JOÃO PAULO DA SILVA

EVOLUTION OF A DNA VIRUS IN THE NATURAL ENVIRONMENT - A JOURNEY THROUGH TIME

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae.*

Orientador: Francisco Murilo Zerbini

Coorientadora: Angélica Maria Nogueira

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RESUMO

SILVA, João Paulo, M.Sc., Universidade Federal de Viçosa, fevereiro de 2020. **Evolução de um virus de DNA em um ambiente natural: Uma viagem através do tempo.** Orientador: Francisco Murilo Zerbini Júnior. Coorientadora: Angélica Maria Nogueira.

Vírus são reconhecidos por apresentarem altas taxas de mutação, resultando em rápida diversificação e aumento do potencial adaptativo. Portanto, vírus constituem modelos interessantes para estudos evolutivos. Begomovírus (família *Geminiviridae*) possuem um genoma composto por uma ou duas moléculas de DNA circular de fita simples encapsidadas em partículas icosaédricas geminadas, e são transmitidos por moscas-brancas do complexo de espécies *Bemisia tabaci*. Os begomovírus causam doenças em várias culturas economicamente importantes. Uma grande diversidade de begomovírus está presente em hospedeiros não-cultivados. Hospedeiros não-cultivados cumprem um importante papel ecológico e epidemiológico, pois são reservatórios de biodiversidade viral, contribuem como fonte de inóculo primário, e constituem ambientes propícios para a recombinação, favorecendo a emergência de novas espécies ou variantes. As populações de begomovírus têm alto grau de variabilidade genética, e apresentam taxas de mutação que se equiparam às de vírus de RNA. Embora alguns estudos tenham abordado a dinâmica temporal de begomovírus, esses estudos foram conduzidos em áreas extensas e em ambientes agrícolas. Sabe-se muito pouco sobre a dinâmica evolutiva dessas populações em pequenas escalas e em ambientes naturais. Neste trabalho, avaliou-se a variabilidade e a estrutura genética da comunidade de begomovírus em *Sida acuta* (Malvaceae) ao longo de oito anos. Amostras foram coletadas de 2011 a 2018 em uma área de aproximadamente 0,1 ha em Viçosa, MG. Três espécies foram detectadas: *Oxalis yellow vien virus* (OxYVV), *Sida yellow leaf curl virus* (SiYLCV), e *Sida micrantha mosaic virus* (SimMV). As populações de OxYVV e SiYLCV estão subdividas em três variantes bem definidas. Além disso encontramos duas estirpes distintas de SimMV. Esses resultados demonstram a heterogeneidade da comunidade de begomovírus, que coexiste como um complexo de espécies e variantes distintas, e revelam o importante papel ecológico de *S. acuta*, que constitui um hospedeiro natural altamente permissivo aos begomovírus. A grande flutuação observada na distribuição das variantes de OxYVV ao longo do tempo reflete o diferencial adaptativo de cada variante e o impacto da seleção sob populações virais em ecossistemas naturais. Ademais, os dados sugerem que a recombinação entre duas variantes de OxYVV foi importante para amenizar o impacto de mutações deletérias. A ausência de sinal temporal nas análises filogenéticas sugere que as populações virais evoluem a taxas lentas em ambientes naturais, ao contrário do que tem sido observado em trabalhos conduzidos em áreas de cultivo submetidas a perturbações antrópicas. Propõe-se que, no ambiente agrícola, a deriva genética é o mecanismo evolutivo proeminente e responsável pelos padrões de segregação ao longo do tempo.

Palavras-chave: Evolução. Diversidade genética de Begomovirus. Analise temporal.

ABSTRACT

SILVA, João Paulo, M.Sc., Universidade Federal de Viçosa, February, 2020. **Evolution of a DNA virus in the natural environment - a journey through time.** Advisor: Francisco Murilo Zerbini Júnior. Co-advisor: Angélica Maria Nogueira.

Viruses are recognized for having high mutation rates, resulting in rapid diversification and increased adaptive potential. For this reason, viruses are interesting models for evolutionary studies. Begomoviruses (family *Geminiviridae*) have a genome composed of one or two molecules of circular, single-stranded DNA (ssDNA) encapsulated in twinned icosahedral particles, and are transmitted by whiteflies of the *Bemisia tabaci* species complex. Begomoviruses cause diseases in several economically important crops. A great diversity of begomoviruses is present in non-cultivated hosts. Non-cultivated hosts play an important ecological and epidemiological role as reservoirs of viral biodiversity, as a source of primary inoculum, and as favorable environments for recombination, favoring the emergence of new species or variants. Begomovirus populations have a high degree of genetic variability, with mutation rates that are equivalent to those of RNA viruses. Although a number of studies have addressed the temporal dynamics of begomoviruses, these studies were conducted in large areas and in the context of agricultural environments. Little is known about the evolutionary dynamics of these populations in small scale and in natural environments. In this work. the variability and genetic structure of the begomovirus community in plants of *Sida acuta* (Malvaceae) was evaluated over a period of eight years. Samples were collected from 2011 to 2018 in an area of approximately 0.1 ha in Viçosa, MG. Three species were detected: *Oxalis yellow vein virus* (OxYVV), *Sida yellow leaf curl virus* (SiYLCV), and *Sida micrantha mosaic virus* (SimMV). The OxYVV and SiYLCV populations are subdivided into three well defined variants. In addition, we found two distinct strains of SimMV. These results highlight the heterogeneity of the begomovirus community, which coexist as a complex of different species and variants, and reveal the important ecological role of *S. acuta*, which constitutes a natural, highly permissive host for begomoviruses. The large fluctuation in the distribution of OxYVV variants over time reflects the adaptive differential of each variant and the impact of selection on viral populations in natural ecosystems. In addition, the data suggests that recombination between OxYVV variants was important to alleviate the impact of harmful mutations. The absence of a temporal signal in phylogenetic analyses suggests that the viral populations evolve at slow rates in natural environments, contrary to what has been observed in studies carried out in cultivated area subjected to anthropic disturbances. We hypothesize that, in crop systems, genetic drift is the predominat evolutionary mechanism responsible for patterns of segregation over time.

Keywords: Evolution. Genetic diversity of Begomovirus. Temporal analysis.

SUMÁRIO

INTRODUÇÃO GERAL

Geminivírus são vírus que infectam plantas e que possuem genoma de DNA circular de fita simples (ssDNA) (Zerbini *et al.*, 2017), responsáveis por grandes perdas econômicas em todo o mundo (Rojas *et al.*, 2018). O intercâmbio de material propagativo vegetal, mudanças nos sistemas de cultivo e a dispersão global de vetores polífagos têm contribuído para a disseminação global dos geminivírus (Rojas *et al.*, 2005; Navas-Castillo *et al.*, 2011; Gilbertson *et al.*, 2015).

A família *Geminiviridae* é composta por nove gêneros: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* e *Turncurtovirus.* O agrupamento é determinado pela organização genômica, relacionamento filogenético, tipo de inseto vetor e gama de hospedeiros (Brown *et al.*, 2012; Varsani *et al.*, 2014; Varsani *et al.*, 2017; Zerbini *et al.*, 2017). É a família mais diversa de vírus de plantas, com 468 espécies oficialmente reconhecidas pelo International Committee on Taxonomy of Viruses (ICTV) em fevereiro de 2020 (talk.ictvonline.org/taxonomy). Altas taxas de mutação e eventos frequentes de recombinação e pseudo-recombinação são responsáveis pela geração de variabilidade genética (Pita *et al.*, 2001; Rojas *et al.*, 2005; Lima *et al.*, 2013; Lefeuvre & Moriones, 2015).

Os begomovírus constituem o gênero mais diversos dentro da família *Geminiviridae*, com 409 espécies reconhecidas pelo ICTV. Begomovírus causam perdas relevantes em culturas de grande importância econômica, principalmente em regiões tropicais e subtropicais (Moriones & Navas-Castillo, 2000; Legg & Fauquet, 2004; Sserubombwe *et al.*, 2008). Por exemplo, o *African cassava mosaic virus* (ACMV) e o *East african cassava mosaic virus* (EACMV) são responsáveis pelo mosaico da mandioca, doença de grande importância no continente africano (Legg & Fauquet, 2004; Sserubombwe *et al.*, 2008), e o *Tomato yellow leaf curl virus* (TYLCV) é responsável por grandes prejuízos na tomaticultura no Mediterrâneo, Américas Central e do Norte, e no Oriente Médio (Moriones & Navas-Castillo, 2000).

Com base na organização genômica e relacionamento filogenético, os begomovírus são agrupados em Novo Mundo (NM; as Américas) e Velho Mundo (VM; África, Eurásia e Oceania) (Rybicki, 1994; Padidam *et al.*, 1999; Paximadis *et al.*, 1999). Begomovírus do NM apresentam genoma bissegmentado, com exceção do *Tomato leaf deformation virus* e *Tomato leaf curl purple vien virus* que apresentam genoma monossegmentado (Melgarejo *et al.*, 2013; Macedo *et al.*, 2017). Os dois componentes genômicos são denominados DNA-A e DNA-B. O DNA-A codifica cinco proteínas, associadas à replicação, encapsidação e supressão das respostas de defesa do hospedeiro (Rojas *et al.*, 2005). O DNA-B codifica duas proteínas responsáveis pelo movimento do vírus do núcleo para o citoplasma, movimento célula-a-célula via plasmodesmas e supressão de respostas de defesa (Noueiry *et al.*, 1994; Sanderfoot & Lazarowitz, 1996; Zorzatto *et al.*, 2015). Os dois componentes compartilham uma região intergênica com cerca de 200 nt denominada região comum (CR), que apresenta alta identidade nucleotídica e na qual está localizada a origem de replicação (Briddon *et al.*, 2010).

Begomovírus do VM podem ser mono- ou bissegmentados. Os bissegmentados apresentam organização genômica semelhante aos do NM. Já os monossegmentados possuem organização genômica similar ao DNA-A dos vírus bissegmentados. Os begomovírus do VM são frequentemente encontrados em associação com DNAs satélites, denominados alfassatélites e betassatélites (Mansoor *et al.*, 2003; Briddon & Stanley, 2006). Há relatos também de associação de begomovírus do NM com alfasatélites, mas até hoje nunca foi relatada sua associação com betassatélites (Paprotka *et al.*, 2010; Romay *et al.*, 2010; Jeske *et al.*, 2014; Ferro *et al.*, 2017).

Os begomovírus são transmitidos de forma persistente-circulativa pela mosca-branca *Bemisia tabaci*, inseto responsável por grandes perdas na agricultura (Stansly & Naranjo, 2010; Navas-Castillo *et al.*, 2011; Gilbertson *et al.*, 2015; Beyene *et al.*, 2016; Ghanim & Czosnek, 2016). *B. tabaci* constitui um complexo que engloba mais de 40 espécies crípticas, definidas com base na divergência do gene mitocondrial citocromo oxidase I (*mtCOI*) (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011; Lee *et al.*, 2013; Mugerwa *et al.*, 2018; Vyskočilová *et al.*, 2018). A emergência de begomovírus tem sido associada à dispersão global de espécies polífagas como *B. tabaci* Middle East-Asia Minor 1 (MEAM1) e *B. tabaci* Mediterranean (MED) (Jones, 2003; Navas-Castillo *et al.*, 2011). No Brasil, a emergência de begomovírus em tomateiro coincidiu com a introdução e rápida disseminação de *B. tabaci* MEAM1 ao longo da década de 1990 (Ribeiro *et al.*, 1998).

Os principais mecanismos geradores de variabilidade genética para os begomovírus são mutação, recombinação e pseudorecombinação (Padidam *et al.*, 1999; Seal *et al.*, 2006; Lefeuvre *et al.*, 2009).

Mutação é o resultado da incorporação incorreta de nucleotídeos na fita nascente de DNA durante o processo de replicação (Smith, 1989; Acosta-Leal *et al.*, 2011). A mutação é o principal mecanismo de geração de variabilidade dos begomovírus (Lima *et al.*, 2017). De fato, os begomovírus apresentam taxas de mutação que se equiparam às de vírus com genoma de RNA (Duffy & Holmes, 2008; Duffy & Holmes, 2009), ao contrário do que se esperaria por serem vírus de DNA e utilizarem a maquinaria de replicação do hospedeiro. As hipóteses mais aceitas para explicar as elevadas taxas de mutação são a possibilidade de não utilizarem o mecanismo de correção de erro do hospedeiro(Sanz *et al.*, 1999) ou a natureza do genoma de fita simples, no qual as bases estariam mais expostas a estresses oxidativos(Monjane *et al.*, 2012). Trabalhos nos quais se explorou a dinâmica temporal de begomovírus indicaram altas taxas de mutação, na ordem de 10^{-4} a 10^{-3} substituições/sítio/ano (Ge *et al.*, 2007; Duffy & Holmes, 2008; Duffy & Holmes, 2009; Harkins *et al.*, 2009).

A recombinação é o processo pelo qual indivíduos diferentes trocam parte de uma molécula de DNA ou RNA entre si (Padidam *et al.*, 1999). O processo de recombinação contribui para o aumento da diversidade genética, do potencial evolutivo e adaptativo (Lefeuvre & Moriones, 2015). O entendimento dos eventos de recombinação é valioso, pois pode ser determinante para o manejo de viroses no campo, visto que variantes recombinantes podem emergir e quebrar a resistência de cultivares (Monci *et al.*, 2002; Awadalla, 2003; Sattar *et al.*, 2013).

Eventos de recombinação têm sido diretamente implicados na emergência de begomovírus em plantas cultivadas. Por exemplo, a epidemia devastadora do mosaico da mandioca em Uganda e países vizinhos causada pelo recombinante EACMV-Ug2 (Zhou *et al.*, 1997; Pita *et al.*, 2001); as epidemias do complexo TYLCV no Mediterrâneo, com o surgimento dos recombinantes *Tomato yellow leaf curl Malaga virus* (TYLCMalV) e *Tomato yellow leaf curl Axarquia virus* (TYLCAxV) em tomateiro na Espanha (Monci *et al.*, 2002; García-Andrés *et al.*, 2006; García-Andrés *et al.*, 2007a; García-Andrés *et al.*, 2007b); e as epidemias de enrolamento das folhas do algodoeiro no Paquistão causadas por um complexo de bwgomovírus incluindo diversos recombinantes (Zhou *et al.*, 1998; Idris & Brown, 2002).

Os mecanismos precisos que controlam a recombinação em begomovírus permanecem desconhecidos (Lefeuvre & Moriones, 2015). No entanto, algumas regiões do genoma estão mais sujeitas a sofrerem recombinação ("hot spots") do que outras ("cold spots") (Stanley, 1995; Fauquet *et al.*, 2005; García-Andrés *et al.*, 2007a; Lefeuvre *et al.*, 2009). A origem de replicação viral é um "hot spot" de recombinação (Gutierrez, 1999; Hanley-Bowdoin *et al.*, 1999). A comparação de sequências de begomovírus mono- e bissegmentados indicou que a região do gene *Rep* que codifica a porção N-terminal da proteína Rep, assim como a região intergênica adjacente, são frequentemente intercambiadas durante a replicação. Também foram identificados sítios frequentes de recombinação localizados na região intergênica entre os genes *CP* e *Ren*, e sítios pouco frequentes na região do genes *CP* e *MP* (Hanley-Bowdoin *et al.*, 1999; Lefeuvre *et al.*, 2007).

Begomovírus bissegmentados estão sujeitos à pseudo-recombinação, que consiste na troca de componentes genômicos entre indivíduos diferentes, sem que haja quebra e religação da molécula de ácido nucleico (Gilbertson *et al.*, 1993; Paplomatas *et al.*, 1994; Sung & Coutts, 1995; Pita *et al.*, 2001; Andrade *et al.*, 2006). A pseudo-recombinação é geralmente observada entre indivíduos da mesma espécie que possuem alta similaridade de sequência na região comum (Lazarowitz, 1991; Von Arnim & Stanley, 1992a; Gilbertson *et al.*, 1993; Stanley, 1995). Diversos estudos têm demonstrado que begomovírus 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 (Von Arnim & Stanley, 1992b; Faria *et al.*, 1994). Essa assimetria indica que a pseudo-recombinação entre begomovírus envolve interações entre fatores virais e do hospedeiro (Hill *et al.*, 1998). A viabilidade de pseudo-recombinantes indica que fatores envolvidos na replicação e movimento são intercambiáveis entre estirpes de uma mesma espécie, ou mesmo entre espécies altamente relacionadas. 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.*, 2006; Bull *et al.*, 2007). Outro fator importante é a conservação da sequência de aminoácidos da proteína Rep, especialmente três aminoácidos do domínio relacionado aos iterons ("iteron-related domain", IRD), que estariam envolvidos diretamente na ligação aos iterons (Arguello-Astorga & Ruiz-Medrano, 2001). Entretanto, a formação de pseudo-recombinantes não depende somente da relação filogenética e conservação dos iterons, já que pseudo-recombinantes infecciosos foram formados entre o DNA-A do TGMV e o DNA-B do ToYSV, que possuem iterons distintos (Andrade *et al.*, 2006).

A nível genético, a evolução pode ser entendida como a variação na frequência de alelos ao longo das gerações (Escriu, 2017). Mudanças na estrutura genética são governadas por duas forças: seleção e deriva genética. Ambas levam à redução da diversidade genética. Isolar o efeito de cada uma é um desafio, visto que elas atuam concomitantemente (Escriu, 2017; Dolan *et al.*, 2018). Essas duas forças estão intimamente relacionadas à adaptabilidade dos indivíduos e ao tamanho efetivo das populações (Dolan *et al.*, 2018). Adaptabilidade ("fitness"), por sua vez, é definida como sendo a capacidade de um indivíduo em contribuir para a próxima geração. O tamanho efetivo da população é um conceito teórico que indica o tamanho de uma população ideal que terá a mesma variabilidade genética da população observável (Smith, 1989; Roossinck, 2008; Escriu, 2017; Dolan *et al.*, 2018).

A deriva genética é uma força evolucionária que leva à flutuação estocástica da frequência de variantes dentro de uma população viral. Gargalos aos quais as populações virais são submetidas resultam em deriva genetica, levando à redução da variabilidade genética pela redução de seu tamanho efetivo (Roossinck, 2008; Escriu, 2017; Dolan *et al.*, 2018). Os gargalos podem ocorrer em diferentes fases do ciclo de vida viral, por exemplo, durante o movimento sistêmico ou durante a transmissão entre hospedeiros (Sacristan *et al.*, 2003; Betancourt *et al.*, 2008). Gargalos genéticos sucessivos somados à ausência de recombinação podem levar ao acúmulo de mutações deletérias, um efeito denominado "catraca de Muller" (Muller, 1964), levando a população a um colapso mutacional. Por outro lado, os gargalos genéticos também são responsáveis pelo efeito fundador, que consiste no estabelecimento de uma nova população por uma pequena fração das variantes da população original (Novella *et al.*, 1995; Wang *et al.*, 2006). Assim, a deriva genética pode resultar tanto na fixação de variantes dentro de uma população, bem como na extinção da população (Fraile *et al.*, 1997).

A redução no tamanho efetivo da população resulta na redução da variabilidade genética, o que por sua vez resulta em uma forte estruturação geográfica e temporal, com uma distribuição aleatória dos alelos no espaço e no tempo (French & Stenger, 2005; Ali *et al.*, 2006). Populações de begomovírus no Brasil apresentam-se geograficamente estruturadas (Rocha *et al.*, 2013; Mar *et al.*, 2017), e um estudo no qual foi explorada a dinâmica populacional de *Euphorbia yellow mosaic virus* (EuYMV) constatou a flutuação aleatória de alelos dentro das subpopulações (Mar *et al.*, 2017). Embora seja difícil particionar a contribuição daderiva e da seleção, esses resultados sugerem um efeito significativo da deriva genética na evolução desses vírus.

A seleção é uma força evolucionária determinística que atua sob populações favorecendo os indivíduos melhor ajustados ao ambiente. Isso é resultado da reprodução diferencial entre as variantes dentro da população, e leva à redução da variabilidade genética. A seleção pode ser diversificadora, quando leva ao aumento da frequência de indivíduos melhor adaptados dentro de uma população, ou purificadora, quando leva à redução da frequência de variantes menos adaptadas (Ali & Roossinck, 2008).

O ambiente é o principal determinante para a ocorrência de seleção. Mudanças nas condições ambientais podem fornecer diferentes oportunidades adaptativas. A seleção natural atua sobre a diversidade fenotípica de genomas mutantes na população para direcionar as populações para o aumento da adaptabilidade (Dolan *et al.*, 2018). Na maior parte das vezes, mutações e eventos de recombinação são deletérios e tendem a ser eliminados da população.

Os vírus experimentam pressões seletivas durante todo seu ciclo de vida, seja durante a interação com os seus hospedeiros, seja durante as interações com seus vetores (Dolan *et al.*, 2018). Para os begomovírus a maioria dos genes está sob seleção negativa, entretanto constatou-se que a porção C-terminal do gene *AC4* tende a estar sob seleção positiva, indicando a evolução independente de diferentes regiões do genoma viral, com os genes sobrepostos *Rep* e *AC4* sob pressões evolutivas distintas (Melgarejo *et al.*, 2013; Yang *et al.*, 2014).

O hospedeiro consiste numa importante fonte de pressão seletiva: hospedeiros diferentes propiciam diferentes oportunidades adaptativas. Evidências de seleção associadas ao hospedeiro vêm sendo documentadas em trabalhos demonstrando diferenciação de populações virais em relação ao hospedeiro. Uma outra fonte de pressão seletiva é a interação entre vírus e vetor. Interações complexas ocorrem entre vírus e vetor e essas podem, por exemplo, resultar no estabelecimento de relações mutualísticas. Por exemplo, insetos virulíferos podem ser impactados, positiva- ou negativamente, em termos de fecundidade, longevidade, comportamentio alimentar e ativação de mecanismos de defesa da planta contra insetos. Os vírus podem se beneficiar disso aumentando sua capacidade de dispersão (Moreno-Delafuente *et al.*, 2013; Luan *et al.*, 2014).

O Brasil é um centro de diversidade de begomovírus. Acredita-se que a emergência dos begomovírus que infectam plantas cultivadas no Brasil seja resultado de saltos de hospedeiros, que proporcionaram uma oportunidade adaptativa para emergência de novos vírus. A constatação da predominância de alguns vírus no campo pode ser devido a diferenças na adaptação ao hospedeiro cultivado ou diferenças na eficiência de transmissão pelo inseto vetor (Rocha *et al.*, 2013).

Os begomovírus economicamente mais importantes estão associados ao feijoeiro e o tomateiro (Faria & Maxwell, 1999; Zerbini *et al.*, 2005). Embora existam relatos de infecção por begomovírus em outras culturas importantes, como a soja (Mello *et al.*, 2000; Mello *et al.*, 2002) e o pimentão (Nozaki *et al.*, 2005), essas não causam grandes danos econômicos. Além das plantas cultivadas, muitas espécies silvestres e/ou invasoras têm sido relatadas como hospedeiras (Faria & Maxwell, 1999; Jovel *et al.*, 2004) (Barbosa *et al.*, 2009; Silva *et al.*, 2010; Fernandes *et al.*, 2011; Silva *et al.*, 2011; Silva *et al.*, 2012; Tavares *et al.*, 2012; Barreto *et al.*, 2013; Pinto *et al.*, 2016; Passos *et al.*, 2017a; Passos *et al.*, 2017b; Quadros *et al.*, 2019). As espécies comumente relatadas como hospedeiras pertencem às famílias Malvaceae, Euphorbiaceae e Fabaceae (Morales & Anderson, 2001). Estudos que abordam a dinâmica evolutiva das populações de begomovírus em plantas não-cultivadas podem fornecer informações importantes sobre o papel epidemiológico desses hospedeiros, atuando como fonte de diversidade viral, contribuindo para o surgimento de novas espécies ou variantes mais agressivas e, principalmente no período de entressafra, contribuindo para a permanência de vírus no campo na ausência do hospedeiro cultivado. Além disso, do ponto de vista ecológico, o estudo de populações virais em hospedeiros não cultivados permite que a evolução viral seja monitorada em ecossistemas naturais na ausência de gargalos impostos pela ação antrópica.

A maior parte dos trabalhos que investigaram a estrutura genética de populações de vírus de plantas foi conduzida avaliando hospedeiros cultivados, ou hospedeiros não-cultivados associados a áreas de cultivo. Em ambos os casos as populações virais estão sujeitas a gargalos genéticos impostos pela ação humana. Pouco se sabe sobre a dinâmica evolutiva de begomovírus em ambientes naturais. Além disso, esses trabalhos amostraram grandes áreas (centenas ou milhares de quilômetros quadrados) em um único ponto no tempo. Estudos em áreas menores e durante vários anos são necessários para verificar se a alta variabilidade genética relatada é um artefato derivado da estratégia de amostragem. A amostragem no mesmo local ao longo de vários anos também permitiria estudos sobre a coexistência de diferentes espécies ou variantes e sobre a evolução das populações virais ao longo do tempo.

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ARTIGO

TEMPORAL DYNAMICS OF A BEGOMOVIRUS COMMUNITY IN THE NATURAL ENVIRONMENT

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Temporal dynamics of a begomovirus community in the natural environment

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ABSTRACT

Viruses have high mutation rates, resulting in rapid diversification and a large adaptive potential. Thus, they are interesting models for evolutionary studies. Begomoviruses (family *Geminiviridae*) have a genome composed of one or two molecules of circular, single-stranded DNA, and cause diseases in several economically important crops. A great diversity of begomoviruses is present in non-cultivated hosts. Although a number of studies have addressed the temporal dynamics of begomoviruses, these studies were conducted in large areas and in the context of agricultural environments. We evaluated the temporal dynamics of a begomovirus community in plants of *Sida acuta* (Malvaceae) over a period of eight years in an area of approximately 0.1 ha. The begomovirus community coexists as a complex of different species and variants: *Oxalis yellow vein virus* (OxYVV), *Sida yellow leaf curl virus* (SiYLCV), and *Sida micrantha mosaic virus* (SimMV), with OxYVV and SiYLCV populations subdivided into three variants. The large fluctuation in the distribution of OxYVV variants over time reflects the adaptive differential of each variant and the impact of selection on viral populations in natural ecosystems. In addition, that recombination between OxYVV variants seems to have alleviated the impact of harmful mutations. The absence of a temporal signal in phylogenetic analyses suggests that the viral populations evolve at slow rates in natural environments. We hypothesize that genetic drift accelerates the evolution of viral populations, leading to segregation patterns over time.

INTRODUCTION

The *Geminiviridae* family is composed of viruses that have one or two genomic components of single-stranded circular ssDNA, encapsidated by a single structural protein forming a twinned icosahedral particle (Zerbini *et al.*, 2017). Based on genomic organization, host range, insect vector and phylogenetic relationship, the family is divided into nine genera- *Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Grablovirus, Eragrovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus* (Zerbini *et al.*, 2017).

The viruses classified in the genus *Begomovirus* have mono- or bisegmented genomes, infect dicotyledonous plants and are transmitted by whiteflies of the cryptic species complex *Bemisia tabaci* (Brown *et al.*, 2015; Zerbini *et al.*, 2017). This group of viruses constitutes one of the most important disease-causing agents in plants, causing significant losses in crops of economic importance such as beans, cassava, cotton and tomato, especially in tropical and subtropical regions (Polston & Anderson, 1997; Moriones & Navas-Castillo, 2000; Morales & Anderson, 2001; Legg & Fauquet, 2004; Inoue-Nagata *et al.*, 2016).

Brazil is considered a center of genetic diversity for begomoviruses, with reports of their detection since the 1940's (Orlando & Silberschmidt, 1946; Costa & Alves, 1950; Costa & Bennett, 1950). The most important diseases caused by begomoviruses in Brazil are the golden mosaic of beans, caused by *Bean golden mosaic virus* (BGMV), and the mosaic and wrinkling of leaves in tomato, caused by a complex of begomovirus species (Inoue-Nagata *et al.*, 2016). Dozens of begomoviruses have been described infecting tomato in Brazil (Fernandes *et al.*, 2006; Castillo-Urquiza *et al.*, 2008; Fernandes *et al.*, 2008; Albuquerque *et al.*, 2012; Macedo *et al.*, 2017; Quadros *et al.*, 2019), however, there is a great variation in their regional distribution and predominance, with only a few species being widely disseminated in the field (Rocha *et al.*, 2013). The predominance of few species could be explained by difference in fitness to tomato or differences in the efficiency of transmission by the whitefly vector (Rocha *et al.*, 2013). Several studies have demonstrated the presence of begomoviruses infecting other crops, such as sweet potato (Albuquerque *et al.*, 2011), passionfruit (Ferreira *et al.*, 2010), okra (Aranha *et al.*, 2011), pepper (Bezerra-Agasie *et al.*, 2006), cotton (Almeida *et al.*, 2013) and soybean (Coco *et al.*, 2013). However, epidemics and losses in these crops due to begomovirus infection have not been observed. In addition, a great diversity of begomoviruses has been reported in non-cultivated plants, especially from the *Fabaceae* and *Malvaceae* families (Faria & Maxwell, 1999; Jovel *et al.*, 2004; Silva *et al.*, 2010b; Silva *et al.*, 2012; Tavares *et al.*, 2012;

Fiallo-Olivé *et al.*, 2015; Pinto *et al.*, 2016; Ferro *et al.*, 2017; Passos *et al.*, 2017a; Passos *et al.*, 2017b; Quadros *et al.*, 2019).

It is believed that the emergence of begomoviruses that infect crop plants (in Brazil and elsewhere) is the result of the horizontal transfer, mediated by the whitefly vector, of indigenous viruses that infect non-cultivated plants. Three observations support this hypothesis. First, begomovirus phylogeny is strongly correlated with geography rather than host (Rybicki, 1994; Rocha *et al.*, 2013). Second, the biological characterization of some species (ToRMV, ToCMoV, ToYSV) confirmed that non-cultivated plants such as *Nicandra physaloides*, *Solanum nigrum* and *Datura stramonium* are hosts (Fernandes *et al.*, 2006; Calegario *et al.*, 2007; Ribeiro *et al.*, 2007). Finally, begomoviruses originally described in non-cultivated plants, such as SiMoV (Fernandes *et al.*, 1999) and SimMV (Jovel *et al.*, 2004), have already been reported to naturally infect crop plants such as beans and tomato (Cotrim *et al.*, 2007; Rocha *et al.*, 2013). Therefore, studies addressing the evolutionary dynamics of begomovirus populations in non-cultivated plants can provide important information about the epidemiological role of these hosts, by acting as a source of viral diversity, by contributing to the emergence of new species or more aggressive variants, and, especially in the off-season, by contributing to the permanence of viruses in the field in the absence of the cultivated host. In addition, from an ecological point of view, the study of viral populations in non-cultivated hosts allows viral evolution to be monitored in natural ecosystems in the absence of bottlenecks imposed by anthropic action.

Geminivirus populations, including begomoviruses, have a high degree of genetic variability, with nucleotide substitutions rates in the order of 10^{-3} substitutions/site/year (Ge *et al.*, 2007; Duffy *et al.*, 2008; Duffy & Holmes, 2009), which is equivalent to that of viruses with an RNA genome. Frequent recombination events (Padidam *et al.*, 1999; Lima *et al.*, 2013; Lefeuvre & Moriones, 2015), the occurrence of pseudo-recombination between viruses with bisegmented genome (Hou *et al.*, 1998; Andrade *et al.*, 2006) and high rates of molecular evolution (Rocha *et al.*, 2013) contribute to the high genetic variability of begomoviruses, increasing their adaptive potential to new environmental conditions and new hosts.

The genetic structure of populations refers to the amount and distribution of genetic variability within and between populations (García-Arenal *et al.*, 2001). Understanding the dynamics of the variability of plant virus populations in cultivated and non-cultivated hosts is important, as it allows us to understand how these populations evolve, with important implications regarding the effectiveness and durability of management strategies (Seal *et al.*, 2006a).

Studies of begomovirus populations in Brazil demonstrate that genetic variability of populations is an intrinsic property of each virus, regardless of whether the host is a cultivated or non-cultivated plant (Lima *et al.*, 2013; Rocha *et al.*, 2013; Ramos-Sobrinho *et al.*, 2014; Mar *et al.*, 2017). In addition, it has been demonstrated that populations of begomoviruses in both types of hosts are structured based on geographical origin (Rocha *et al.*, 2013; Mar *et al.*, 2017).

Much of the work on the evolutionary dynamics of begomovirus populations conducted in Brazil and elsewhere has sampled large areas (hundreds or thousands of square kilometers) at a single point in time. Studies in smaller areas and during several years are necessary to verify whether the high genetic variability that has been reported is an artifact derived from the sampling strategy. Sampling at the same location over several years would also allow studies on the coexistence of different species or variants and on the evolution of viral populations over time. This work aimed to evaluate the molecular evolution of a population of begomoviruses infecting the non-cultivated host *Sida acuta*, in an area of approximately 0.1 ha during eight years, with minimal human interference.

MATERIALS AND METHODS

Sample collection

Samples of *Sida acuta* plants showings typical symptoms of begomovirus infection were collected in a 1,000 m^2 fragment of Atlantic Forest undergoing regeneration in Viçosa, MG (Figure 1). Sampling was carried out from 2011 to 2018, with the plants being collected in December of each year, excepted in 2015 when there was no collection. Data of collection, geographic coordinates and an image of each sample were recorded. The collected samples were identified and stored as herbarium samples at room temperature at the Laboratório de Virologia Vegetal e Molecular of Universidade Federal de Viçosa (UFV).

Cloning and sequencing of viral genomes

Total DNA was extracted according to Doyle & Doyle (1987) and used as a template for the amplification of complete genomes using rolling-circle amplification (RCA; (Inoue-Nagata *et al.*, 2004). The RCA products were cleaved with different restriction enzymes and analyzed on agarose gels (0.8%). Aliquots of cleavage reactions that showed a fragment of approximately 2,600 nucleotides, corresponding to the DNA-A or DNA-B components of bipartite begomoviruses, were cloned into the pBLUESCRIPT-KS+ plasmid vector

(Stratagene), previously cleaved with the same enzyme and dephosphorylated. Recombinant plasmids were transformed into *Escherichia coli* by electroporation (Sambrook & Russel, 2001), and clones were completely sequenced at Macrogen Inc. (Seoul, South Korea).

Assembly and comparison of sequences

The sequences of the genomic components were assembled using Geneious 8.1 (Kearse *et al.*, 2012), and arranged to start from the first nucleotide of the cleavage site at the origin of replication (TAATATT//AC). The sequences were initially analyzed using the BLASTn algorithm (Altschul *et al.*, 1990) to determine the viral species with the highest sequence identities. The sequences of these species were retrieved from GenBank and used to calculate pairwise sequence identities with the Species Demarcation Tool program (Muhire *et al.*, 2013). Analysis of the genomic organization was performed using the Fangorn Forest method implemented in Geminivirus Data Warehouse (Silva *et al.*, 2017).

Phylogenetic analysis

Multiple sequence alignments were obtained for the full-length DNA-A and for nucleotide sequences corresponding to the *CP* and *Rep* genes, using the MUSCLE algorithm implemented in the MEGAX program (Kumar *et al.*, 2018).

Phylogenetic trees were built using Bayesian inference with the MrBayes 3.0b4 program (Ronquist & Huelsenbeck, 2003), using the models selected with MrModeltest 2.2 (Nylander, 2004) in the Akaike Information Criterion (AIC). The analysis was performed using 20 million generations and the first 2 million generations were excluded. Phylogenetic trees were visualized using FigTree (tree.bio.ed.ac.uk/software/figtree/).

Genetic structure and variability of viral populations

Nucleotide polymorphism analyses within and between subpopulations were performed using DnaSP v. 5 (Rozas *et al.*, 2003). Estimates of genetic variability were obtained with the following descriptors- nucleotide diversity (π), number of haplotypes (h) and haplotypic diversity (Hd). The partition of genetic variability and inferences about the genetic structure of the population were based on Wright's F fixation index (Wright, 1951).

Recombination analysis

To investigate the occurrence of recombination events, multiple alignments of full-length DNA-A sequences were analyzed with the Rdp, Geneconv, Bootscan, Maximum $\chi 2$, Chimaera, Siscan and 3Seq methods implemented in the RDP4 package (Martin *et al.*, 2015). Statistical significance was inferred by *P*-values lower than a Bonferroni-corrected cut-off of 0.05. Only recombination events detected by at least four methods were considered reliable.

Selection analysis

Sites under negative or positive selection in the *CP*, *Rep*, *TrAP*, *Ren* and *AC4* genes were identified using three maximum likelihood-based methods- Single Likelihood Ancestor Counting (SLAC), Fixed Effects Likelihood (FEL) and Random Effects Likelihood (REL) (Kosakovsky-Pond & Frost, 2005b), implemented in the DataMonkey server (Kosakovsky-Pond & Frost, 2005a). The average ratio of synonymous to non-synonymous substitutions (dN/dS) was estimated using the SLAC method.

Detection of species and their variants, mixed infections and pseudorecombination

To verify the frequency of species or variants, the occurrence of mixed infections and possible pseudorecombination, a restriction map was constructed using *Msp*I, a four-base cutter restriction enzyme which generates different restriction patterns for the DNA-A and DNA-B of each species or variant.

RESULTS

Sida acuta is a permissive host for begomoviruses

A total of 220 samples of *S. acuta* were collected between 2011 and 2018, of which 184 were positive for infection by a begomovirus based on the restriction pattern of RCA products obtained from total DNA extracts. From the positive samples, a data set of 231 sequences was built, of which 167 sequences correspond to a DNA-A component and 64 sequences correspond to a DNA-B component (Table 1).

BLAST*n* analysis indicated that 125 DNA-A sequences share at least 92.6% identity with *Oxalis yellow vein virus* (OxYVV), and therefore constitute isolates of this species according to the species demarcation criteria defined by the *Geminiviridae* Study Group of the ICTV (Brown *et al.*, 2015). An additional 36 DNA-A sequences share at least 96.5% identity with *Sida yellow leaf curl virus* (SiYLCV), and 6 DNA-A sequences share at least 97.4% identity with *Sida micrantha mosaic virus* (SimMV). Thus, three different viruses were detected in the area during the eight years of sample collection.

Pairwise identity comparisons using SDT indicated the segregation of OxYVV isolates into three distinct groups (Figure 2; Table 2). Isolates within each group display 99-100% identity, while the identity between isolates in different groups ranges from 95 to 96%. The SiYLCV population can also be subdivided into three variants, with isolates within the same group displaying 98-100% identity, and the identity between isolates from different groups ranging from 96.9 to 97.8%. For both species, the three groups cannot be classified as distinct strains based on the ICTV criteria, as the cut-off value for strain demarcation is 94% (Brown *et al.*, 2015). Therefore, they will be referred to in this work as variants. Interestingly, two strains of SimMV were found coexisting in the area, with isolates displaying 93.3-100% sequence identity (Figure 2; Table 2).

These results indicate that *S. acuta* is a permissive host for begomoviruses, capable of harboring several distinct viruses. This can contribute to the emergence of new species or variants. Therefore, this host has an important role from ecological and epidemiological perspectives.

Recombination analysys

Two putative recombination events were detected in the DNA-A data set (167 sequences). An interspecific recombination event was detected in the OxYVV isolate BR-Vic303.2-2014, with SiYLCV-[BR-Vic501-2016] as the major parent and SimMV-[BR-Vic608-2018] as the minor parent (Table 3). An intraspecific event was detected in the SimMV isolate BR-Vic628-2018, with the major parent unknown and SimMV-[BR-Vic608-2018] as the minor parent (Table 3). Both events had breakpoints located between the *Rep* gene and the common region. No recombination event was detected in a SiYLVC isolate, and no intraspecific recombination was detected in the OxYVV and SiYLCV isolates.

The begomovirus population in *S. acuta* coexists as a complex of different species and variants

The phylogenetic tree based on the complete sequences of OxYVV DNA-A displays three well-defined clusters, with no evidence of temporal segregation of the isolates in either one of the three clades (Figure 3). Together with the results of pairwise sequence comparisons, the OxYVV isolates can therefore be divided into three variants, named OxYVV-I, OxYVV-II and OxYVV-III. Variant OxYVV-I has the largest number of isolates, followed by variant OxYVV-III and finally OxYVV-II. Corroborating the proposed division, genetic differentiation between each variant was high, with an Nst value of 0.958 between OxYVV-I and OxYVV-III, 0.915 between OxYVV-I and OxYVV-II, and 0.933 between OxYVV-II and OxYVV-III.

The SiYLCV population has a similar pattern of subdivision, with three phylogenetic clades that correspond to the same three groups identified in the pairwise sequence comparisons (Figure 3). The three variants were named based on their distribution pattern, with variant SiYLCV-I predominating followed by variants SiYLCV-II and SiYLCV-III. Due to the small size of the data set, it was not possible to calculate genetic differentiation indexes between the three SiYLCV variants.

Phylogenetic analysis were also performed for the *CP* and *Rep* genes. For OxYVV isolates, the *Rep* gene tree displays the same topology as the DNA-A tree, with three clades corresponding to variants OxYVV-I, OxYVV-II and OxYVV-III (Figure 4). However, the *CP* gene tree displays a slightly different topology. Although it also contains the same three clades corresponding to the three variants, the clade corresponding to variant OxYVV-III clusters closer to OxYVV-I than to OxYVV-II (Figure 5), different from the DNA-A and *Rep* gene trees. Furthermore, the isolate BR-Vic309-14, belonging to group/variant OxYVV-III in the pairwise sequence comparisons and in the DNA-A and *CP* trees, clustered with OxYVV-I in the *Rep* tree (Figures 2-5). Although no intraspecific recombination events were detected among OxYVV isolates, the clustering pattern of the BR-Vic309-14 isolate suggests a recombinant origin for thise isolate. The failure to detected intraspecific recombination events could be due to the high sequence identity among isolates, which makes it difficult to detect recombinant blocks by the methods implemented in the RDP package.

For SiYLCV isolates, the *CP* gene tree displays the same topology as the DNA-A tree, with the same three clades corresponding to the three variants (Figure 5). However, the *Rep* tree displays two clades, one correspnding to SiYLCV-III and the other including both SiYLCV-I and SiYLCV-II isolates (Figure 4), suggesting that individuals from these two variants share a closely related *Rep* gene, maybe due to recombination. As for OxYVV, the failure to detect intraspecific recombination events in the SiYLCV data set could be due to the high degree of sequence identity among the isolates.

Genetic variability analysis

Variability descriptors were calculated for each species and also for each OxYVV variant (Table 4). The results showed that the OxYVV population, although subdivided into three variants, has low genetic variability (π=0,01633; Table 4). Likewise, the SiYLCV population, also divided into three variants, presents even lower variability (π=0,00917; Table

4). The genetic variability of OxYVV variants I, II and III, when analyzed separately, is even lower compared to the variability of the entire population, which is in accordance with the proposed subdivision. Comparison between variants was not possible for SiYLCV due to the small number of sequences of the less predominant variants.

Non-synonymous substitutions in the *CP* and *Rep* genes mark the difference between OxYVV variants

Seventeen non-synonymous substitutions were identified in the *CP* gene. A single sub-institution, changing a tyrosine (hydrophilic) to a phenylalanine (hydrophobic), was able to demarcate the difference between the OxYVV-II variant and variants OxYVV-I and OxYVV-III (Figure 6A). The other 16 substitutions occurred at low frequencies, appearing in one or a few sequences.

The Rep protein showed a greater number of non-synonymous substitutions, totaling 50, most of them occurring in unique sequences. Eleven mutations were relevant to differentiate the OxYVV-II variant from the others, with the exception of the isolate BR-Vic309-14, which has a Rep that is more related to OxYVV- I likely due to a recombination event between OxYVV-I and OxYVV-II. Seven amino acids made it possible to separate the OxYVV-I variant from the others. Lastly, the differences between the OxYVV-I and OxYVV-II variants are due to four substitutions. Three substitutions were identified within conserved domains associated with replication. No fixation pattern was observed over time (Figure 6B).

Selection analysis

We calculated the ratio of synonymous to non-synonymous substitutions for each coding region of the OxYVV and SiYLCV genomes. In all cases the dN/dS ratio was <1, indicating that all regions of the OxYVV are under negative (purifying) selection (Table 5). Using the SLAC method, sites under negative selection were detected for most of the genes in the OxYVV DNA-A, except *Trap*. With the FEL method it was possible to identify several sites under negative selection for all OxYVV DNA-A genes. Evidence of positive selection was identified by the REL method in the OxYVV *Rep* and *TrAP* genes (Table 5).

For SiYVV the dN/dS values were also <1, again indicating that all genes of the DNA-A are under the effect of purifying selection (Table 5). The SLAC method identified a single one site under negative selection, in the *Rep* gene. With the FEL method several sites in four DNA-A genes were identified as under negative selection. Evidence of positive selection was identified by the REL method (Table 5).

Due to the limited number of sequences, it was not possible to perform these analyses for the SimMV data set.

Temporal dynamics of the viral community in *Sida acuta*

Based on RFLP patterns (Figure 8), it was possible to identify each of the species and their respective variants in the context of single and mixed infection, as well as pseudorecombinants among OxYVV variants. Pseudorecombination was not identified among SiYLCV isolates, and SimMV was only detected in single infection.

To verify the temporal dynamics of the viral community that infects *S. acuta* in the sampled area, the proportion between individuals of each species (OxYVV, SiYLCV and SimMV) and their respective variants, in each year, were calculated based on the number of sequences and RFLP patterns (Figure 7). In the first year (2011) only OxYVV was found, subdivided into three variants. Interestingly, OxYVV-I was prevalent over almost the entire sampling period compared to variants OxYVV-II and OxYVV-III, except in 2016, when its frequency was drastically reduced and variant OxYVV-II was the most frequent. OxYVV-III was always present in a lower frequency compared to OxYVV-I, and although it was more prevalent than OxYVV-II in 2011, its frequency gradually reduced during the sampling period until disappearing in 2014. A single OxYVV-III isolate was found in 2017, and again it was not detected in 2018. OxYVV-II was found only in 2011, 2012 and 2016, always at a very low frequency (Figure 7 and Table 6). Therefore, 2011 and 2012 were the only years in which all three OxYVV variants were detected.

SiYLCV appeared in the second year of collection (2012) at a low frequency, but in 2016 it surpassed OxYVV in terms of frequency. The two species were balanced in 2017, and in 2018 OxYVV was again the species with the highest frequency. The two SimMV strains appear only in 2018 at a low frequency (Figure 7 and Table 6).

DISCUSSION

Understanding the temporal dynamics of complex populations, the conflicting demands established between different variants competing for the same resources, and the evolutionary forces that ensure that viral populations are structured in the form of complexes of variants, provides us with important information on the potential risks of epidemic fractures, emergence of new diseases and reemergence of known diseases, besides providing the basis for effective and durable management strategies (Seal *et al.*, 2006a; Escriu, 2017; Rojas *et al.*, 2018; Nurtay *et al.*, 2019).

In this work, we investigated the temporal evolutionary dynamics of a begomovirus population in a natural ecosystem. Systematic samplings were carried out over eight years in a small area in the municipality of Viçosa (MG), where *Sida acuta* plants, showing typical symptoms of begomovirus infection, were collected. Although some studies have already been carried out addressing the evolutionary dynamics of begomovirus populations in non-cultivated plants (Lima *et al.*, 2013; Rocha *et al.*, 2013; Ramos-Sobrinho *et al.*, 2014; Mar *et al.*, 2017), the time dynamics in a small scale has been little explored, and little is known about how these populations behave over time.

Remarkably, even in such a small area, three different viral species were identified, two of which are subdivided into three variants. This suggests that *Sida acuta* constitutes a "mixing vessel" type of host, which can harbor several distinct species of begomovirus (García-Arenal & Zerbini, 2019). Such hosts are important both from evolutionary and epidemiological perspectives. Housing a great viral biodiversity, they constitute a permissive environment for mixed viral infections and offer the opportunity for recombination events, contributing to the emergence of new species or variants and representing a potential risk for agricultural systems (Zhou *et al.*, 1997; Pita *et al.*, 2001; Idris & Brown, 2002; Monci *et al.*, 2002; García-Andrés *et al.*, 2007b).

Viral populations are heterogeneous and exist as complexes made up of different variants generated by diversifying processes such as mutation and recombination. Generally, one variant predominates and other variants are maintained at low frequencies by selection (García-Arenal *et al.*, 2001; Nurtay *et al.*, 2019). We observed this behavior for the OxYVV, SiYLCV and SiMMV populations from *S. acuta*, each comprised of three variants or strains that coexist in time but which are present at different frequencies. Furthermore, changes in the composition of the population were observed over time.

In most years we observed the prevalence of variant OxYVV-I over the others. We also observed the disappearance of the OxYVV-III variant after 2014. The OxYVV-II variant was detected at low frequencies in almost all years, except in 2016, when it overcame the OxYVV-I variant in terms of frequency. The general prevalence of the OxYVV-I variant may be the result of an adaptive difference, which would also explain the low frequency of the other two variants and the disappearance of the OxYVV-III variant.

We also observed events of pseudorecombination occurring between the OxYVV variants. We detected DNA-A components of OxYVV-I replicating together with DNA-Bs of both OxYVV-I and OxYVV-III, that is, a mix of DNA-B components. We also detected two distinct DNA-A components, OxYVV-I and OxYVV-III, sharing a single DNA-B, in this case from OxYVV-III. Pseudorecombination between OxYVV-I and OxYVV-III variants was found, but the frequency of detection of OxYVV-I DNA-A associated with OxYVV-III DNA-B was more frequent than the reciprocal combination. This observation deserves to be better studied to understand if this phenomenon occurs at random or if there is any mechanism that directs it in this direction.

SiYLCV was first detected in 2012, when a single isolate was obtained. However, a sudden change in the composition of species in the begomovirus community occurred in 2016, when SiYLCV became prevalent over OxYVV. The most plausible explanation for this phenomenon is the occurrence of a random event that would have caused a disturbance in the system, leading to a reduction in the effective size of the OxYVV population and creating an adaptive opportunity for the emergence of SiYLCV. It has been proposed that random events such as genetic bottlenecks can lead to a change in the composition of a population, due to a reduction in haplotypes close to fixation that would favor suboptimal haplotypes (Zwart & Elena, 2015; Kutnjak *et al.*, 2017). Corroborating this hypothesis is the fact that the OxYVV-II variant was detected at a higher frequency than the OxYVV-I variant in 2016. Moreover, in the following years, the number of OxYVV-I individuals increased and the number of OxYVV-II individuals decreased, indicating a tendency of the system to return to the initial equilibrium. However, the time series after the disturbance is still short, so that inferences about the resilience of the systems have little experimental support. Samples continue to be collected at the same location (the 2019 samples are being processed), and more robust information about will certainly be generated over the next few years.

We can speculate that the emergence of SiMMV in 2018 is probably the result of migratory processes, since it was the first year that this species was detected in this area. However, we cannot rule out the hypothesis that this species was present in the area at a low frequency, making its detection difficult. In this second hypothetical scenario, possible genetic bottlenecks may have contributed to the emergence of this species at the site, supporting our hypothesis presented above.

The incongruence observed between the phylogenies of the CP and Rep genes for both OxYVV and SiYLCV suggests their recombinant origin, with a possible intraspecific recombination event. However, no intraspecific recombination event was detected wih the RDP package. It should be taken into account that recombination events between sequences with high identity (>95% among OxYVV isolates and >96.9% among SiYLCV isolates) are difficult (if not impossible) to detect with RDP. Considering the occurrence of mixed infection with variants of the same virus (for example, in sample 4, infected by OxYVV-I and OxYVV-III; Table 1), the possibility of recombination should be considered.

Among the non-synonymous mutations observed in the *Rep* protein region detected only in the OxYVV-II and OxYVV-III variants, three are located in functional motifs: $E_{318}D$ in motif III (the catalytic site for DNA cleavage), $G_{670}S$ located in the Walker A (NTP-binding) motif, and $V_{781}I$ located in the Walker B motif. Although all three changes involve an exchange for amino acids of the same class, we speculate that these mutations have incurred adaptive costs for the OXYVV-II and OXYVV-III variants, which could explain their lower frequencies and the disappearance of the OXYVV-III variant.

It is intriguing how the OxYVV-II variant, which has always been present at a low frequency, has managed to maintain itself. It is possible that the recombination event from which it originated conferred it a slight adaptive advantage that allowed it to remain within the population even in low frequency. Since the OxYVV-II variant shares part of the genome of the (possibly more adapted) OxYVV-I variant and part of the (possibly less adapted) OxYVV-III, we speculate that its presence over time is the result of an epstatic effect of a OxYVV-I gene on a OxYVV-III gene, reducing the latter's disadvantageous effect and causing this variant to remain within the population for a period longer than one of its parents (OxYVV-III), which was eliminated from the population.

Begomoviruses constitute an interesting model for evolutionary studies due to their high evolutionary rates (Ge *et al.*, 2007; Duffy & Holmes, 2008; Duffy & Holmes, 2009). We used OxYVV in a natural ecosystem as a model to study evolution. Contrary to what was observed for EACMV (Duffy & Holmes, 2009), it was not possible to observe a temporal sign of segregation in our phylogenetic analysis. However, the EACMV studies were conducted in cultivated environments, in which the viral populations are subjected to successive bottlenecks. It is possible that the OxYVV population is evolving at slower rates due to the greater genetic stability of the environment where it is located, suffering little influence from bottlenecks imposed by anthropic action, unlike what occurs in agricultural systems. Although no time-based structuring was detected, the data indicates the occurrence of a change in the composition of the OxYVV population over time, suggesting an effect of selection as the main evolutionary force acting in natural systems. A corolary of our results would be that in areas where viral populations are subject to successive bottlenecks, the main evolutionary force driving their evolution would be genetic drift. Further studies will be carried out to test this hypothesis.

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Table 1. List of *Sida acuta* samples collected in Viçosa, MG, from 2011 to 2018, and the corresponding begomovirus clones obtained from each sample.

Sample	**Year**	**Isolate**	**Component**	**Enzyme**	**Species/ variant**
01	2011	BR-Vic01D5C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic01D1B	DNA-B	*Bam*HI	OxYVV-I
03	2011	BR-Vic03D1C	DNA-A	*Cla*I	OxYVV-II
		BR-Vic03D3C	DNA-A	*Cla*I	OxYVV-II
		BR-Vic03D1B	DNA-B	*Bam*HI	OxYVV-II
		BR-Vic03D1B1	DNA-B	*Bam*HI	OxYVV-II
		BR-Vic03D2B	DNA-B	*Bam*HI	OxYVV-II
04	2011	BR-Vic04D1C	DNA-A	*Cla*I	OxYVV-III
		BR-Vic04D1EV	DNA-A	*Eco*RV	OxYVV-III
		BR-Vic04D3C	DNA-A	*Cla*I	OxYVV-I
05	2011	BR-Vic05D1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic05D3B	DNA-B	*Bam*HI	OxYVV-I
06	2011	BR-Vic06D1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic06D2EV	DNA-A	*Eco*RV	OxYVV-I
		BR-Vic06D1B	DNA-B	*Bam*HI	OxYVV-I
07	2011	BR-Vic07D1C1	DNA-A	*Cla*I	OxYVV-I
		BR-Vic07D2C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic07D1B	DNA-B	*Bam*HI	OxYVV-I
		BR-Vic07D2B	DNA-B	*Bam*HI	OxYVV-I
09	2011	BR-Vic09D1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic09D3EV	DNA-A	*Eco*RV	OxYVV-I
		BR-Vic09D1B	DNA-B	*Bam*HI	OxYVV-I
10	2011	BR-Vic10D3C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic10D1B	DNA-B	*Bam*HI	OxYVV-I
12	2011	BR-Vic12D1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic12D2C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic12D1B	DNA-B	*Bam*HI	OxYVV-I
13	2011	BR-Vic13D1B	DNA-B	*Bam*HI	n.d.
		BR-Vic13D1B1	DNA-B	*Bam*HI	n.d.
14	2011	BR-Vic14D1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic14D2B	DNA-B	*Bam*HI	OxYVV-I
15	2011	BR-Vic15D2C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic15D2C1	DNA-A	*Cla*I	OxYVV-I
		BR-Vic15D5C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic15D1B	DNA-B	*Bam*HI	OxYVV-I

		BR-Vic15D1C	DNA-B	*Cla*I	OxYVV-I
16	2011	BR-Vic16D2C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic16D2C1	DNA-B	*Cla*I	OxYVV-I
		BR-Vic16D3C	DNA-B	*Cla*I	OxYVV-I
18	2011	BR-Vic18D1EV	DNA-A	*Eco*RV	OxYVV-III
		BR-Vic18D1C	DNA-B	*Cla*I	OxYVV-III
19	2011	BR-Vic19D1C	DNA-A	*Cla*I	OxYVV-III
		BR-Vic19D2C	DNA-A	*Cla*I	OxYVV-III
		BR-Vic19D1B	DNA-B	*Bam*HI	OxYVV-III
20	2011	BR-Vic20D1C	DNA-B	*Cla*I	n.d.
23	2011	BR-Vic23D4C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic23D1B	DNA-B	*Bam*HI	OxYVV-I
24	2011	BR-Vic24D1C	DNA-A	*Cla*I	OxYVV-III
27	2011	BR-Vic27D1C	DNA-B	*Cla*I	n.d.
32	2011	BR-Vic32D2B	DNA-B	*Bam*HI	n.d.
36	2011	BR-Vic36D1C	DNA-A	*Cla*I	OxYVV-III
40	2011	BR-Vic40D2C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic40D1C	DNA-B	*Cla*I	OxYVV-I
41	2011	BR-Vic41D3C	DNA-A	*Cla*I	OxYVV-III
		BR-Vic41D3C1	DNA-B	*Cla*I	OxYVV-III
42	2011	BR-Vic42D1C	DNA-B	*Cla*I	n.d.
		BR-Vic42D2C	DNA-B	*Cla*I	n.d.
44	2011	BR-Vic44D1B	DNA-B	*Bam*HI	n.d.
		BR-Vic44D3B	DNA-B	*Bam*HI	n.d.
45	2011	BR-Vic45D1EV	DNA-A	*Eco*RV	OxYVV-I
46	2011	BR-Vic46D1B	DNA-B	*Bam*HI	n.d.
		BR-Vic46D2C	DNA-B	*Cla*I	n.d.
47	2011	BR-Vic47D1C	DNA-A	*Cla*I	OxYVV-III
		BR-Vic47D2C	DNA-A	*Cla*I	OxYVV-III
104	2012	BR-Vic104-4EV	DNA-A	*Eco*RV	OxYVV-I
105	2012	BR-Vic105-3EV	DNA-A	*Eco*RV	OxYVV-I
106	2012	BR-Vic106-1EV	DNA-A	*Eco*RV	OxYVV-I
		BR-Vic106-5EV	DNA-A	*Eco*RV	OxYVV-I
111	2012	BR-Vic111-1EV	DNA-A	*Eco*RV	OxYVV-I
		BR-Vic111-2EV	DNA-A	*Eco*RV	OxYVV-I
112	2012	BR-Vic112-1EV	DNA-A	*Eco*RV	OxYVV-III
114	2012	BR-Vic114-1C	DNA-A	*Cla*I	OxYVV-II
116	2012	BR-Vic116-5EV	DNA-A	*Eco*RV	OxYVV-III
117	2012	BR-Vic117-3EV	DNA-A	*Eco*RV	OxYVV-III

119	2012	BR-Vic119-1EV	DNA-A	*Eco*RV	OxYVV-I
120	2012	BR-Vic120-3EV	DNA-A	*Eco*RV	OxYVV-I
123	2012	BR-Vic123-2EV	DNA-A	*Eco*RV	OxYVV-I
125	2012	BR-Vic125-2EV	DNA-A	*Eco*RV	SiYLCV
126	2012	BR-Vic126-1EV	DNA-A	*Eco*RV	OxYVV-I
		BR-Vic126-2EV	DNA-B	*Eco*RV	OxYVV-I
128	2012	BR-Vic128-4EV	DNA-A	*Eco*RV	OxYVV-I
131	2012	BR-Vic131-1EV	DNA-A	*Eco*RV	OxYVV-I
132	2012	BR-Vic132-2EV	DNA-A	*Eco*RV	OxYVV-I
133	2012	BR-Vic133-6C	DNA-A	*Cla*I	OxYVV-I
136	2012	BR-Vic136-1-C	DNA-A	*Cla*I	OxYVV-III
201	2013	BR-Vic201-1C	DNA-A	*Cla*I	OxYVV-III
206	2013	BR-Vic206-PT	DNA-A	*Pte*I	OxYVV-I
211	2013	BR-Vic211-2C	DNA-A	*Cla*I	OxYVV-I
212	2013	BR-Vic212-1BS	DNA-A	*Bss*HII	OxYVV-I
215	2013	BR-Vic215-1BS	DNA-A	*Bss*HII	OxYVV-I
218	2013	BR-Vic218-1C	DNA-A	*Cla*I	OxYVV-I
220	2013	BR-Vic220-1BS	DNA-A	*Bss*HII	OxYVV-I
225	2013	BR-Vic225-4BS	DNA-A	*Bss*HII	OxYVV-I
226	2013	BR-Vic226-1C	DNA-A	*Cla*I	OxYVV-I
227	2013	BR-Vic227-4BS	DNA-A	*Bss*HII	OxYVV-I
228	2013	BR-Vic228-1C	DNA-A	*Cla*I	OxYVV-I
229	2013	BR-Vic229-2C	DNA-A	*Cla*I	OxYVV-I
231	2013	BR-Vic231-2BS	DNA-A	*Bss*HII	OxYVV-III
232	2013	BR-Vic232-2BS	DNA-A	*Bss*HII	OxYVV-I
233	2013	BR-Vic233-1C	DNA-A	*Cla*I	OxYVV-I
237	2013	BR-Vic237-1BS	DAN-A	*Bss*HII	OxYVV-I
253	2013	BR-Vic253-2BS	DNA-A	*Bss*HII	OxYVV-I
256	2013	BR-Vic256-1BS	DNA-A	*Bss*HII	OxYVV-I
301	2014	BR-Vic301-1PT	DNA-B	*Pte*I	n.d.
303	2014	BR-Vic303-1BS	DNA-A	*Bss*HII	OxYVV-I
		BR-Vic303-2-BS	DNA-A	*Bss*HII	OxYVV-I
304	2014	BR-Vic304-6C	DNA-B	*Cla*I	n.d.
307	2014	BR-Vic307-1BS	DNA-A	*Bss*HII	OxYVV-I
309	2014	BR-Vic309-1PT	DNA-A	*Pte*I	OxYVV-III
310	2014	BR-Vic310-1C	DNA-A	*Cla*I	OxYVV-I
		BR-Vic310-2C	DNA-B	*Cla*I	OxYVV-I
311	2014	BR-Vic311-2-BS	DNA-A	*Bss*HII	OxYVV-I

		BR-Vic311-6-BS	DNA-A	*Bss*HII	OxYVV-I
312	2014	BR-Vic312-2BS	DNA-A	*Bss*HII	OxYVV-I
313	2014	BR-Vic313-2BS	DNA-A	*Bss*HII	OxYVV-I
315	2014	BR-Vic315-1C	DNA-A	*Cla*I	OxYVV-I
317	2014	BR-Vic317-2BS	DNA-A	*Bss*HII	OxYVV-I
318	2014	BR-Vic318-1BS	DNA-A	*Bss*HII	OxYVV-I
319	2014	BR-Vic319-2BS	DNA-A	*Bss*HII	OxYVV-I
320	2014	BR-Vic320-2BS	DNA-A	*Bss*HII	OxYVV-I
321	2014	BR-Vic321-10BS	DNA-A	*Bss*HII	OxYVV-I
323	2014	BR-Vic323-3C	DNA-B	*Cla*I	OxYVV-I
		BR-Vic323-6C	DNA-A	*Cla*I	OxYVV-I
325	2014	BR-Vic325-6PT	DNA-A	*Pte*I	OxYVV-I
326	2014	BR-Vic326-1BS	DNA-A	*Bss*HII	OxYVV-I
		BR-Vic326-6BS	DNA-A	*Bss*HII	OxYVV-I
327	2014	BR-Vic327-1BS	DNA-A	*Bss*HII	OxYVV-I
329	2014	BR-Vic329-4BS	DNA-A	*Bss*HII	OxYVV-I
331	2014	BR-Vic331-6PT	DNA-A	*Pte*I	OxYVV-I
334	2014	BR-Vic334-19EV	DNA-A	*Eco*RV	OxYVV-I
335	2014	BR-Vic335-3PT	DNA-A	*Pte*I	OxYVV-I
337	2014	BR-Vic337-5PT	DNA-A	*Pte*I	OxYVV-I
338	2014	BR-Vic338-1PT	DNA-A	*Pte*I	OxYVV-I
401	2016	BR-Vic401-1BS	DNA-A	*Bss*HII	OxYVV-II
403	2016	BR-Vic403-5BS	DNA-A	*Bss*HII	SIYLCV
404	2016	BR-Vic404-1BS	DNA-B	*Bss*HII	n.d.
		BR-Vic404-3PT	DNA-B	*Bss*HII	n.d.
		BR-Vic404-9BS	DNA-B	*Bss*HII	n.d.
405	2016	BR-Vic405-1PT	DNA-A	*Pte*I	OxYVV-II
406	2016	BR-Vic406-2BS	DNA-A	*Bss*HII	OxYVV-II
407	2016	BR-Vic407-18C	DNA-A	*Cla*I	OxYVV-II
408	2016	BR-Vic408-1PT	DNA-A	*Pte*I	OxYVV-II
409	2016	BR-Vic409-2PT	DNA-A	*Pte*I	OxYVV-I
		BR-Vic409-3PT	DNA-A	*Pte*I	OxYVV-I
		BR-Vic409-6PT	DNA-A	*Pte*I	OxYVV-I
410	2019	BR-Vic410-13PT	DNA-A	*Pte*I	OxYVV-I
411	2016	BR-Vic411-3PT	DNA-B	*Pte*I	n.d.
412	2016	BR-Vic412-12EV	DNA-A	*Eco*RV	SiYLCV
414	2016	BR-Vic414-16PT	DNA-B	*Pte*I	n.d.
415	2016	BR-Vic415-5BS	DNA-A	*Bss*HII	SiYLCV
		BR-Vic415-7BS	DNA-A	*Bss*HII	SiYLCV

416	2016	BR-Vic416-2BS	DNA-A	*Bss*HII	SiYLCV
		BR-Vic416-PT	DNA-A	*Pte*I	SiYLCV
418	2016	BR-Vic418-2BS	DNA-B	*Bss*HII	n.d.
419	2016	BR-Vic419-5PT	DNA-B	*Pte*I	n.d.
		BR-Vic419-7PT	DNA-B	*Pte*I	n.d.
421	2016	BR-Vic421-3EV	DNA-A	*Eco*RV	SiYLCV
423	2016	BR-Vic423-1PT	DNA-A	*Pte*I	SiYLCV
		BR-Vic423-4PT	DNA-B	*Pte*I	SiYLCV
424	2016	BR-Vic424-1BS	DNA-A	*Bss*HII	SiYLCV
426	2016	BR-Vic426-1EV	DNA-A	*Eco*RV	SiYLCV
428	2016	BR-Vic428-1PT	DNA-A	*Pte*I	SiYLCV
		BR-Vic428-6PT	DNA-A	*Pte*I	SiYLCV
429	2016	BR-Vic429-6BS	DNA-A	*Bss*HII	SiYLCV
		BR-Vic429-6BS	DNA-A	*Bss*HII	SiYLCV
501	2017	BR-Vic501-18PT	DNA-A	*Pte*I	SiYLCV
502	2017	BR-Vic502-9PT	DNA-A	*Pte*I	SiYLCV
504	2017	BR-Vic504-19PT	DNA-A	*Pte*I	SiYLCV
505	2017	BR-Vic505-2PT	DNA-B	*Pte*I	SiYLCV
		BR-Vic505-7PT	DNA-A	*Pte*I	SiYLCV
506	2017	BR-Vic506-1BS	DNA-B	*Bss*HII	n.d.
507	2017	BR-Vic507-1PT	DNA-A	*Pte*I	SiYLCV
508	2017	BR-Vic508-8EV	DNA-A	*Eco*RV	OxYVV-I
510	2017	BR-Vic510-2PT	DNA-B	*Pte*I	n.d.
511	2017	BR-Vic511-19PT	DNA-A	*Pte*I	SiYLCV
512		BR-Vic512-5PT	DNA-A	*Pte*I	SiYLCV
514	2017	BR-Vic514-3PT	DNA-A	*Pte*I	OxYVV-II
515	2017	BR-Vic515-1PT	DNA-A	*Pte*I	OxYVV-I
516	2017	BR-Vic516-6EV	DNA-A	*Eco*RV	SiYLCV
		BR-Vic516-10EV			
517	2017	BR-Vic517-9PT	DNA-A	*Pte*I	OxYVV-I
518	2017	BR-Vic518-1PT	DNA-A	*Pte*I	OxYVV-I
520	2017	BR-Vic520-4PT	DNA-A	*Pte*I	OxYVV-I
522	2017	BR-Vic522-3PT	DNA-A	*Pte*I	OxYVV-I
		BR-Vic522-15PT	DNA-A	*Pte*I	OxYVV-I
524	2017	BR-Vic524-2PT	DNA-A	*Pte*I	OxYVV-I
525	2017	BR-Vic525-1PT	DNA-B	*Pte*I	n.d.
527	2017	BR-Vic527-5PT	DNA-A	*Pte*I	OxYVV-I
		BR-Vic527-15PT	DNA-A	*Pte*I	OxYVV-I
529	2017	BR-Vic529-1PT	DNA-A	*Pte*I	OxYVV-I

530		BR-Vic530-1PT	DNA-A	*Pte*I	SiYLCV
		BR-Vic530-8PT	DNA-A	*Pte*I	SiYLCV
531	2017	BR-Vic531-1EV	DNA-A	*Eco*RV	SiYLCV
603	2018	BR-Vic603-5PT	DNA-B	*Pte*I	SiYLCV
604	2018	BR-Vic603-28PT	DNA-A	*Pte*I	SiYLCV
607	2018	BR-Vic607-7PT	DNA-B	*Pte*I	n.d.
608	2018	BR-Vic608-22PT	DNA-A	*Pte*I	SimMV
609	2018	BR-Vic609-1PT	DNA-B	*Pte*I	n.d.
610	2018	BR-Vic-610-1PT	DNA-B	*Pte*I	n.d.
612	2018	BR-Vic612-7PT	DNA-B	*Pte*I	n.d.
615	2018	BR-Vic615-8PT	DNA-A	*Pte*I	SiYLCV
618	2018	BR-Vic618-4PT	DNA-A	*Pte*I	SimMV
		BR-Vic618-6PT	DNA-A	*Pte*I	SimMV
619	2018	BR-Vic619-1PT	DNA-A	*Pte*I	OxYVV-I
620	2018	BR-Vic620-7PT	DNA-A	*Pte*I	OxYVV-I
622	2018	BR-Vic622-2PT	DNA-B	*Pte*I	SiYLCV
		BR-Vic622-17PT	DNA-B	*Pte*I	SiYLCV
		BR-Vic622-24PT	DNA-A	*Pte*I	SiYLCV
623	2018	BR-Vic623-5PT	DNA-A	*Pte*I	OxYVV-I
624	2018	BR-Vic624-8PT	DNA-A	*Pte*I	OxYVV-I
625	2018	BR-Vic625-15PT	DNA-A	*Pte*I	OxYVV-I
626	2018	BR-Vic626-6PT	DNA-A	*Pte*I	SiYLCV-3
		BR-Vic626-7PT	DNA-A	*Pte*I	SiYLCV-3
		BR-Vic626-8PT	DNA-A	*Pte*I	SiYLCV-3
627	2018	BR-Vic627-15PT	DNA-A	*Pte*I	SimMV
628	2018	BR-Vic628-8PT	DNA-A	*Pte*I	SimMV
630	2018	BR-Vic630-4PT	DNA-A	*Pte*I	OxYVV-I
631	2018	BR-Vic631-13PT	DNA-A	*Pte*I	OxYVV-I
632	2018	BR-Vic632-20PT	DNA-B	*Pte*I	n.d.
		BR-Vic632-27PT	DNA-B	*Pte*I	n.d.
634	2018	BR-Vic634-3PT	DNA-A	*Pte*I	OxYVV-I
636	2018	BR-Vic636-9PT	DNA-A	*Pte*I	OxYVV-I
637	2018	BR-Vic637-36PT	DNA-A	*Pte*I	SiYLCV
		BR-Vic637-2PT	DNA-B	*Pte*I	SiYLCV
639	2018	BR-Vic639-10PT	DNA-A	*Pte*I	SimMV

Table 2. List of clones of each begomovirus and their respective variants, obtained from *Sida acuta* samples collected in Viçosa, MG, from 2011 to 2018.

Year	**OxYVV I**	**OxYVV II**	**OxYVV III**	**SiYLCV I**	**SiYLCV II**	**SiYLCV III**	**SimMV 1**	**SimMV 2**	**Total/ Year**
2011	19	2	10	-	-	-	-	-	31
2012	14	1	4	1	-	-	-	-	20
2013	16	-	2	-	-	-	-	-	18
2014	25	-	1	-	-	-	-	-	26
2016	4	6	-	12	2	-	-	-	24
2017	11	1	-	7	5	-	-	-	24
2018	9	-	-	6	-	3	4	2	24
Total/ Variant	98	10	17	26	7	3	4	2	167

Table 3. Summary of recombination events detected in the begomovirus population infecting *Sida acuta* in Viçosa, MG. The analysis was performed using complete DNA-A sequences.

	Breakpoints			Parents		Detection methods							
Event Number	End	Begin	Recombinant Sequence	Minor Parent	Major Parent	RDP	GENECONV	Bootscan	Maxchi	Chimaera	SiSscan	PhylPro	3Seq
1	123	2036	OxYVV 303.2-14	SimMV 608-18	SiYLCV 501-16	9,868e^{-10}	1,086e^{-7}	NS	1,649e^{-9}	1,550e^{-4}	4,966e^{-25}	NS	5,592e^{-25}
2	112	2245	SimMV 628-18	Unknown (SimMV 618.1-18)	SimMV 608-18	1,323e^{-3}	9,938e^{-5}	NS	2,559e^{-9}	3,542e^{-6}	1,566e^{-19}	NS	6,737e^{-7}

Table 4. Genetic variability indices of the begomovirus populations infecting *Sida acuta* in Viçosa, MG. N, number of isolates; H, number of haplotypes; Hd, haplotypic diversity; π, nucleotide diversity.

Population	**N**	**H**	**Hd**	π
OxYVV-I	98	74	0,983	0,00225
OxYVV-II	10	9	0,978	0,00298
OxYVV-III	17	15	0,978	0,00181
OxYVV total	125	97	0,988	0,01633
SiYLCV	36	29	0,987	0,00917

Table 5. Proportion of non-synonymous to synonymous substitutions for DNA-A genes of OxYVV and SiYLCV isolates, and sites under positive or negative selection according to the three methods used (SLAC, FEL and REL).

				OxYVV			
Gene	dN/dS	SLAC		FEL		REL	
		Positive	Negative	Positive	Negative	Positive	Negative
CP	0.174	-	72, 249	-	32, 38, 47, 55, 71, 72, 73, 86, 132, 154, 158, 162, 171, 172, 177, 197, 209, 210, 245, 249, 250	-	-
Rep	0.302	-	70, 157, 247	-	39, 43, 46, 57, 70, 116, 137, 144, 146, 151, 154, 157, 175, 179, 184, 186, 191, 201, 202, 220, 223, 243, 251, 259, 263, 268, 274, 283, 288, 314 321, 324, 332, 347	142, 214	-
TrAP	0.747	-	-	-	2, 8, 40, 64, 121	14, 94, 119, 124	-
Ren	0.441	-	74	-	7, 49, 73, 74 102, 103, 117, 123	-	-
AC4	0.703	-	81	-	25, 58, 81	-	7, 25, 45, 48, 58, 70, 81
				SiYLCV			
Gene	dN/dS	SLAC		FEL		REL	
		Positive	Negative	Positive	Negative	Positive	Negative
CP	0.138	-	-	-	36, 80, 83, 94, 104, 118,127, 136,146, 151, 155, 173, 208, 214,224, 249	10, 32, 33, 57, 61, 78, 81, 90, 117	-
Rep	0.040	-	52	-	12, 13, 23, 52, 53, 57, 125, 187, 248, 259325, 332	-	12, 13, 23, 25, 43, 52, 53, 57, 12, 187, 19, 218, 243, 248, 259, 323, 325, 332, 34
TrAP	0.411	-	-	-	42, 99, 102	-	-
Ren	0.355	-	-	-	106, 130	-	-
AC4	0.734	-	-	-	-	-	-

Table 6. Number of *Sida acuta* samples infected with each OxYVV variant, SiYLCV variant and SimMV strain in single infection, mixed infection and pseudo-recombination based on RCA-RFLP analysis.

Species-variant/strain	**2011**	**2012**	**2013**	**2014**	**2016**	**2017**	**2018**
OxYVV-I	19	14	16	25	4	12	9
OxYVV-II	2	1	-	-	6	1	-
OxYVV-III	13	4	3	1	-	-	-
SiYLCV-I	-	1	-	-	12	7	6
SiYLCV-II	-	-	-	-	2	5	-
SiYLCV-III	-	-	-	-	-	-	3
SimMV-1	-	-	-	-	-	-	3
SimMV-2	-	-	-	-	-	-	2
OxYVVnd	4	-	1	-	3	3	2
SiYLCVnd	-	2	2	3	4	2	1
Mix OxYVVnd and OxYVV-III	3	1	1	-	-	-	-
Mix OxYVVnd SiYLCVnd	-	-	-	-	3	1	1
DNA-A of OxYVV-I replicating DNA-B of OxYVV-I and OxYVV-III	3	3	3	8	4	3	3
DNA-A of OxYVV-I replicating DNA-B of OxYVV-III	4	1	2	-	-	-	-
DNA-A of OxYVV-I and OxYVV-III replicating DNA-B of OxYVV-III	3	1	1	1	-	-	-
DNA-A of OxYVV-III replicating DNA-B and OxYVV-I	-	1	-	-	-	-	-

nd: variant identification not possible due to the same RCA-RFLP pattern.

Figure legends

Figure 1. A. Non-symptomatic *Sida acuta* plant. **B.** *Sida acuta* plant infected with a begomovirus displaying yellow mosaic symptoms.

Figure 2. Pairwise nucleotide sequence identity matrix of the begomovirus isolates obtained in this work (DNA-A). The vertical bar at the left indicates the species, strains and variants identified based on the demarcation criteria established by the *Geminiviridae* Study Group of the ICTV {Brown, 2015 #19477}.

Figure 3. Phylogenetic tree based on the DNA-A nucleotide sequence of the begomovirus isolates obtained in this work. Filled circles at the nodes represent posterior probabilities from 0.90 to 1, and open circles represent posterior probabilities from 0.50 to 0.89. Viral isolates classified as members of the species *Oxalis yellow vein virus* (OxYVV) are represented in blue, *Sida yellow leaf curl virus* (SiYLCV) in green, and *Sida micrantha mosaic virus* (SimMV) in yellow. The colored bar at the right indicates the species, strains and variants identified based on the demarcation criteria established by the *Geminiviridae* Study Group of the ICTV {Brown, 2015 #19477}.

Figure 4. Phylogenetic tree based on the nucleotide sequence of the *CP* gene of the begomovirus isolates obtained in this work. Filled circles at the tree nodes represent posterior probabilities from 0.90 to 1, and open circles represent posterior probabilities from 0.50 to 0.89. Viral isolates classified as members of the species *Oxalis yellow vein virus* (OxYVV) are represented in blue, *Sida yellow leaf curl virus* (SiYLCV) in green, and *Sida micrantha mosaic virus* (SimMV) in yellow. The colored bar at the right indicates the species, strains and variants identified based on the demarcation criteria established by the *Geminiviridae* Study Group of the ICTV {Brown, 2015 #19477}.

Figure 5. Phylogenetic tree based on the nucleotide sequence of the *Rep* gene of the begomovirus isolates obtained in this work. Filled circles at the tree nodes represent posterior probabilities from 0.90 to 1, and open circles represent posterior probabilities from 0.50 to 0.89. Viral isolates classified as members of the species *Oxalis yellow vein virus* (OxYVV) are represented in blue, *Sida yellow leaf curl virus* (SiYLCV) in green, and *Sida micrantha mosaic virus* (SimMV) in yellow. The colored bar at the right indicates the species, strains and variants

identified based on the demarcation criteria established by the *Geminiviridae* Study Group of the ICTV {Brown, 2015 #19477}.

Figure 6. A. Schematic representation of the OxYVV CP protein. The yellow boxes represent non-synonymous sites that allow differentiating amongst the variants. Letters represent amino acid changes. **B.** Schematic representation of the OxYVV Rep protein. The yellow boxes represent non-synonymous sites that allow differentiating amongst the variants. Pink boxes represent conserved motifs in the coding sequence. Letters represent amino acid changes; U represents any hydrophobic amino acid and X represents any amino acid. The numbers represent amino acid positions in the protein sequence. The letters in parentheses represent amino acids present in each variant (OxYVV-I/II/III).

Figure 7. Relative proportions of sequenced isolates belonging to each species and variant, by year of sampling. n, number of isolates sampled in each year.

Figure 8. *In silico Msp*I restriction patterns for the begomovirus species and variants detected in this work, and examples of the identification of each species and variant in single and mixed infection. Variants OxYVV-I and -II have the same restriction pattern and therefore could not be differentiated using this assay. **23**, plant infected by OxYVV-I or -II in single infection. **24**, plant infected by OxYVV-III in single infection. **04**, plant infected by OxYVV-I or -II and OxYVV-III (mixed infection). **16** and **112**, plants ifnected by pseudorecombinants between OxYVV-I or II and OxYVV-III. **415**, **505** and **626**, plants infected by SiYLCV-I, -II and -III, respectively, in single infection. ND, pattern not determined *in silico* due to lack of DNA-B sequence.

Figure 1

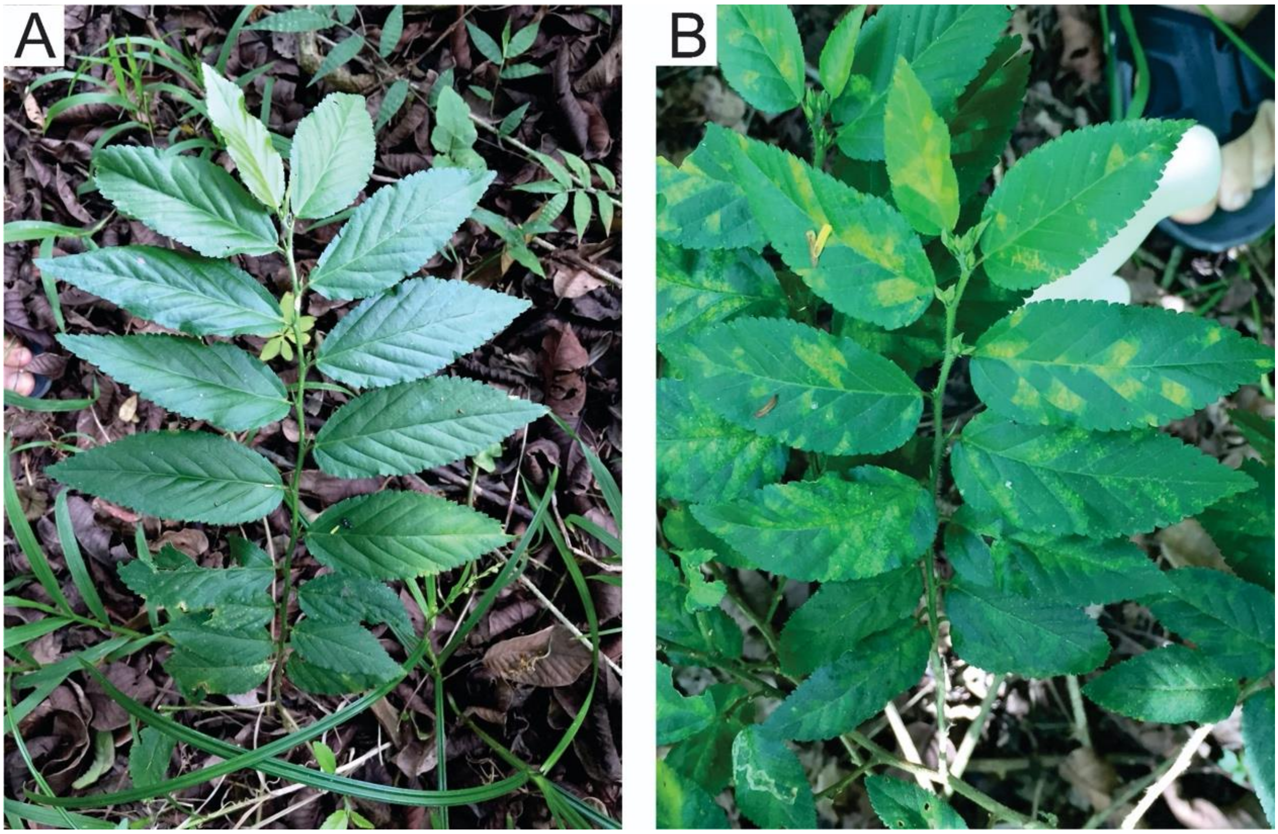


Figure 2

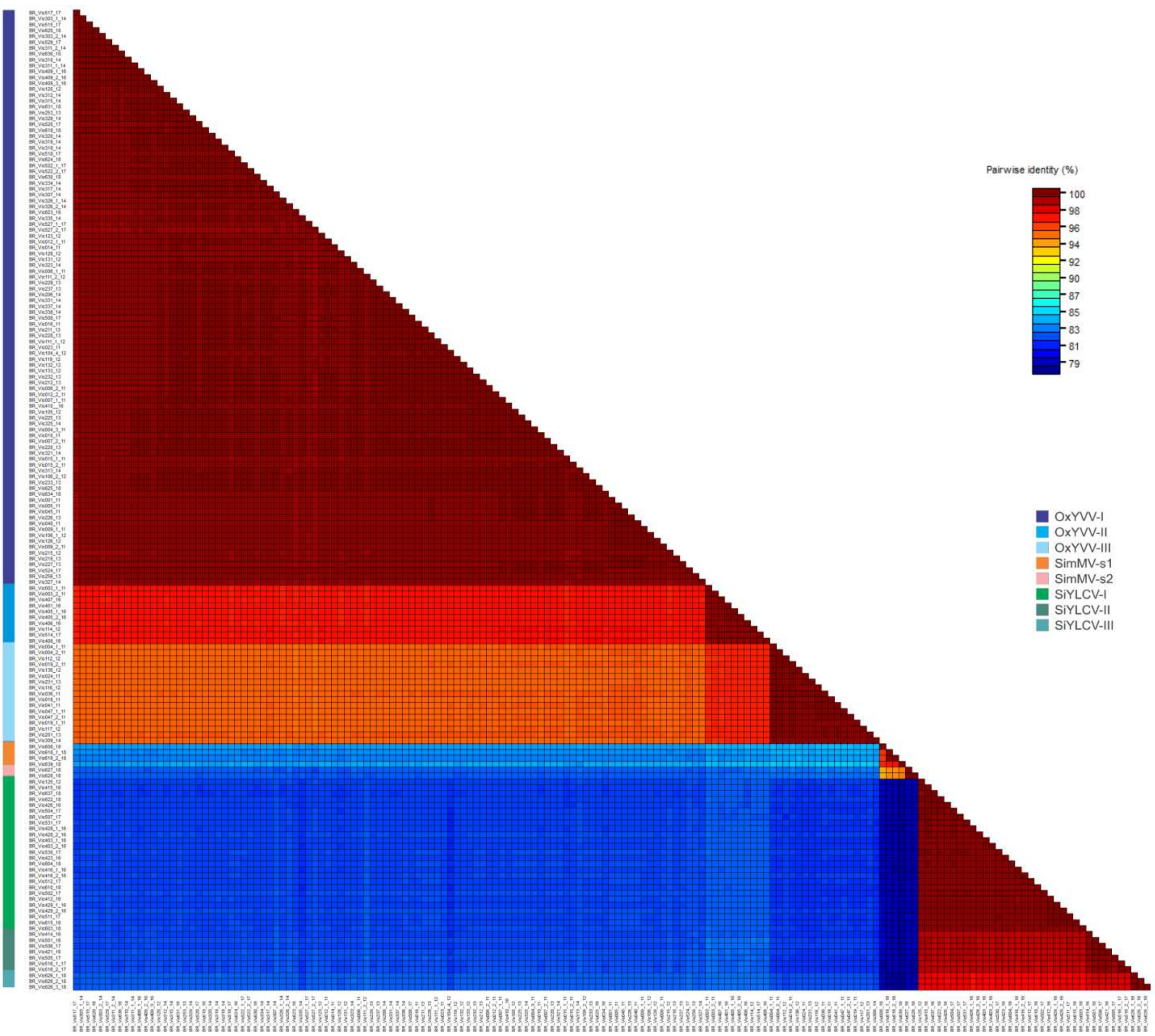
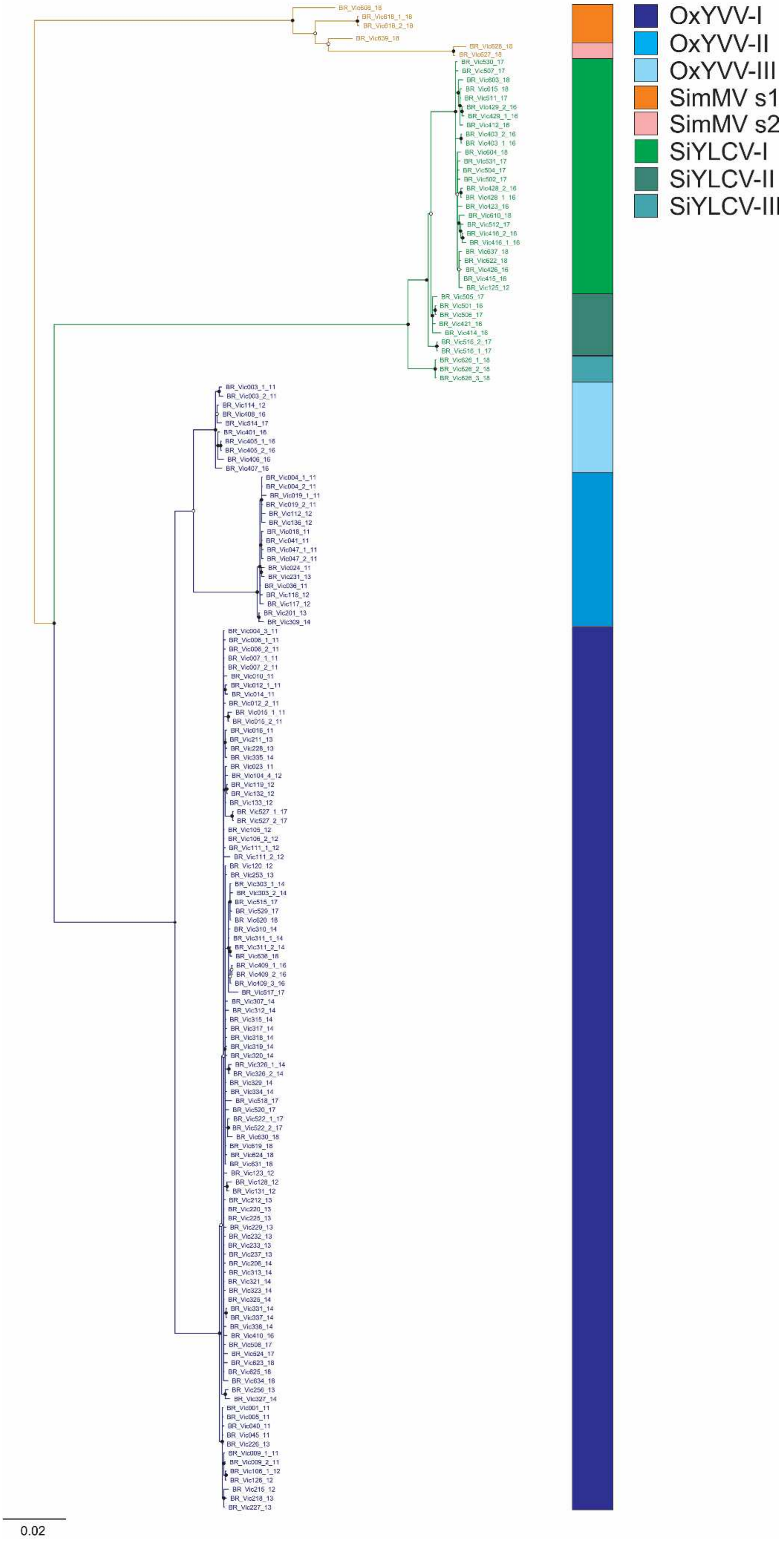

Pairwise identity (%)
100
98
96
94
92
90
87
85
83
81
79
OxYVV-I
OxYVV-II
OxYVV-III
SimMV-s1
SimMV-s2
SiYLCV-I
SiYLCV-II
SiYLCV-III

Figure 3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OxYVV-III
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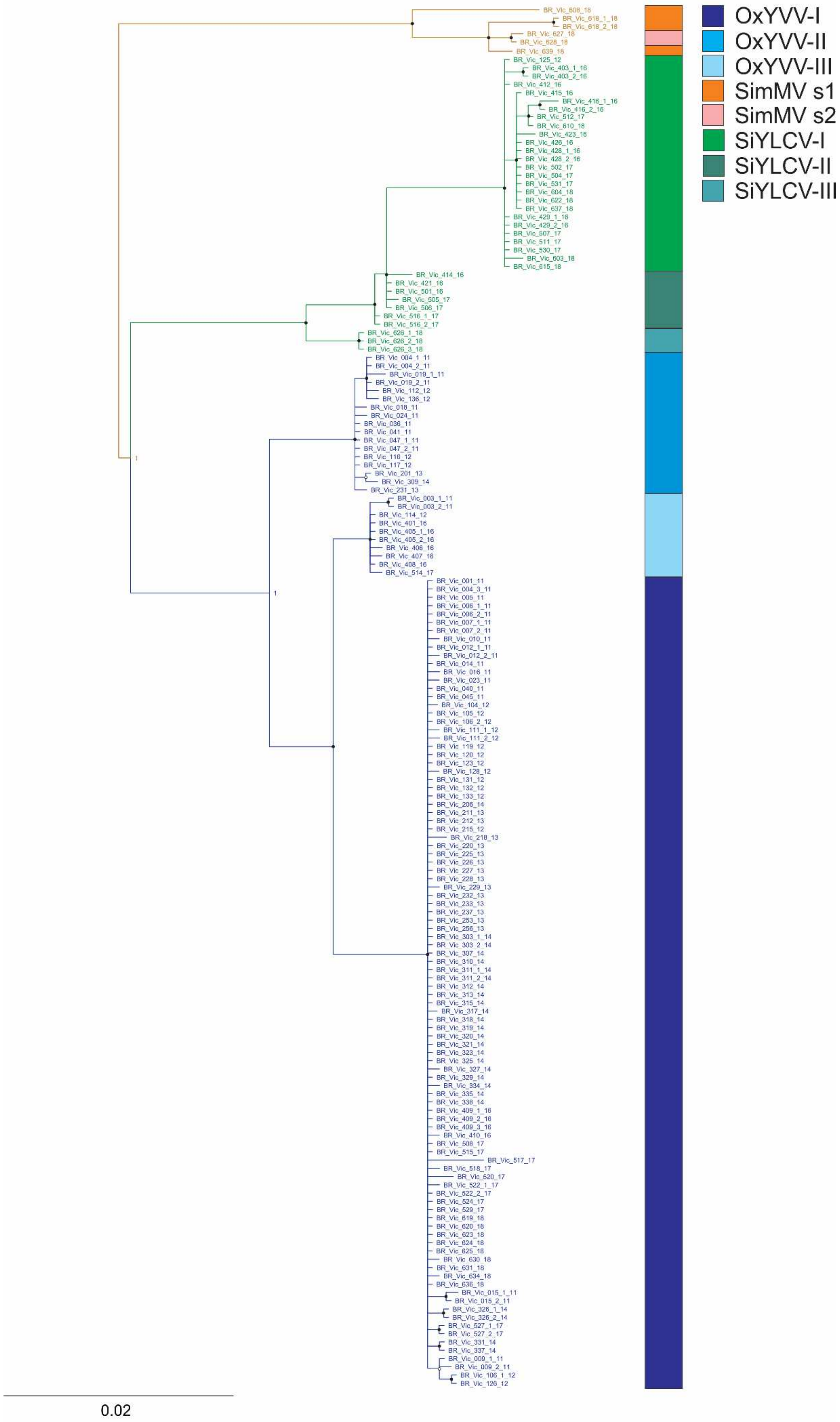
SimMV s2
SiYLCV-I
SiYLCV-II
SiYLCV-III

Figure 4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OxYVV-III
SimMV s1
SimMV s2
SiYLCV-I
SiYLCV-II
SiYLCV-III
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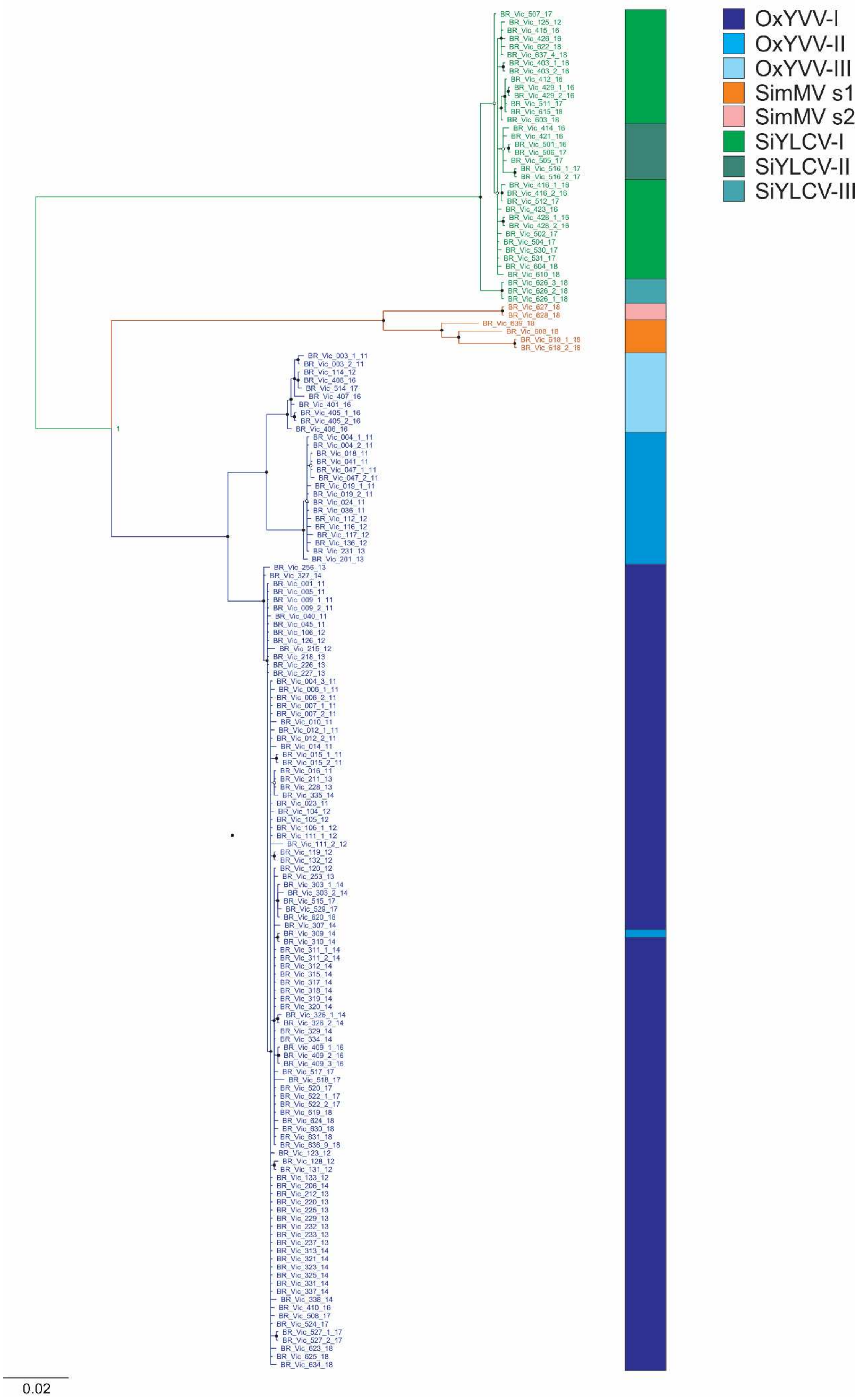
Figure 5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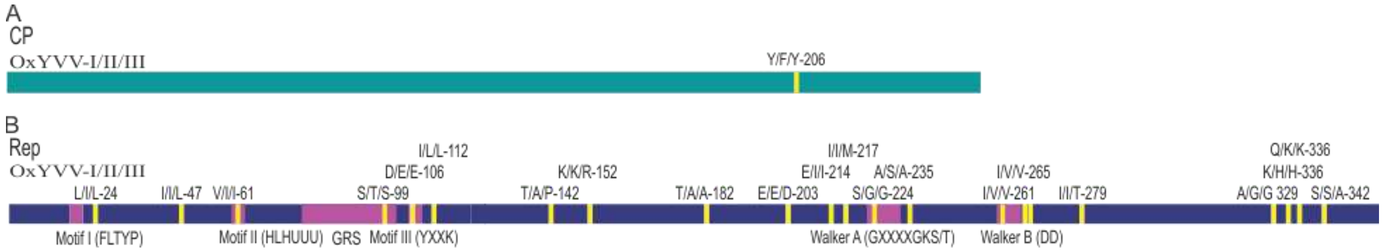
OxYVV-I
OxYVV-II
OxYVV-III
SimMV s1
SimMV s2
SiYLCV-I
SiYLCV-II
SiYLCV-III
0.02

Figure 6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OxYVV-I/II/III
Y/F/Y-206
B
Rep
OxYVV-I/II/III
I/L/L-112
D/E/E-106
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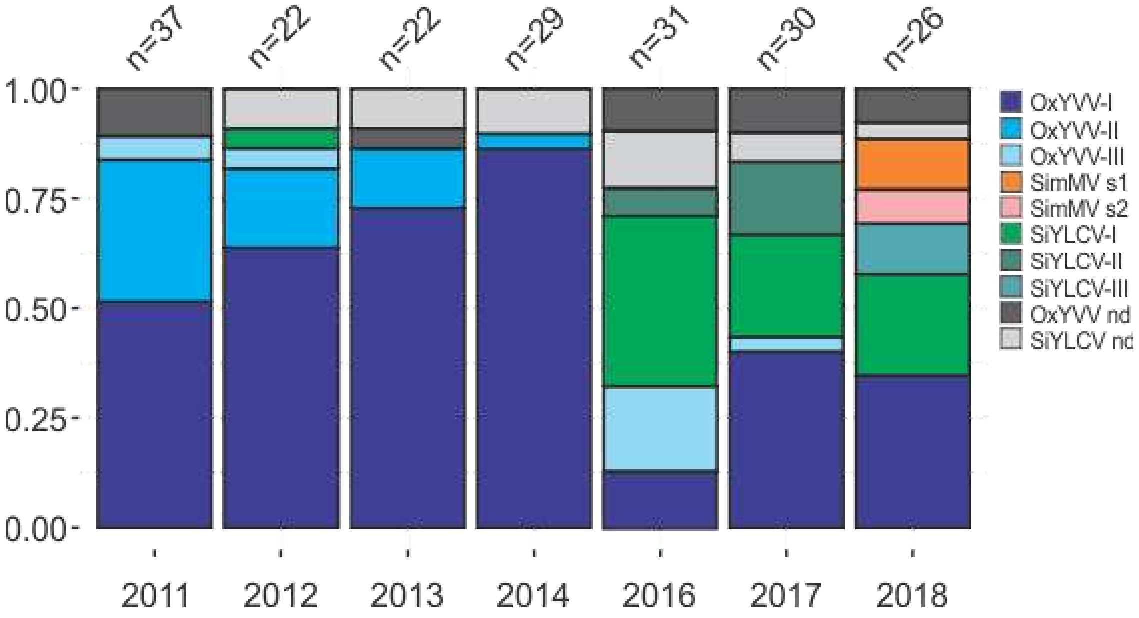
I/I/M-217
E/I/I-214
A/S/A-235
I/V/V-265
Q/K/K-336
K/H/H-336
L/I/L-24
I/I/L-47
V/I/I-61
S/T/S-99
T/A/P-142
T/A/A-182
E/E/D-203
S/G/G-224
I/V/V-261
I/I/T-279
A/G/G 329
S/S/A-342
Motif I (FLTYP)
Motif II (HLHUUU)
GRS
Motif III (YXXK)
Walker A (GXXXXGKS/T)
Walker B (DD)

Figure 7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n=22
n=29
n=31
n=30
n=26
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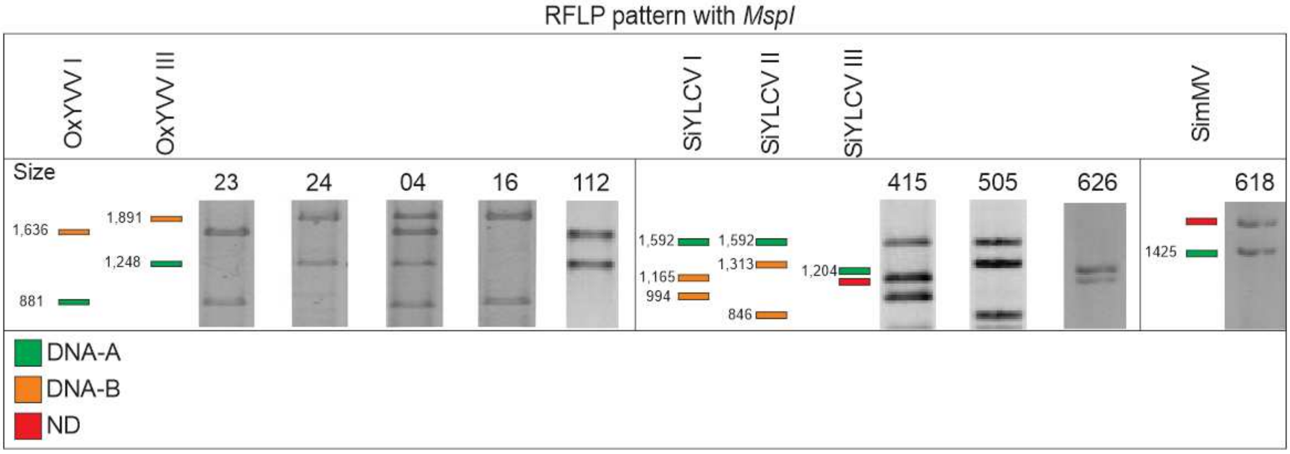
0.75
0.50
0.25
0.00
2011
2012
2013
2014
2016
2017
2018
OxYVV-I
OxYVV-II
OxYVV-III
SimMV s1
SimMV s2
SiYLCV-I
SiYLCV-II
SiYLCV-III
OxYVV nd
SiYLCV nd

Figure 8


RFLP pattern with *MspI*
OxYVV I
OxYVV III
SiYLCV I
SiYLCV II
SiYLCV III
SimMV
Size
23
24
04
16
112
415
505
626
618
1,636
881
1,891
1,248
1,592
1,165
994
1,592
1,313
846
1,204
1425
DNA-A
DNA-B
ND