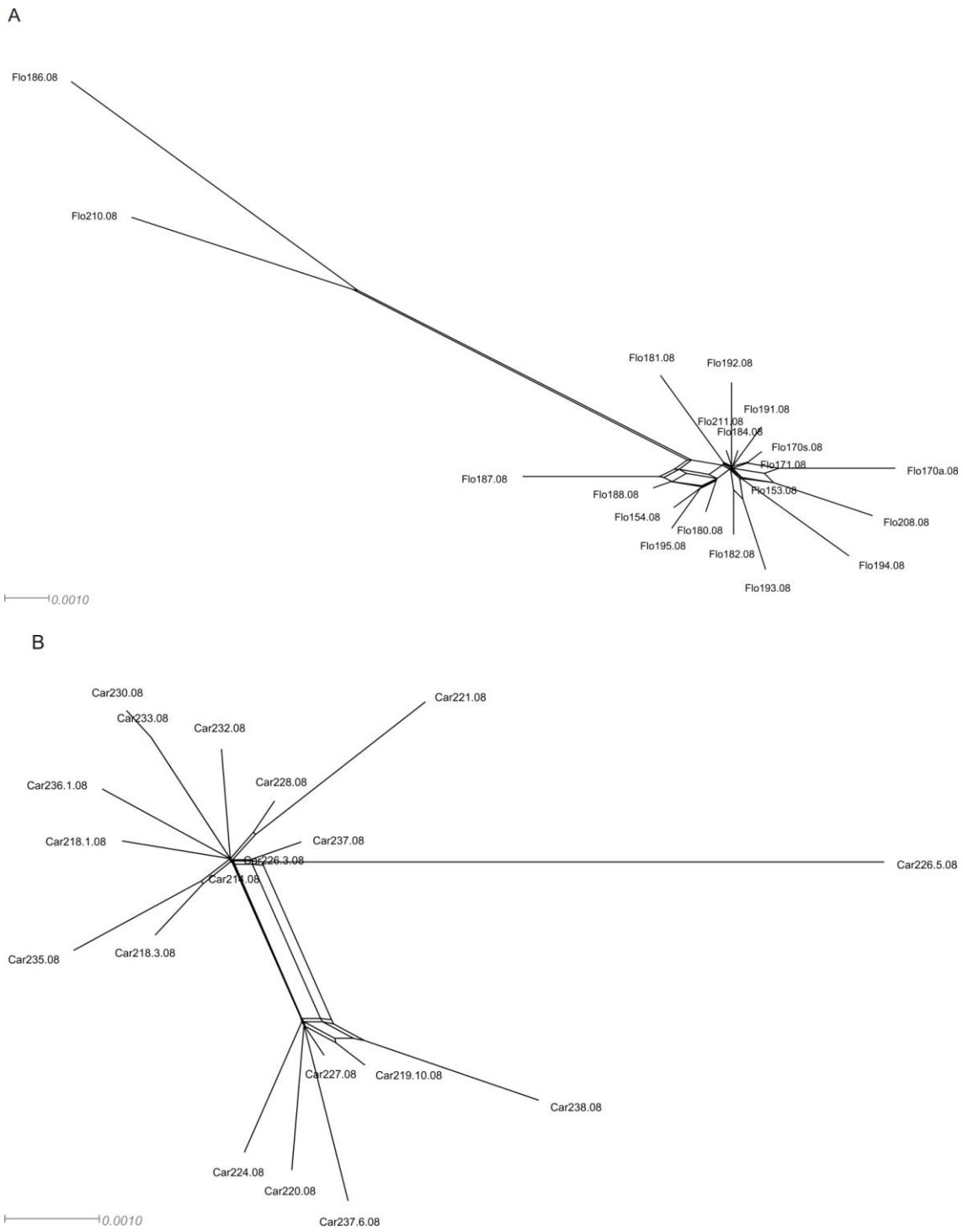
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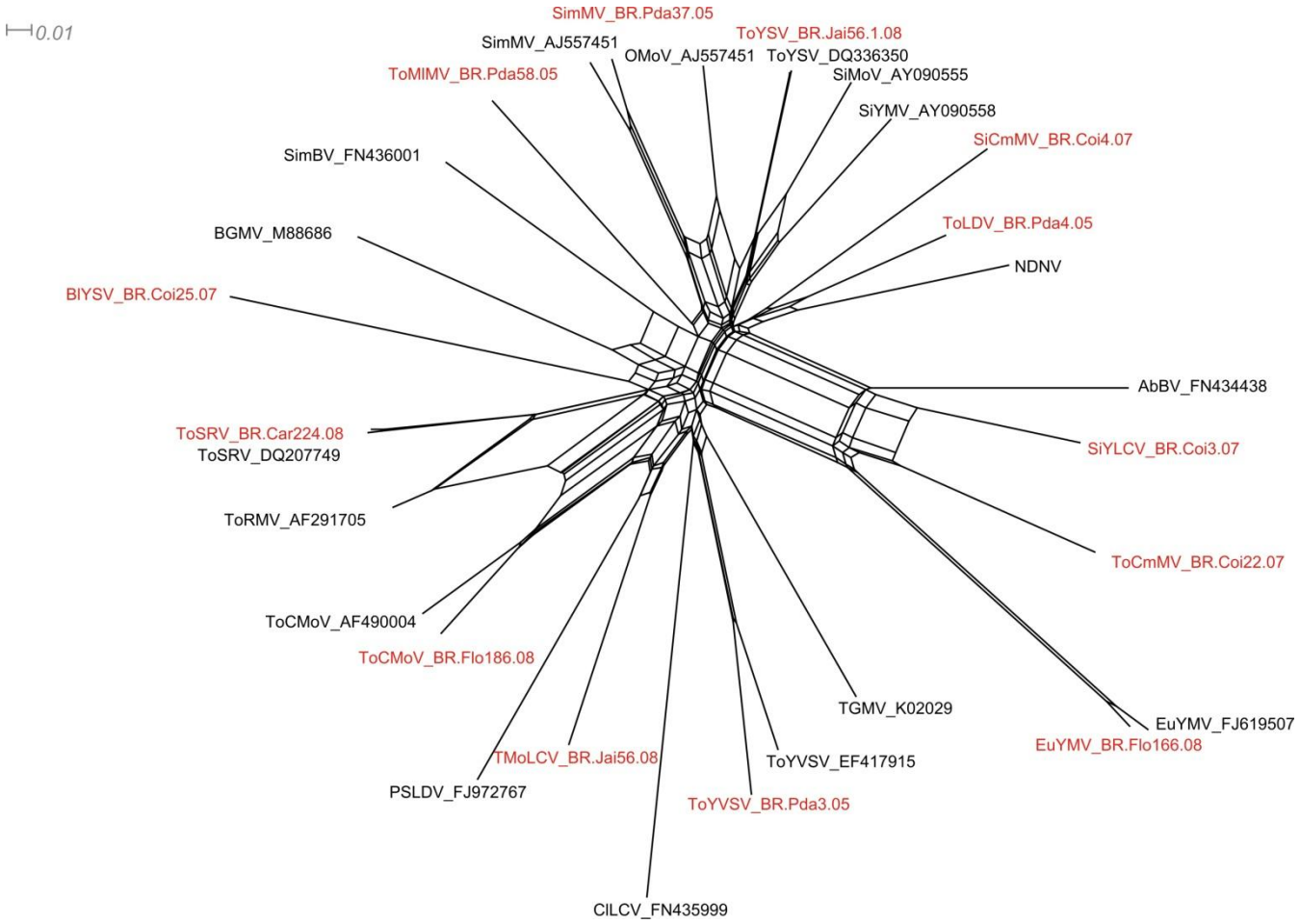
Figure 4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SimMV_AJ557451
ToYSV_BR.Jai56.1.08
OMoV_AJ557451
ToYSV_DQ336350
ToMIMV_BR.Pda58.05
SiMoV_AY090555
SiYMV_AY090558
SiCmMV_BR.Coi4.07
SimBV_FN436001
ToLDV_BR.Pda4.05
BGMV_M88686
NDNV
BlYSV_BR.Coi25.07
AbBV_FN434438
ToSRV_BR.Car224.08
ToSRV_DQ207749
SiYLCV_BR.Coi3.07
ToRMV_AF291705
ToCmMV_BR.Coi22.07
ToCMoV_AF490004
ToCMoV_BR.Flo186.08
TGMV_K02029
EuYMV_FJ619507
EuYMV_BR.Flo166.08
TMoLCV_BR.Jai56.08
ToYVSV_EF417915
PSLDV_FJ972767
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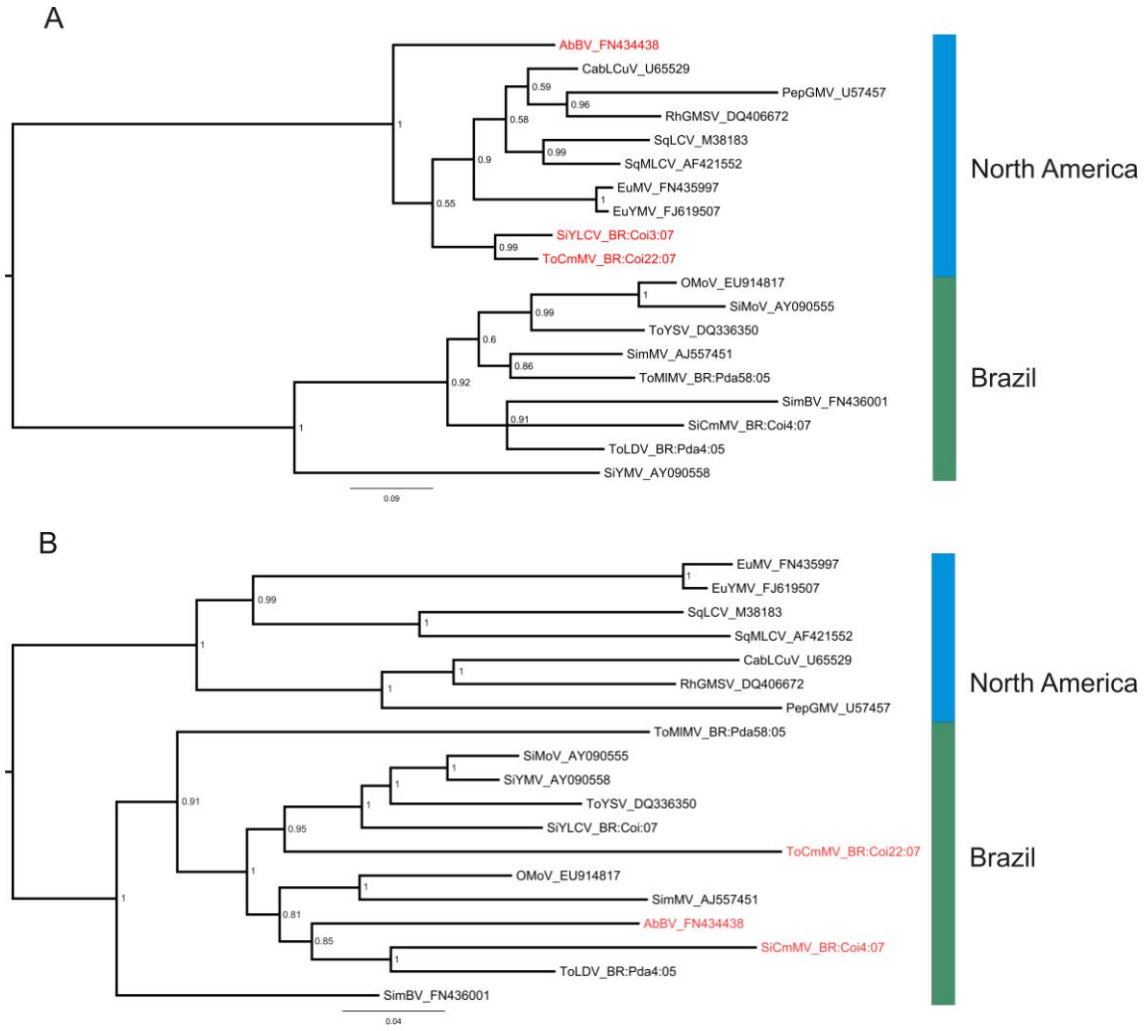
ClLCV_FN435999

Figure 5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CabLCuV_U65529
PepGMV_U57457
RhGMSV_DQ406672
SqLCV_M38183
SqMLCV_AF421552
EuMV_FN435997
EuYMV_FJ619507
SiYLCV_BR:Coi3:07
ToCmMV_BR:Coi22:07
OMoV_EU914817
SiMoV_AY090555
ToYSV_DQ336350
SimMV_AJ557451
ToMIMV_BR:Pda58:05
SimBV_FN436001
SiCmMV_BR:Coi4:07
ToLDV_BR:Pda4:05
SiYMV_AY090558
0.09
North America
Brazil
B
EuMV_FN435997
EuYMV_FJ619507
SqLCV_M38183
SqMLCV_AF421552
CabLCuV_U65529
RhGMSV_DQ406672
PepGMV_U57457
ToMIMV_BR:Pda58:05
SiMoV_AY090555
SiYMV_AY090558
ToYSV_DQ336350
SiYLCV_BR:Coi:07
ToCmMV_BR:Coi22:07
OMoV_EU914817
SimMV_AJ557451
AbBV_FN434438
SiCmMV_BR:Coi4:07
ToLDV_BR:Pda4:05
SimBV_FN436001
0.04
North America
Brazil

Figure 6

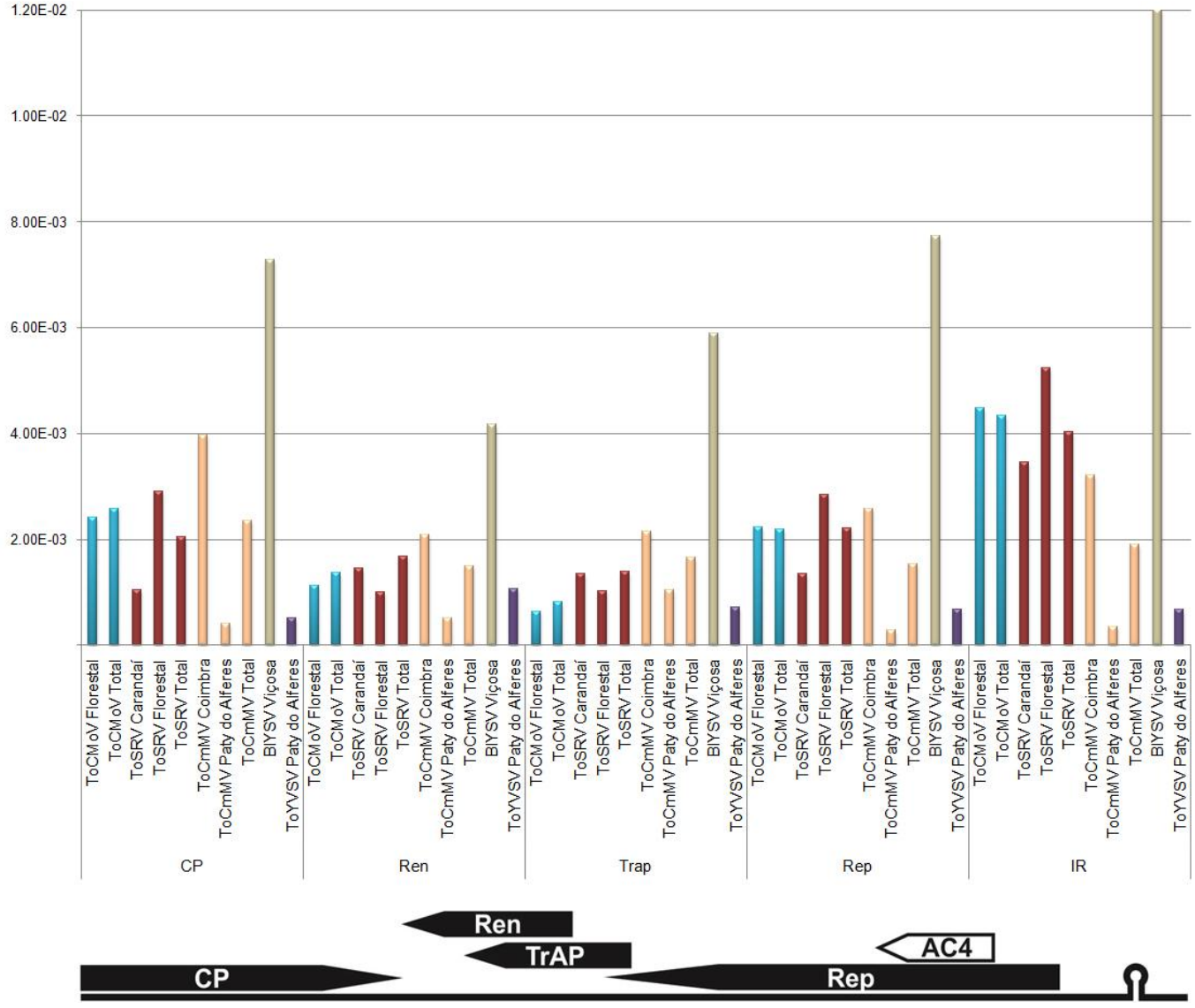
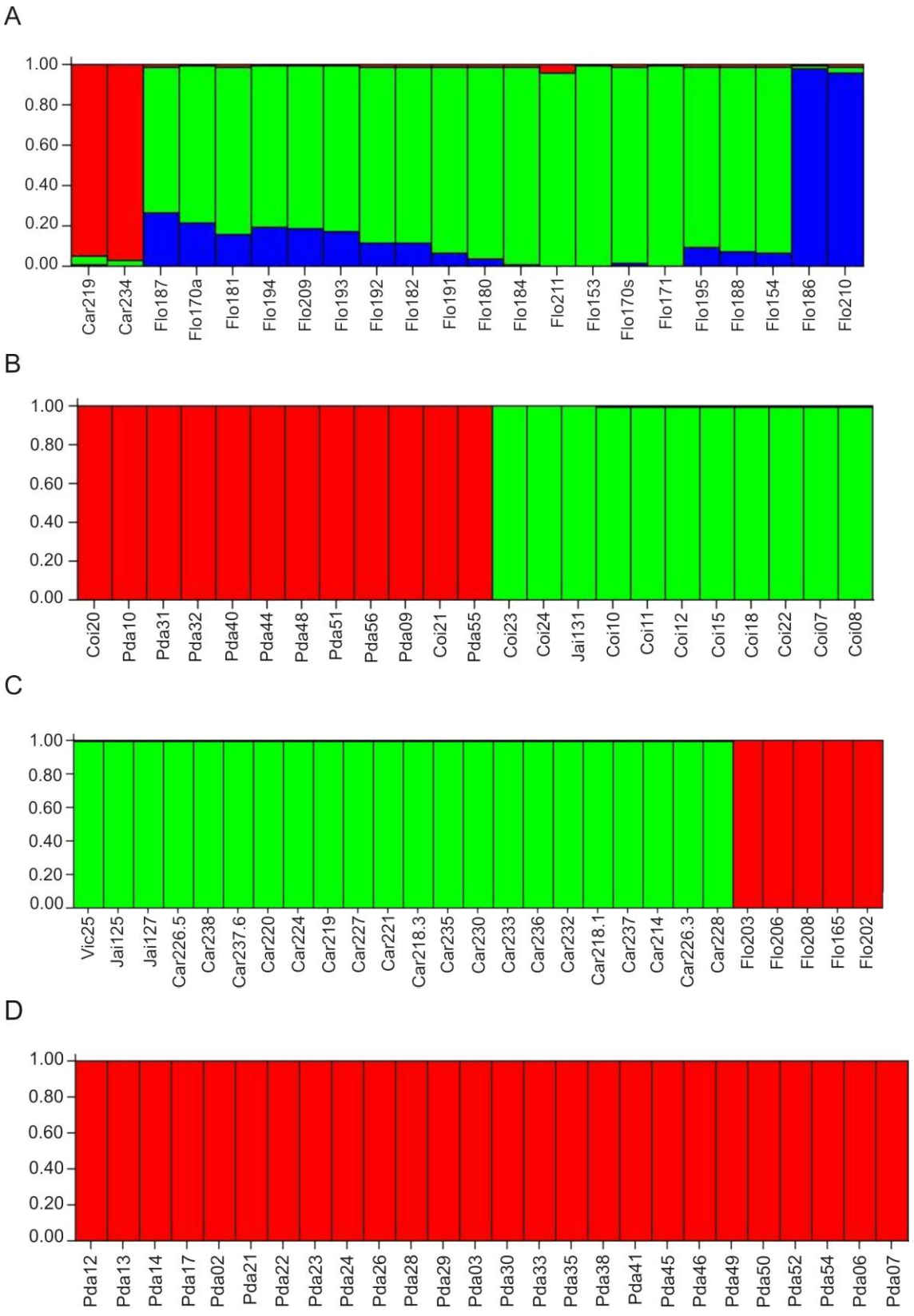

1.20E-02
1.00E-02
8.00E-03
6.00E-03
4.00E-03
2.00E-03
ToCMoV Florestal
ToCMoV Total
ToSRV Carandaí
ToSRV Florestal
ToSRV Total
ToCmMV Coimbra
ToCmMV Paty do Alferes
ToCmMV Total
BlYSV Viçosa
ToYVSV Paty do Alferes
CP
Ren
Trap
Rep
IR
Ren
TrAP
AC4
CP
Rep

Figure 7


A
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0.80
0.60
0.40
0.20
0.00
Car219
Car234
Flo187
Flo170a
Flo181
Flo194
Flo209
Flo193
Flo192
Flo182
Flo191
Flo180
Flo184
Flo211
Flo153
Flo170s
Flo171
Flo195
Flo188
Flo154
Flo186
Flo210
B
1.00
0.80
0.60
0.40
0.20
0.00
Coi20
Pda10
Pda31
Pda32
Pda40
Pda44
Pda48
Pda51
Pda56
Pda09
Coi21
Pda55
Coi23
Coi24
Jai131
Coi10
Coi11
Coi12
Coi15
Coi18
Coi22
Coi07
Coi08
C
1.00
0.80
0.60
0.40
0.20
0.00
Vic25
Jai125
Jai127
Car226.5
Car238
Car237.6
Car220
Car224
Car219
Car227
Car221
Car218.3
Car235
Car230
Car233
Car236
Car232
Car218.1
Car237
Car214
Car226.3
Car228
Flo203
Flo206
Flo208
Flo165
Flo202
D
1.00
0.80
0.60
0.40
0.20
0.00
Pda12
Pda13
Pda14
Pda17
Pda02
Pda21
Pda22
Pda23
Pda24
Pda26
Pda28
Pda29
Pda03
Pda30
Pda33
Pda35
Pda38
Pda41
Pda45
Pda46
Pda49
Pda50
Pda52
Pda54
Pda06
Pda07

Conclusões gerais

- Um estudo em larga escala foi conduzido para determinar a estrutura e a variabilidade genética de populações de begomovírus associados com tomateiros e plantas daninhas em regiões produtoras de tomate do sudeste do Brasil.
- Foi detectada a presença de treze espécies de begomovírus no campo.
- A sequência completa de nucleotídeos do DNA-A do Tomato mottle leaf curl virus (ToMoLCV) foi determinada pela primeira vez. A análise da sequência indica relacionamento filogenético com begomovírus do Brasil e da America Central, e que o ToMoLCV é um possível recombinante entre o ToCMoV e um vírus desconhecido. Os resultados apoiam a classificação do ToMoLCV como uma espécie do gênero *Begomovirus*.
- Vírus originalmente detectados em tomateiro foram encontrados em plantas daninhas, e vice e versa.
- Os vírus que infectam plantas daninhas são geneticamente mais variáveis que vírus que infectam tomate, e em todos os casos o DNA-B é mais variável que o DNA-A.
- A análise filogenética indicou uma divisão local entre as populações de begomovírus.
- Foi confirmada a ocorrência frequente de recombinação entre begomovírus brasileiros, com vírus de plantas daninhas frequentemente identificados como parentais de vírus de tomateiro, mas não vice e versa.