FURTHER MOLECULAR CHARACTERIZATION OF WEED-ASSOCIATED BEGOMOVIRUSES IN BRAZIL WITH AN EMPHASIS ON *Sida* spp.[1]

Caracterização Molecular Adicional de Begomovírus Associados a Plantas Daninhas no Brasil, com Ênfase em ***Sida*** *spp.*

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ABSTRACT - Begomoviruses are whitefly-transmitted, single-stranded DNA viruses that are often associated with weed plants. The aim of this study was to further characterize the diversity of begomoviruses infecting weeds (mostly *Sida* spp.) in Brazil. Total DNA was extracted from weed samples collected in Viçosa (Minas Gerais state) and in some municipalities of Alagoas state in 2009 and 2010. Viral genomes were amplified by RCA, cloned and sequenced. A total of 26 DNA-A clones were obtained. Sequence analysis indicated the presence of 10 begomoviruses. All viral isolates from *Blainvillea rhomboidea* belonged to the same species, *Blainvillea* yellow spot virus (BlYSV), thereby suggesting that BlYSV may be the only begomovirus present in this weed species. Four isolates represent new species, for which the following names are proposed: *Sida* yellow blotch virus (SiYBV), *Sida* yellow net virus (SiYNV), *Sida* mottle Alagoas virus (SiMoAV) and *Sida* yellow mosaic Alagoas virus (SiYMAV). Recombination events were detected among the SiYBV isolates and in the SiYNV isolate. These results constitute further evidence of the high species diversity of begomoviruses in *Sida* spp. However, the role of this weed species as a source of begomoviruses infecting crop plants remains to be determined.

Keywords: geminivirus, recombination, *Blainvillea rhomboidea*, *Sida micrantha*, *Sida urens*, *Sida santaremnensis*

RESUMO - Begomovírus são vírus de DNA circular fita simples transmitidos por mosca branca, os quais são frequentemente associados com plantas daninhas. O objetivo deste trabalho foi caracterizar a diversidade de begomovírus infectando plantas daninhas (principalmente ***Sida*** *spp.) no Brasil. DNA total foi extraído a partir de plantas daninhas coletadas em Viçosa (Minas Gerais) e em alguns municípios do estado de Alagoas em 2009 e 2010. Os genomas virais foram amplificados por RCA, clonados e sequenciados. Um total de 26 clones de DNA-A foram obtidos. A análise das sequências indicou a presença de dez diferentes begomovírus. Todos os isolados originários de* ***Blainvillea rhomboidea*** *pertencem a uma única espécie viral,* ***Blainvillea*** *yellow spot virus (BlYSV), sugerindo que o BlYSV pode ser o único begomovírus presente nesta espécie de planta invasora. Quatro isolados representam espécies novas, para as quais os seguintes nomes são propostos:* ***Sida*** *yellow blotch virus (SiYBV),* ***Sida*** *yellow net virus (SiYNV),* ***Sida*** *mottle Alagoas virus (SiMoAV) e* ***Sida*** *yellow mosaic Alagoas virus (SiYMAV). Eventos de recombinação foram detectados entre isolados de SiYBV e no isolado de SiYNV. Estes resultados constituem uma evidência adicional da alta diversidade de espécies de begomovírus em* ***Sida*** *spp. Contudo, o possível papel dessas plantas daninhas como fonte de begomovírus para plantas cultivadas ainda permanece indeterminado.*

Palavras-chave: geminivírus, recombinação, *Blainvillea rhomboidea*, *Sida micrantha*, *Sida urens*, *Sida santaremnensis*.

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INTRODUCTION

Viruses belonging to the family *Geminiviridae* have a genome comprised of circular ssDNA encapsidated in a twinned icosahedral capsid (Rojas et al., 2005). The family is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocovirus* and *Begomovirus*) according to the insect vector, host range, genome organization and phylogeny (Brown et al., 2011). Viruses classified within the genus *Begomovirus* are transmitted by whiteflies (*Bemisia tabaci* - Homoptera: Aleyrodidae) in a persistent circulative manner and infect dicotyledonous plants. Begomovirus species that occur in the "Old World" have only one genomic component and are often associated with a DNA satellite known as DNA beta or betasatellite (Mansoor et al., 2003). Begomoviruses found in the "New World" have two components known as DNA-A and DNA-B. The DNA-A contains genes involved in the replication and encapsidation of viral progeny, while the DNA-B contains genes responsible for intra- and intercellular movement (Brown et al., 2011). Both components are required for systemic infection.

The identification of a begomovirus species is based on the determination of the complete nucleotide sequence of the DNA-A. The *Geminiviridae* Study Group of the ICTV has established a species demarcation threshold of 89% identity for the full-length DNA-A (Brown et al., 2011). Recently, Inoue-Nagata et al. (2004) developed a simple method for cloning full-length begomovirus genomic components using rolling-circle amplification (RCA). This method has facilitated the cloning and sequencing of genomes for a large number of isolates, allowing studies on viral genetic variability on a population scale (Lefeuvre et al., 2007b; Owor et al., 2007; Harkins et al., 2009; Varsani et al., 2009; Prasanna et al., 2010; Silva et al., 2011a,b).

Sida spp. has been described as a host of several begomoviruses throughout the Americas (Frischmuth et al., 1997; Hofer et al., 1997; Roye et al., 1997; Echemendía et al., 2004; Fiallo-Olive et al., 2010, 2011) including Brazil (Castillo-Urquiza et al., 2008). Despite reports of begomoviruses infecting weeds in Brazil dating as far back as the 1950s (Costa & Bennett, 1950), it was only recently that the molecular characterization of these isolates has started to receive attention (Jovel et al., 2004; Jeske et al., 2010). Silva et al. (2011a, b) observed a high species diversity in begomoviruses infecting leguminous weeds such as *Macroptilium* spp., while a single viral species was detected in the weed *Cleome affinis* (although with a high degree of intraspecies genetic variability). The characterization of viral populations infecting weeds provides important information about the ecological and evolutionary aspects of these viruses, and may also provide clues as to whether they can infect and become established in crop plants.

The aim of this study was to characterize the diversity of begomoviruses infecting economically important weeds in Brazil, with a special focus on *Sida* spp., as a step to assess their importance as sources of novel viruses for crop plants.

MATERIAL AND METHODS

Sample collection and storage

Weed samples were collected in locations throughout the states of Alagoas (AL) and Minas Gerais (MG) in 2009 and 2010. Plants displaying symptoms of mosaic, yellowing and stunting typical of begomovirus infection were preferentially collected. Samples were press-dried and stored at room temperature as herbarium-like specimens.

DNA amplification and cloning

DNA was extracted from dried leaves according to Doyle & Doyle (1987). Full-length viral genomes were amplified by rolling-circle amplification (RCA) (Inoue-Nagata et al., 2004), cloned in pBLUESCRIPT KS+ (Stratagene) after monomerization with the restriction enzymes *Apa* I, *Bam*H I, *Eco*R V, *Hin*d III, *Pst* I, *Sac* I or *Kpn* I, and sequenced commercially (Macrogen Inc., Seoul, South Korea) by primer walking.

Sequence comparisons and phylogenetic analysis

DNA-A nucleotide sequences were initially submitted to a BLAST search for preliminary

species assignment based on the 89% threshold level established by the *Geminiviridae* Study Group of the ICTV (Brown et al., 2011). Additional pairwise nucleotide sequence comparisons were made with DNAMan v. 6.0 using the Optimal Alignment option with the following parameters: Ktuple = 2, Gap penalty = 7, Gap open = 10, Gap extension = 5. The nucleotide sequences of begomoviruses used in the phylogenetic analysis (isolates obtained in the current study and begomoviruses previously described in the Americas, including Brazil; Table 1) were aligned using the MUSCLE module in Mega 5.05 (Tamura et al., 2011). A phylogenetic tree based on the DNA-A alignment was constructed with Mega 5.05 using the neighbor-joining method and the Tamura-Nei nucleotide substitution model. Bootstrap analysis (5,000 replications) was carried out to verify the significance of each tree branch.

Recombination analysis

Phylogenetic network analysis was performed with the neighbor-net method implemented in the program SplitsTree4 (Huson & Bryant, 2006). Analysis of potential recombination events was carried out using the Recombination Detection Program (RDP) v. 3.0 (Martin et al., 2010) using default parameters. Recombination analysis included viruses that represented new species obtained in the current study and begomoviruses previously described in Brazil.

RESULTS

Sequence comparisons and phylogenetic analysis

A total of 67 samples were collected: 10 samples of *B. rhomboidea* (family Asteraceae) and 10 of *Sida* spp. (family Malvaceae) in different municipalities of AL, and 47 samples of *Sida* spp. in Viçosa, MG. A total of 26 DNA-A clones were obtained: 11 from MG and 15 from AL. BLAST analysis and pairwise sequence comparisons of the DNA-A clones indicated the presence of 10 begomovirus species (Table 2; Figure 1).

The clone BR:Vic1:10 obtained from *S. santaremnensis* corresponds to an isolate of *Sida yellow mosaic virus* (SiYMV), with a 97.5% identity with SiYMV access number AY090558. Clones BR:Vic3:10, BR:Vic4:10, BR:Vic5:10 and BR:Vic8:10 obtained from *S. micrantha* (syn. *Sidastrum micranthum*) correspond to isolates of *Sida* common mosaic virus (SiCmMV), with a 94-97.1% identity with SiCmMV EU710751. Clones BR:Vic6:10 and BR:Vic7:10 from *S. urens* correspond to isolates of Tomato mild mosaic virus (ToMlMV), with a 95.1% identity with ToMlMV EU710752. The clone BR:Vic9:10 obtained from *S. santaremnensis* corresponds to an isolate of *Euphorbia* yellow mosaic virus (EuYMV), with a 96.4% identity with EuYMV FJ619507. Clones BR:Vic10:10 and BR:Vic11:10 obtained from *S. santaremnensis* correspond to isolates of *Sida mottle virus* (SiMoV), with a 95.4% identity with SiMoV AY090555. Clones BR:Rla3:10, BR:Rla4:10, BR:Rla5:10, BR:Jun1:09, BR:Lim1:09 and BR:Rla6:09 obtained from *B. rhomboidea* correspond to isolates of *Blainvillea* yellow spot virus (BlYSV), with a 92.1-95.3% identity with BlYSV EU710756.

The clone BR:Vic2:10 obtained from *S. micrantha* (Figure 2A) represents a novel species that is most closely related to *Tomato yellow spot virus* (ToYSV) and SiMoV (DQ336350 and AY090555, with 87.3 and 87.2% identity, respectively) for which the name *Sida* yellow net virus (SiYNV) is proposed. Clones BR:Vsa1:10 and BR:Vsa2:10, obtained from *S. urens*, and BR:Mar1:10, BR:Mar2:10 and BR:Mar3:10 obtained from *Sida* sp. (Figure 2B), also correspond to a new species that is most closely related to Tomato leaf distortion virus (ToLDV EU710749, with 83.3-83.6% identity) for which the name *Sida* mottle Alagoas virus (SiMoAV) is proposed. A third new species is represented by clones BR:Rla1:10, BR:Rla2:10 and BR:Chp1:10 obtained from *S. urens* (Figure 2C), most closely related to SiMoAV (with 83.5-88.4% identity) for which the name *Sida* yellow blotch virus (SiYBV) is proposed. The clone BR:Vsa3:09 obtained from *S. urens* (Figure 2D) also corresponds to a new species most closely related to SiCmMV (EU710751, with 79.9% identity) for which the name *Sida* yellow mosaic Alagoas virus (SiYMAV) is proposed.

Isolates of the four novel species display 76.5-88.4% sequence identity among

Table 1 - Begomoviruses used for pairwise sequence comparisons, phylogenetic and recombination analyses

Virus	Acronym	GenBank access	Virus	Acronym	GenBank access
From Brazil			*Euphobia mosaic virus*	EuMV	AF068642
Abutilon Brazil virus	AbBV	FN434438	*Macroptilium golden mosaic virus*	MaGMV	EF645647
Bean golden mosaic virus	BGMV	M88686	*Macroptilium mosaic Puerto Rico virus*	MaMPR	AF449192
Blainvillea yellow spot virus	BlYSV	EU710756	*Macroptilium yellow mosaic Florida virus*	MaYMFV	AY044135
Centrosema yellow spot virus	CenYSV	JN419002	*Macroptilium yellow mosaic virus*	MaYMV	AJ344452
Cleome leaf crumple virus	ClLCrV	FN35999	*Melon chlorotic leaf curl virus*	MCLCuV	AF325497
Euphorbia yellow mosaic virus	EuYMV	FJ619507	*Merremia mosaic virus*	MeMV	AF068636
Macroptilium yellow net virus	MaYNV	JN418998	*Okra yellow mosaic Mexico virus*	OYMMV	DQ022611
Macroptilium yellow spot virus	MaYSV	JN419005	*Okra yellow mottle Iguala virus*	OYMoIV	AY751753
Macroptilium yellow vein virus	MaYVV	JN419021	*Pepper golden mosaic virus*	PepGMV	AF149227
Okra mottle virus	OMoV	EU914817	*Pepper huasteco yellow vein virus*	PHYVV	AY044162
Passionfruit severe leaf distortion virus	PSLDV	FJ972767	*Potato yellow mosaic Panama virus*	PYMPV	Y15034
Sida common mosaic virus	SiCmMV	EU710751	*Potato yellow mosaic virus*	PYMV	AY120882
Sida Brazil virus	SiBV	FN436001	*Potato yellow mosaic Trinidad virus*	PYMTV	AF039031
Sida micrantha mosaic virus	SiMMV	AJ557451	*Rhyncosia golden mosaic Sinaloa virus*	RhGMSV	DQ406672
Sida mottle virus	SiMoV	AY090555	*Rhyncosia golden mosaic virus*	RhGMV	AF239671
Sida yellow leaf curl virus	SiYLCV	EU710750	*Rhyncosia rugose golden mosaic virus*	RhRGMV	HM236360
Sida yellow mosaic virus	SiYMV	AY090558	*Sida golden mosaic Costa Rica virus*	SGMCRV	X99550
Soybean blistering mosaic virus	SoBlMV	EF016486	*Sida golden mosaic Honduras virus*	SGMHV	Y11097
Tomato chlorotic mottle virus	ToCMoV	AF490004	*Sida golden mosaic virus*	SGMV	AF049336
Tomato common mosaic virus	ToCmMV	EU710754	*Sida golden yellow vein virus*	SiGYVV	AJ577395
Tomato golden mosaic virus	TGMV	K02029	*Sida yellow mosaic Yucatan virus*	SiYMYuV	DQ875872
Tomato leaf distortion virus	ToLDV	EU710749	*Sida yellow vein virus*	SiYVV	Y11099
Tomato mild mosaic virus	ToMlMV	EU710752	*Squash leaf curl virus*	SqLCV	AF256203
Tomato rugose mosaic virus	ToRMV	AF291705	*Squash mild leaf curl virus*	SqMLCV	AF421552
Tomato severe rugose virus	ToSRV	AY029750	*Tobacco leaf curl Cuba virus*	TbLCuCUV	AM050143
Tomato yellow spot virus	ToYSV	DQ336350	*Tomato Chino La Paz virus*	ToChLPV	AY339618
Tomato yellow vein streak virus	ToYVSV	EF417915	*Tomato golden motlle virus*	ToGMoV	DQ520943
From other countries in the Americas			*Tomato mosaic Havana virus*	ToMHV	EF088197
Abutilon mosaic virus	AbMV	HQ588899	*Tomato mottle Taino virus*	ToMoTV	AF012300
Bean calico mosaic virus	BCaMV	AF110189	*Tomato mottle virus*	ToMoV	AY965900
Bean dwarf mosaic virus	BDMV	M88179	*Tomato mild yellow leaf curl Aragua virus*	ToMYLCAV	AY927277
Bean golden yellow mosaic virus	BGYMV	AF173555	Tomato yellow leaf distortion virus	ToYLDV	FJ174698
Cabbage leaf curl virus	CaLCuV	DQ178612	*Tomato yellow margin leaf curl virus*	TYMLCV	AY508993
Chino del tomate virus	CdTV	AF101476	*Tomato severe leaf curl virus*	ToSLCV	AF130415
Cotton leaf crumple virus	CLCrV	AF480940	Tobacco yellow crinkle virus	TYCV	FJ213931
Corchorus yellow spot virus	CoYSV	DQ875868	*Wissadula* golden mosaic virus	WGMV	DQ395343
Curcubit leaf crumple virus	CuLCrV	AF224760	**Outgroup**		
Desmodium leaf distortion virus	DesLDV	DQ875870	*Tomato leaf curl New Delhi virus*	TLCNDV	U15015

themselves (Figure 1) and the genomes of all the new species show a typical bipartite, New World begomovirus organization, with five ORFs in the DNA-A. Some isolates contained an additional AC5 ORF (Table 3). The common regions (CR) have the conserved nonanucleotide (5'TAATATT/AC3') as part of a stem-loop structure at the origin of replication.

In order to determine the phylogenetic relationship among these begomoviruses, a phylogenetic tree based on complete DNA-A nucleotide sequences was constructed using the neighbor-joining method (Figure 3). The weed-infecting begomoviruses were placed in three major clusters within the tree. The EuYMV isolate obtained from *S. santaremnensis* was placed in Cluster I,

Table 2 - Taxonomic classification of begomovirus isolates obtained from weed samples collected in Minas Gerais and Alagoas states, Brazil, in 2009 and 2010. Viral species being reported for the first time in this study are underlined

Clone	Location and date of collection	Host	Specie
	Minas Gerais		
BR:Vic1:10	Viçosa, Nov/2010	*Sida santaremnensis*	*Sida yellow mosaic virus* - SiYMV
BR:Vic2:10	Viçosa, Nov/2010	*Sida micrantha*	*Sida* yellow net virus - SiYNV
BR:Vic3:10	Viçosa, Nov/2010	*Sida micrantha*	*Sida* common mosaic virus - SiCmMV
BR:Vic4:10	Viçosa, Nov/2010	*Sida micrantha*	*Sida* common mosaic virus - SiCmMV
BR:Vic5:10	Viçosa, Nov/2010	*Sida micrantha*	*Sida* common mosaic virus - SiCmMV
BR:Vic6:10, BR:Vic7:10	Viçosa, Jul/2010	*Sida urens*	Tomato mild mosaic virus - ToMiMV
BR:Vic8:10	Viçosa, Nov/2010	*Sida micrantha*	*Sida* common mosaic virus - SiCmMV
BR:Vic9:10	Viçosa, Nov/2010	*Sida santaremnensis*	*Euphorbia* yellow mosaic virus - EuYMV
BR:Vic10:10, BR:Vic11:10	Viçosa, Nov/2010	*Sida santaremnensis*	*Sida mottle virus* - SiMoV
	Alagoas		
BR:Rla1:10, BR:Rla2:10	Rio Largo, Jan/2010	*Sida urens*	*Sida* yellow blotch virus - SiYBV
BR:Vsa3:10	Viçosa, Jan/2010	*Sida urens*	*Sida* yellow mosaic Alagoas virus - SiYMAV
BR:Vsa1:10, BR:Vsa2:10	Viçosa, Oct/210	*Sida urens*	*Sida* mottle Alagoas virus - SiMoAV
BR:Mar1:10, BR:Mar2:10, BR:Mar3:10	Maragogi, Feb/2010	*Sida* sp.	*Sida* mottle Alagoas virus – SiMoAV
BR:Chp1:10	Chã Preta, Feb/2010	*Sida urens*	*Sida* yellow blotch virus - SiYBV
BR:Rla3:10, BR:Rla4:10, BR:Rla5:10	Rio Largo, Jan /2010	*Blainvillea rhomboidea*	*Blainvillea* yellow spot virus - BlYSV
BR:Jun1:09	Junqueiro, Nov/2009	*Blainvillea rhomboidea*	*Blainvillea* yellow spot virus - BlYSV
BR:Lim1:09	Limoeiro, Nov/2009	*Blainvillea rhomboidea*	*Blainvillea* yellow spot virus - BlYSV
BR:Rla6:09	Rio Largo, Nov/2009	*Blainvillea rhomboidea*	*Blainvillea* yellow spot virus - BlYSV

together with begomovirus species mostly from Central and North America. Cluster II includes the BlYSV isolates obtained from *B. rhomboidea*, which formed a monophyletic branch supported by a bootstrap value of 99%. Isolates of the new species SiMoAV and SiYBV, cloned from *S. urens*, formed distinct branches in Cluster III supported by a bootstrap value of 98%. Cluster III also included the new species SiYMAV and SiYNV and the isolates of SiCmMV, SiMoV, SiYMV and ToMlMV.

Recombination analysis

Phylogenetic relationships inferred by neighbor-net analysis based on a data set consisting of the new begomovirus species described here and other Brazilian begomoviruses revealed clear evidence of multiple recombination events (Figure 4). Strong evidence for recombination was found in Cluster II, containing the three SiYBV isolates, the two SiMoV isolates and the SiYNV isolate. Weaker evidence was observed in Clusters I, III, and IV.

To further investigate these putative recombination signals, the same set of sequences was analyzed using the RDP3 package. To omit unreliable signals, only recombination events supported by at least four different methods were considered. Two putative recombination events evidenced by the neighbor-net analysis were strongly supported by RDP. BR:Rla1:10 and BR:Rla2:10 (SiYBV) were identified as recombinants, with BR:Chp1:10 (also SiYBV) and one unknown virus as putative parents and recombination breakpoints at the Rep ORF and the common region (CR) (Table 4). A second recombination event was detected for BR:Vic2:10 (SiYNV), with BR:Vsa2:10 (SiMoAV) and SiMoV as putative parents and breakpoints also at the Rep ORF and the CR (Table 4).

DISCUSSION

The incidence and severity of the diseases caused by begomoviruses in economically important crops such as beans, tomatoes and peppers has increased significantly in Brazil and other countries in the Americas, due to the explosion of *Bemisia tabaci* populations since the 1980s (Morales, 2006). In particular, the B biotype of *B. tabaci* colonizes a wide range

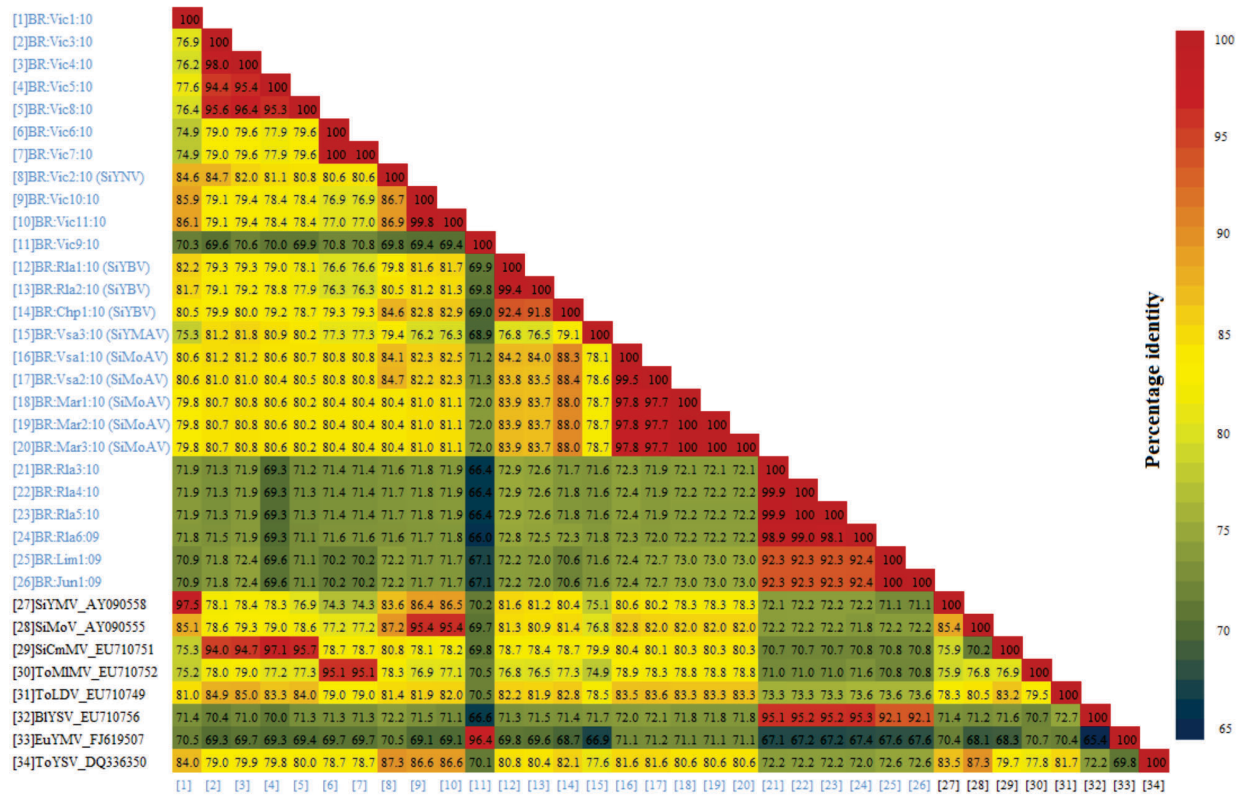

Figure 1 **-** Two-dimensional plot representing pairwise nucleotide sequence comparisons of the full-length DNA-A of the begomoviruses described in this work and other Brazilian begomoviruses.

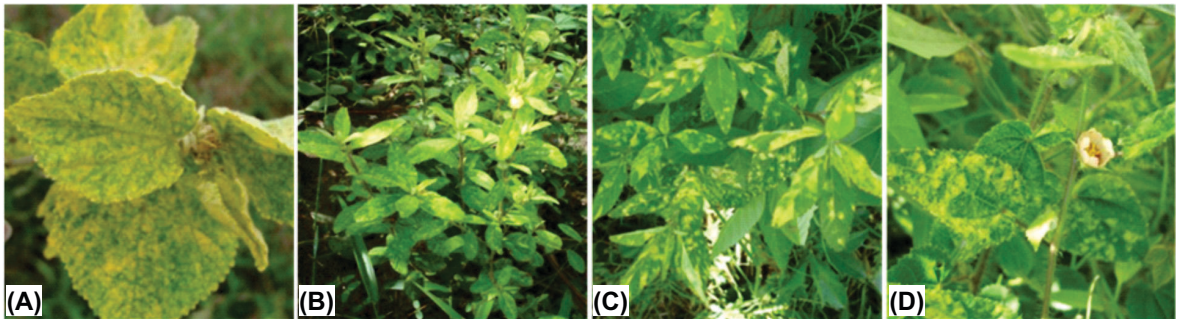

Figure 2 **-** Symptoms in weed samples from which new begomoviruses were obtained. (A) Reticulate yellow mosaic in the *Sida micrantha* sample from which isolate BR:Vic2:10 (*Sida* yellow net virus, SiYNV) was obtained; (B) Yellow mottle in the *Sida* sp. sample from which isolate BR:Vsa1:10 (*Sida* mottle Alagoas virus, SiMoAV) was obtained; (C) Yellow blotch symptoms in the *Sida urens* sample from which isolate BR:Rla1:10 (*Sida* yellow blotch virus, SiYBV) was obtained; (D) Yellow mosaic in the *Sida urens* sample from which isolate BR:Vsa3:09 (*Sida* yellow mosaic Alagoas virus, SiYMAV) was obtained.

of plants and is highly mobile, being able to fly over short distances or traveling up to several kilometers when assisted by the wind (Byrne, 1999). These characteristics facilitate the transmission of indigenous begomoviruses to new cultivated hosts, increasing the probability of novel, recombinant variants arising from mixed infections (Ribeiro et al., 2007). The current study investigated the species diversity of begomoviruses infecting malvaceous and compositae weeds in Brazil to assess the importance of these hosts as begomovirus reservoirs and as sources of novel viruses to crop plants.

A total of 10 begomovirus species were found out of 26 DNA-A clones. Nine species were obtained from malvaceous samples, including four new species: SiYNV isolated from *S. micrantha*, SiYBV and SiYMAV from *S. urens* and SiMoAV from *S. urens* and *Sida* sp. In addition, isolates of five previously described viruses were found. The total number of new species was greater in AL (three) than in MG (one). This is probably due to the fact that surveys have been carried out previously in MG (Castillo-Urquiza et al., 2008), but not in AL. In sharp contrast to what was observed for malvaceous weeds, *Blainvillea rhomboidea*, it seems, hosts only BlYSV. Silva et al. (2011a,b) reported analogous results, with *Macroptilium* spp. as a natural reservoir of different begomovirus species, while *Cleome affinis* hosts only *Cleome* leaf crumple virus (ClLCrV). Therefore, it seems that while some wild hosts harbor a rich diversity of virus species, others are infected by only one or two species. Whether or not this has significance in terms of the emergence of novel viruses in crop species still remains to be determined. However, it is reasonable to speculate that in those wild species harboring different viruses, the probability of recombination or pseudo-recombination events generating novel viruses would be much higher.

Results from phylogenetic analysis based on DNA-A sequences indicated that EuYMV (BR:Vic9:10) grouped with species that are phylogenetically closer to begomoviruses found in Central and North America rather than South America (including Brazil). The fact that some "Brazilian" viruses cluster with "Central American" viruses has been previously observed (Castillo-Urquiza et al., 2008; Silva et al., 2011a) and suggests a common origin for these two begomovirus lineages. The significance of this observation lies in the fact that begomoviruses in Central and South America are clearly segregated, and have never mixed. For example, *Bean golden mosaic virus* (BGMV) occurs only in South America and has never been reported north of the Equator, while the exact opposite is true for *Bean golden yellow mosaic virus* (BGYMV). This cluster of South and Central American viruses, which includes EuYMV, ToCmMV, SiYLCV and *Abutilon* Brazil virus (AbBV) is, therefore, unique.

Several studies have shown that recombination plays an important role in the generation of genetic variability in begomoviruses in Brazil (Galvão et al., 2003; Inoue-Nagata et al., 2006; Ribeiro et al., 2007; Silva et al., 2011a,b) and worldwide (Pita et al., 2001; Monci et al., 2002; García-Andrés et al., 2006, 2007a, b). Recombination analysis showed the occurrence of inter- and intraspecific recombination events in the Rep, CP and CR of the viral isolates detected in *Sida* spp. Lefeuvre et al. (2007a) proposed that coding regions are less susceptible to recombination. However, the regions encoding the Rep and CP of begomoviruses have been shown to be recombination hotspots (García-Andrés et al., 2007b; Lefeuvre et al., 2007b; Silva et al., 2011a,b), in agreement with the results obtained in this work.

Table 3 - Open reading frames, with their respective amino acid (aa) length, detected in the DNA-A of the begomovirus species described in this work

Viral species/Clones	cp	rep	ren	trap	ac4	ac5
Blainvillea yellow spot virus (BlYSV) - [BR:Rla3:10]	251 aa	358 aa	132 aa	131 aa	85 aa	181 aa
Euphorbia yellow mosaic virus (EuYMV) - [BR:Vic9:10]	249 aa	359 aa	132 aa	129 aa	120 aa	179 aa
Sida common mosaic virus (SiCmMV) - [BR:Vic3:10]	249 aa	359 aa	132 aa	129 aa	155 aa	-
Sida mottle Alagoas virus (SiMoAV) - [BR:Vsa1:10]	251 aa	361 aa	132 aa	129 aa	85 aa	-
Sida mottle virus (SiMoV) - [BR:Vic10:10]	250 aa	359 aa	132 aa	129 aa	87 aa	-
Sida yellow blotch virus (SiYBV) - [BR:Rla1:10]	251 aa	358 aa	132 aa	129 aa	85 aa	96 aa
Sida yellow mosaic Alagoas virus (SiYMAV) - [BR:Vsa3:10]	251 aa	358 aa	132 aa	129 aa	85 aa	105 aa
Sida yellow mosaic virus (SiYMV) - [BR:Vic1:10]	251 aa	358 aa	132 aa	129 aa	85 aa	-
Sida yellow net virus (SiYNV) - [BR:Vic2:10]	259 aa	359 aa	132 aa	129 aa	87 aa	-
Tomato mild mosaic virus (ToMlMV) - [BR:Vic6:10]	249 aa	361 aa	132 aa	129 aa	85 aa	-

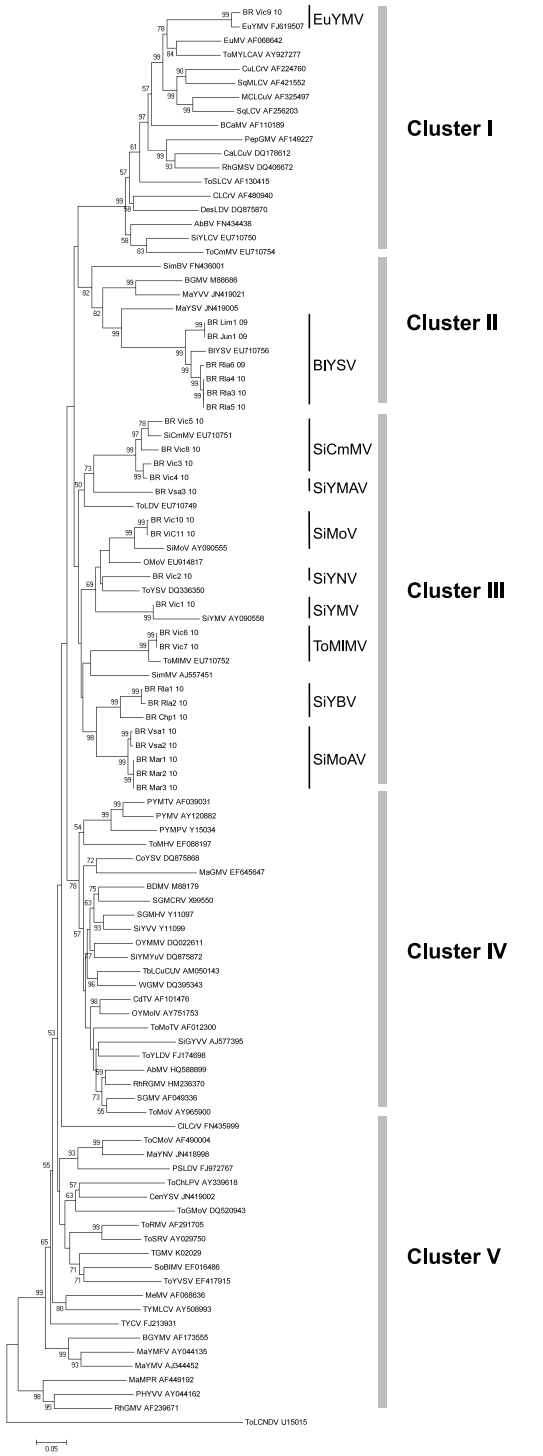

Figure 3 - Phylogenetic tree based on the alignment of complete DNA-A sequences of the begomovirus isolates from weeds collected in Minas Gerais and Alagoas states and other begomoviruses from the Americas, including Brazil. *Tomato leaf curl New Delhi virus* (ToLCNDV) was used as an outgroup. The tree was obtained with MEGA 5.05 using the neighbor-joining method, and the significance of each branch was verified by bootstrap analysis (5,000 replications).

Our findings indicate that *Sida* spp. (including the synonym *Sidastrum micranthum*) are natural reservoirs of several begomoviruses in Brazil, similarly to what has been observed in Central America and the Caribbean (Frischmuth et al., 1997; Hofer et al., 1997; Roye et al., 1997; Echemendía et al., 2004; Fiallo-Olive et al., 2010; Fiallo-Olivé et al., 2011). However, the true role of these species in the epidemiology of begomovirus diseases in crop plants is still unclear. Hofer et al. (1997) described *Sida golden mosaic Costa Rica virus* (SiGMCRV), which is capable of infecting tomatoes and beans under experimental conditions. Durham et al. (2010) described an isolate of *Sida golden mosaic virus* (SiGMV) in Florida that is capable of naturally infecting beans. Roye et al. (1997) described SiGMV infecting *Sida* sp. and *Macroptilium lathyroides* in Jamaica, but none of these viruses was associated with crops such as beans, tomatoes and peppers in that country.

Begomovirus epidemics in Brazil occur mostly in beans and tomatoes, and to a lesser extent in peppers (Faria et al., 2000; Zerbini et al., 2005; Nozaki et al., 2010). Beans are infected almost exclusively by BGMV, which has a host range limited to leguminous species. In tomatoes, a large number of viral species have been described (Zerbini et al., 2005). Some of them (such as ToMlMV) have also been detected in *Sida* spp., while viruses such as *Sida micrantha mosaic virus* (SiMMV) have been detected in tomatoes (Castillo-Urquiza et al., 2010). Based on these observations, we propose three hypotheses regarding the epidemiological significance of *Sida* spp. as a source of viruses for solanaceous crops such as tomatoes and peppers. First, viruses in *Sida* could be poorly adapted to solanaceous plants and thereby *Sida* spp. would not be relevant to epidemics in tomatoes, with only the occasional detection of *Sida* viruses in tomato and vice versa. According to this hypothesis, the source of the tomato viruses would be other unidentified (possibly symptomless) wild hosts. In this regard, it is noteworthy that *Tomato severe rugose virus* (ToSRV) has been reported to infect solanaceous weeds such as *Nicandra physaloides* in the field (Barbosa et al., 2009). Second, *Sida* spp. could be the source of begomoviruses to solanaceous crops, with the viruses currently found in tomato having

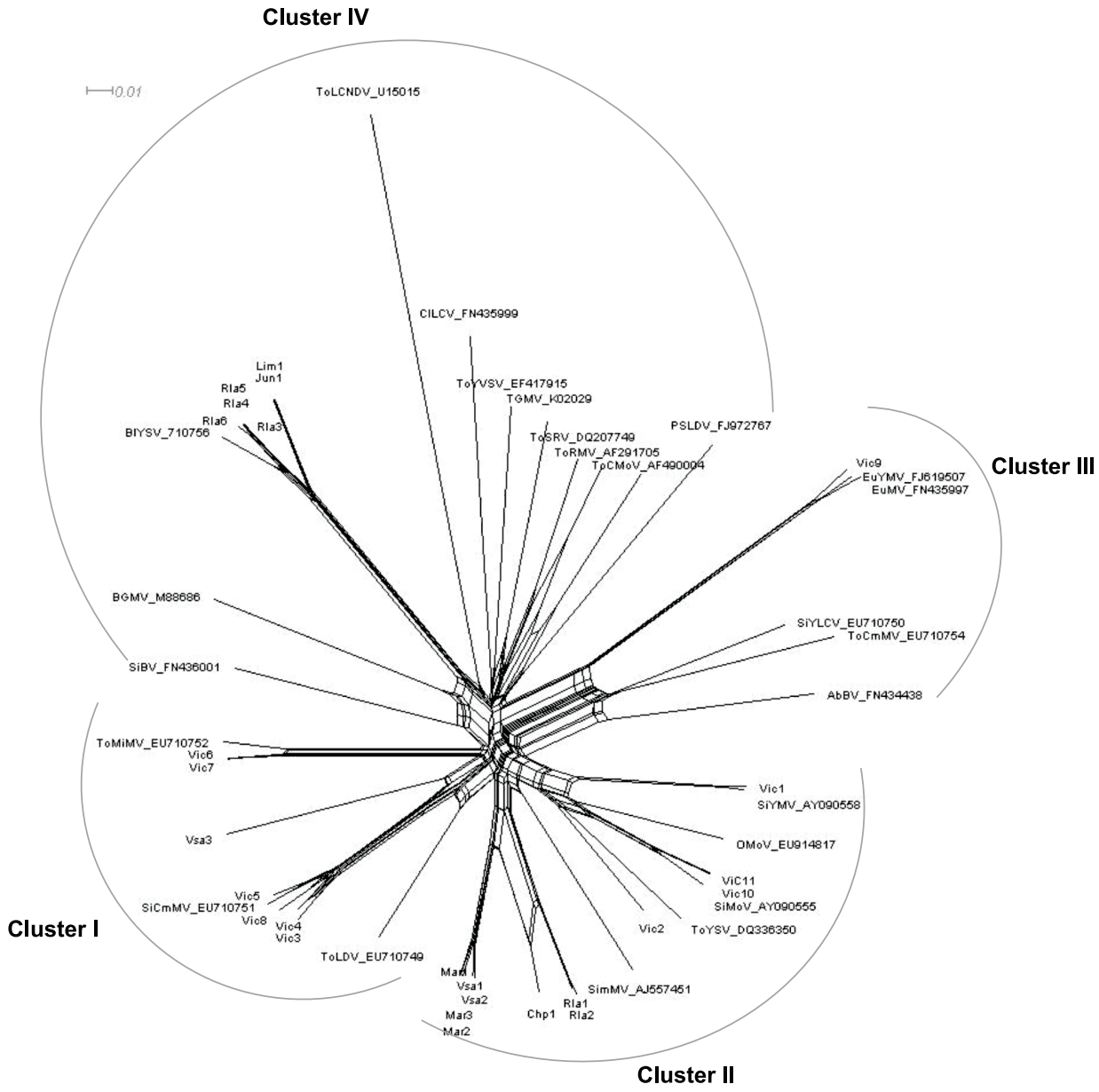

Figure 4 - Phylogenetic evidence of recombination among begomovirus isolates from weeds described in this work and other begomoviruses previously described in Brazil. Neighbor-net analysis was carried out using SplitsTree 4. The formation of a reticular network rather than a single bifurcated tree is evidence of recombination.

Table 4 - Recombination events detected among begomoviruses from weeds described in this work and previously described begomoviruses from Brazil

Clone	Parent		Breakpoint		p Value						
	Major	Minor	Initial	End	R[2/]	G	B	M	C	S	3S
BR:Rla1:10	BR:Chp1:10	Unknown[1/]	2001	64	$1,9 \times 10^{-25}$	$2,7 \times 10^{-25}$	$2,5 \times 10^{-25}$	$2,2 \times 10^{-19}$	$2,7 \times 10^{-21}$	$2,0 \times 10^{-22}$	-[3/]
BR:Rla2:10	BR:Chp1:10	Unknown	2001	2648	$1,9 \times 10^{-25}$	$2,7 \times 10^{-25}$	$2,5 \times 10^{-25}$	$2,2 \times 10^{-19}$	$2,7 \times 10^{-21}$	$2,0 \times 10^{-22}$	-
BR:Vic2:10	BR:Vsa2:10	SiMoV	37	2217	-	-	-	$8,1 \times 10^{-07}$	$6,9 \times 10^{-07}$	$3,4 \times 10^{-18}$	$2,1 \times 10^{-18}$

[1/] No parent identified. [2/] Programs: R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; C, CHIMAERA; S, SisScan; 3S, 3SEQ. [3/] Recombination event not detected.

evolved from viruses in *Sida* spp. Support for this hypothesis comes from the aforementioned detection of ToMlMV and SiMMV in both tomato and *Sida* spp. Also noteworthy is ToYSV, which is closely related to begomovirus species from *Sida* spp. (Andrade et al., 2006). However, these three viruses have been detected only sporadically in tomatoes, which would favor the first hypothesis. Third, and more intriguingly, *Sida* spp. could be infected by heterogeneous begomovirus populations, with rare variants that would not be easily detected by RCA-based cloning. Upon transmission of these heterogeneous populations to tomato plants by the insect vector, the rare variants would become predominant (and thus would be detected at high frequency) due to their better adaptation to the new host. The deep sequencing of viral populations in naturally-infected *Sida* and tomatoes could provide support for this hypothesis.

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