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Scientific Note

Molecular Characterization of a Novel *Begomovirus* Infecting Malva (*Gaya guerkeana* K. Schum.)

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Abstract

The genus *Begomovirus* (family *Geminirvidae*) comprises circular single-stranded DNA viruses that infect both cultivated and non-cultivated hosts, being transmitted by the insect vector *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). This study aimed to conduct the molecular characterization of a novel begomovirus infecting the non-cultivated plant *Gaya guerkeana* K. Schum. (Malvaceae). A leaf sample of *G. guerkeana* was collected in the State of Pernambuco, Brazil, in 2010. Viral genomic components were amplified via RCA, cloned, and commercially sequenced. The full-length DNA-A sequence was used for phylogenetic analysis and pairwise comparisons with other previously reported begomoviruses. The begomovirus DNA-A from *G. guerkeana* was most closely related to the *Sida mottle Alagoas virus* (SiMoAV, JX871386), having 84.8% nucleotide identity. Using the ≥ 91% identity criteria for DNA-A established by the Geminivirus Study Group of the International Committee on Taxonomy of Viruses, this isolate was considered a putative new begomovirus species, and the name *Gaya* yellow mosaic virus (GaYMV) is suggested. The GaYMV isolate grouped with SiMoAV and other begomoviruses infecting *Sida* spp. from the State of Alagoas, Brazil. This is the first report of a begomovirus infecting *G. guerkeana*, indicating that this non-cultivated plant can act as a reservoir/source of begomoviruses.

Keywords: alternative host, *Gaya guerkeana*, geminivirus, phylogeny

The family *Geminiviridae* encompasses several species of phytoviruses that have a genome composed of circular single-stranded DNA (ssDNA) encapsidated in geminated quasi-icosahedral particles (Brown *et al.*, 2012). The family consists of the genera *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus* and *Turncurtovirus*, classified according to insect vector type, host range, genomic organization and phylogenetic relationship (Varsani et al., 2017; Zerbini et al., 2017). *Begomoviruses* are transmitted by the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and are widely spread in tropical and subtropical regions, where they are considered a limiting factor for the production of economically important crops (Morales, 2010; Navas-Castillo *et al.*, 2011). In Brazil, bean (*Phaseolus* spp.) and tomato (*Solanum lycopersicon* L.) crops are the most severely affected (Morales, 2010; Navas-Castillo *et al.*, 2011).

Besides infecting cultivated plants, begomoviruses are found in a wide range of wild/non-cultivated hosts, especially those belonging to the families Malvaceae, Euphorbiaceae, and Fabaceae (Morales, 2010). Non-cultivated plants may act as alternative begomovirus hosts when cultivated species do not exist in the field or as a source of novel begomoviruses that arise

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via recombination in cases of mixed infections (Barreto *et al.*, 2013; Rocha *et al.*, 2013).

In this context, this work aimed at the molecular characterization of a novel begomovirus infecting the non-cultivated plant *Gaya guerkeana*.

A leaf sample of *G. guerkeana* presenting symptoms indicative of begomovirus infection (yellowing and mosaic) was collected in Caruaru, Pernambuco (PE) in 2010 (Figure 1), and the total DNA was extracted from plant tissue, according to the protocol described by Doyle & Doyle (1987). Viral genomic components were amplified via *Rolling Circle Amplification* (RCA) according to the method described by Inoue-Nagata *et al.*, (2004). Aliquots of the amplification were individually submitted to cleavage through *Bam*HI and *Hind*III endonucleases. The products of the cleavage reactions containing fragments of approximately 2600 nucleotides, corresponding to one copy of each genomic component, were used for binding to the pBluescript KS+ vector (Stratagene), previously linearized through the same enzyme and dephosphorylated. Potential recombinant plasmids were used to transform ultracompetent cells of *Escherichia coli* DH5α strain by the heat shock method. The obtained clones were commercially sequenced via *primer walking*.

Figure 1. Mosaic symptoms in *Gaya guerkeana* K. Schum. (Malvaceae) infected with the Gaya yellow mosaic virus (GaYMV), a putative new begomovirus.

Contigs of the complete viral genomic component were assembled using the DNAMAN software version 6 (Lynnon Biosoft Corporation). The obtained sequence was initially analyzed through the BLAST*n* algorithm and the non-redundant nucleotide database GenBank to determine the viral species with the highest percentage of sequence identity. Similar sequences from GenBank were used for species demarcation of the novel begomovirus isolate using the *Sequence Demarcation Tool* v. 1.2 (Muhire *et al.*, 2013).

Multiple nucleotide sequence alignments for the DNA-A dataset of the *G. guerkeana* isolate and other begomoviruses from Brazil were prepared using the MUSCLE algorithm and manually adjusted in the MEGA6 package (Tamura *et al.*, 2013). Bayesian Inference for DNA-A was performed on the CIPRES *web portal* (https://www.phylo.org/) using MrBayes v. 3.2.3 (Ronquist *et al.*, 2012). The GTR+G+I model of evolution was used for the GaYMV DNA-A dataset. The analysis was run for 10 million generations using four chains and sampling at every 1,000 generations for a total of 10,000 trees. The first 2,500 trees were discarded as a *burn-in* phase. Posterior probabilities (Rannala & Yang, 1996) were determined from a consensus tree *majority-rule* generated with the 7,500 remaining trees. Trees were viewed and edited using the software FigTree v. 1.4 (ztreebio.ed.acuk/software/figtree) and Inkscape (https://inkscape.org/pt/).

A clone (BR-Car1-12A) was obtained from the *G. guerkeana* sample collected in Caruaru (PE). BLAST*n* analysis showed that the genomic component corresponded to DNA-A. Using pairwise comparisons and the ≥91% identity criteria for complete DNA-A sequences established by the *Geminiviridae Study Group* of *International Committee on Taxonomy of Viruses* (ICTV) for the demarcation of species within the *Begomovirus* genus (Brown *et al.*, 2015), the clone obtained herein was classified as a putative new species of *Begomovirus*. BR-Car1-12A was more closely related to the *Sida mottle Alagoas virus* (SiMoAV, JX871386), displaying an 84.8% nucleotide identity, and the name *Gaya* yellow mosaic virus (GaYMV) is proposed.

GaYMV presented a genomic organization typical of New World begomoviruses, with five ORFs in DNA-A. The conserved nonanucleotide (5' TAATATT/AC 3') was detected in the common region, which is part of the stem-loop that contains the viral

replication origin. We also identified repeated GGGGG/GGGGG sequences (*iterons*), which are essential to initiate viral replication.

In the Bayesian phylogenetic tree, GaYMV grouped with SiMoAV, *Sida mosaic Alagoas virus* (SiMAV), and *Sida yellow blotch virus* (SiYBV), all obtained from *Sida* spp. from the State of Alagoas (Tavares *et al*., 2012; Wyant *et al*., 2012) (Figure 2). These results agree with those found in pairwise comparison analysis, in which the BR-Car1-12A isolate has a higher identity percentage with the SiMoAV species.

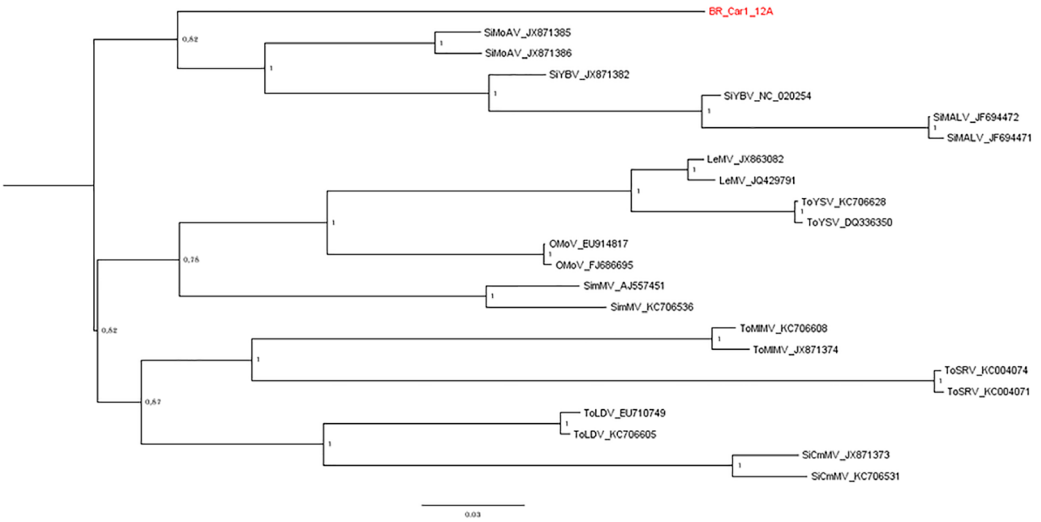

Figure 2. Phylogenetic analysis of Bayesian inference based on DNA-A from the *Gaya* yellow mosaic virus (GaYMV; highlighted in red) and other begomoviruses from Brazil.

Several studies have shown that, as it occurs with cultivated plants, the diversity of begomovirus species associated with weeds is high, especially those belonging to the families Malvaceae, Solanaceae and Fabaceae (Castillo-Urquiza *et al*., 2008; Silva *et al*., 2012; Tavares *et al*., 2012). Standing out among the malvae, members of the genera *Sida* and *Abutilon* show wide begomovirus genetic diversity, and some cultivated species are also infected by this virus group (Castillo-Urquiza, *et al*., 2008; Tavares *et al*., 2012; Wyant *et al*., 2012; Albuquerque *et al*., 2013).

In Brazil, *Sida* spp. have been frequently described as hosts for several begomovirus species, and may play an important epidemiological role as a source of begomovirus for cultivated species, especially tomato plants (Castillo-Urquiza *et al*., 2008; Tavares *et al*., 2012). This hypothesis is reinforced by the detection of *Sida micranta mosaic virus*, SiMoV and *Tomato mild mosaic virus* occurring in both tomato plants and *Sida* spp. in field conditions (Castillo-Urquiza *et al.*, 2007; Cotrim *et al.*, 2007; Rocha *et al.*, 2013).

Although this is the first report of a begomovirus infecting the wild species *G. guerkeana* (Malvaceae), this result indicates that this uncultivated plant may constitute an important reservoir and source of novel begomoviruses. However, additional studies are needed to evaluate the epidemiological role of these hosts for begomovirus outbreaks in cultivated species.

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