

First report of *Passion fruit green spot virus* infecting hibiscus plants

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Hibiscus (*Hibiscus rosa-sinensis*, family Malvaceae) are bushy evergreen shrubs widely distributed in tropical regions of the world. They can host viroids and DNA and RNA viruses from several families, including *Brevipalpus*-transmitted viruses (Kitajima et al., 2010). Fifteen leaf samples from different hibiscus plants showing dark green spots, and/or irregular green rings or necrotic ringspots, were collected in Brazil and Paraguay between 2012 and 2020 (Figure 1). Ultrathin sections from lesions were examined in a JEM 1011 (JEOL, Japan) transmission electron microscope. The presence of bacilliform particles (50 × 100 nm) resembling virions of the genus *Cilevirus* were observed in the cytoplasm of parenchymal cells of all samples (Figures 2a and 2b). Additionally, rod-like particles (40 × 100 nm), consistent with the genus *Dichorhavirus*, were also identified in two samples, SBO01 and Cmp01 (Figure 2c).

RNA extracts were prepared from individual lesions (c. 100 mg) using TRIzol™ reagent (Life Technologies, USA) from which cDNA solutions were prepared using a GoScript™ Reverse Transcriptase kit (Promega, USA) and a mix of random primers. The presence of viral genomes was tested by PCR using cDNA as a template and GoTaq® G2 Green Master Mix (Promega). A set of ten primer pairs for a range of cileviruses and dichorhaviruses was used (Table 1). Amplicons of the expected size were obtained in all 15 samples using four different tests for detection of the *Passion fruit green spot virus* (PfGSV, *Cile-*

virus) and in samples SBO01 and Cmp01 for *Clerodendrum chlorotic spot virus* (CICSV, *Dichorhavirus*) using one test. Amplicons from 12 samples were sequenced and representative sequences were submitted to GenBank (Accession Nos. OM_938963-OM_938974). The identity of the sequences was confirmed by comparing them with reference sequences using ClustalX; the amplicons had >98% nucleotide sequence identity with either PfGSV or CICSV reference sequences (PfGSV RNA1 & RNA2: Accession Nos. NC_055653 & NC_055652, and CICSV RNA2: NC_043649). The phylogenetic analysis didn't reveal a clearly defined segregation of the isolates by host or geographic origin (Figure 3).

PfGSV and CICSV are transmitted by *Brevipalpus yothersi* (Freitas-Astúa et al., 2018). CICSV is recurrently detected in several ornamental species growing in municipal and private gardens of the central, southern, and south-eastern regions of Brazil (Kitajima et al., 2008). Different strains of PfGSV have been detected in symptomatic passion fruit (*Passiflora* spp.) in two of the largest fruit-producing areas (Ramos-González et al., 2020). Hibiscus may act as reservoir of PfGSV, which could be of particularly concern for the passionfruit crop. The Brazilian passionfruit harvest represents c. 65% of worldwide production and the country is the world's largest exporter of passionfruit as fresh fruit, preserved or in concentrated juice.

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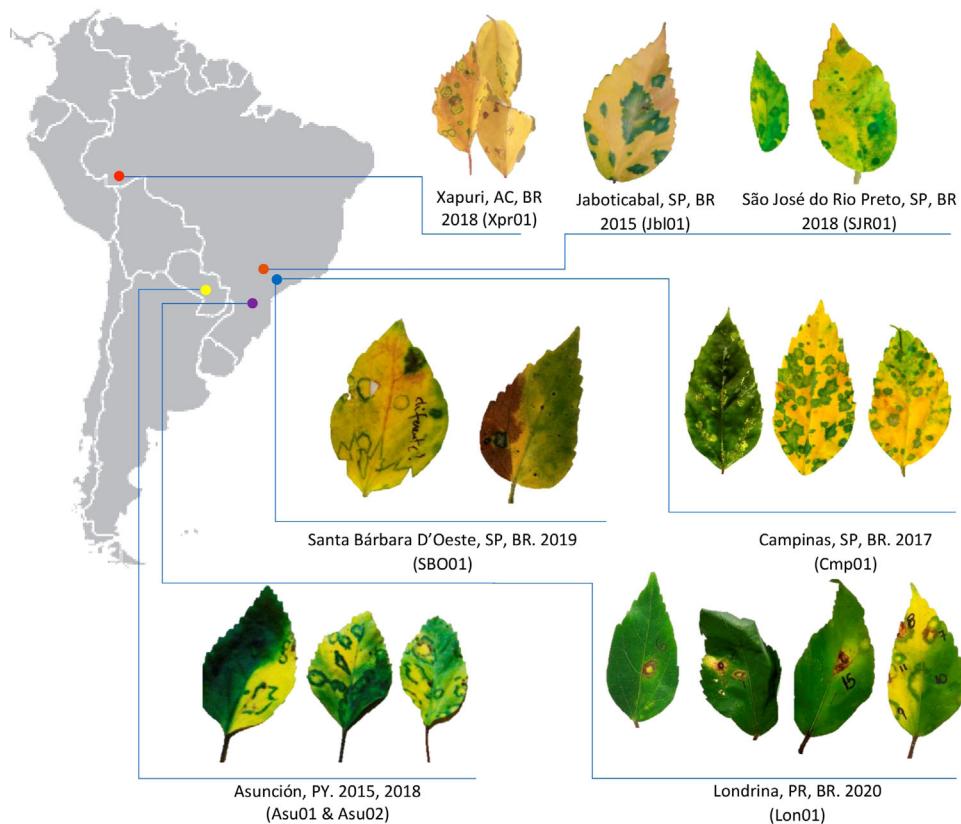


FIGURE 1 Green rings and dark green spots in leaves from hibiscus (*Hibiscus rosa-sinensis*) samples collected in Brazil (BR) and Paraguay (PY) between 2012–2020. The name of each isolate is shown in brackets

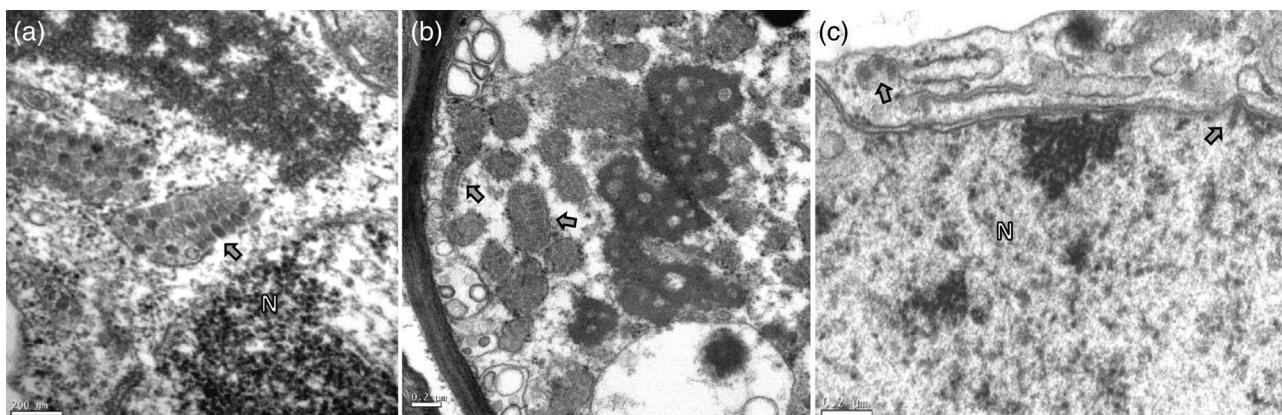


FIGURE 2 Transmission electron micrographs of ultrathin sections of symptomatic leaf samples of hibiscus plants collected in the State of São Paulo, Brazil, samples Prb02 (A), Jbl01 (B), and SBO01 (C). Short, bacilliform, membrane-bounded particles, presumably particles of cileviruses, were observed contained in cisternae of the endoplasmic reticulum (A and B). Rod-shaped viruses, presumably dichorhavirus particles, were detected bound to inner membrane of the nuclear envelope and elements of the endoplasmic reticulum (C). Arrows indicate virus particles; N: nucleus

TABLE 1 List of primers used in this study for detection of South American kitavirids, and the dichorhaviruses *Clerodendrum chlorotic spot virus*, *Citrus leprosis virus N* and *Orchid fleck virus*

Virus	ORF target	Primer sequence (5'-3')	T _a ¹ (°C)	Amplicon size (bp)	Reference
<i>Citrus leprosis virus C</i>	p24	F: CGCAGTTCTAATAACACC R: GGGAGTTCACTAAAGC	54	322	Compiled in Ramos-González et al. (2022)
<i>Citrus leprosis virus C2</i>	p29	F: ATGAGTAACATTGTCGTTTCGTTG R: TCACTCTCTGTTCATCAACCTGTT	56	795	
<i>Passion fruit green spot virus</i>	RdRp ²	F: ATTATCGTTTACGGTTA R: CGAATGCCCTGACACAAC	56	322	
	p29	F: ACACCAAGAGTACTATCGATC R: CATCAAGTGGAGCAAGTT	54	452	
	mp	F: CGATATTGATCAATCCGTT R: CACCTAAAATTCGAGGGTT	56	245	
	p24	F: TTCATCGCAAGTCTGTACACCT R: CTGTTGTGCCAAATCATCAA	56	299	
<i>Solanum violifolium ringspot virus</i>	p31	F: CACGTGTTTCAGCAGAA R: ACCTCTGGTCATCGACT	54	490	
<i>Ligustrum chlorotic spot virus</i>	p23	F: TCGGATTGATTGTCCTGTG R: AAACCGGATTGAATTATATG	54	420	
<i>Ligustrum leprosis virus</i>	RdRp	F: AAAACCCACACTTCTGATG R: TTGCACTCGAATAACAAGAC	54	303	
<i>Clerodendrum chlorotic spot virus</i>	G	F: AGGCCCATGATTAGAT R: AACAGGCTGGTCTGA	56	577	Ramos-González et al. (2018)

¹Annealing temperature. ²Fragments used in the phylogenetic analysis.

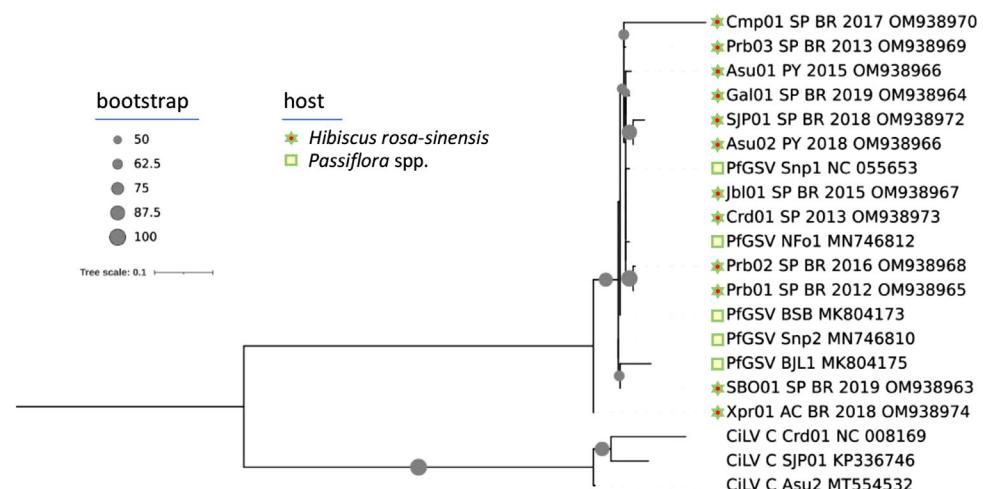


FIGURE 3 Phylogenetic tree of *Passion fruit green spot virus* (PfGSV) isolates using the maximum likelihood method. Multiple sequence alignment including 320 nucleotides of the RdRp ORF were obtained using MAFFT and the tree was obtained using IQ-tree. Nucleotide sequences of partial RdRp ORF of three isolates of *Citrus leprosis virus C* (CiLV-C) were used as an outgroup. The nomenclature of hibiscus isolates is as follows: city of collection and number of the isolate,_state,_country (BR: Brazil, PY: Paraguay) and GenBank Accession No

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