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https://doi.org/10.1590/1678-992X-2023-0078

ISSN 1678-992X

SCIENTIA AGRICOLA

Plant Pathology

First occurrence of bidens mottle virus in Brazil: biological and molecular characterization of isolates infecting *Zinnia* sp. and *Bidens pilosa*

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Edited by: Alice Kazuko Inoue-Nagata

Received April 11, 2023
Accepted July 04, 2023

ABSTRACT: *Zinnia* sp. and hairy beggartick (*Bidens pilosa*) plants exhibiting symptoms of possible virus infection were found in the municipality of Santa Bárbara d'Oeste, São Paulo State, Brazil. Flexuous filamentous particles and cytoplasmatic inclusions typical of potyvirus infection were observed by transmission electron microscopy, respectively, in leaf extracts and cells of symptomatic leaves. Infection of both plants with bidens mottle virus (BiMoV) was confirmed by RT-PCR using potyvirus universal primers, followed by nucleotide sequencing of the amplicons. The nearly complete genome sequence of the Brazilian isolate, named BiMoV-BR, is 9700 nucleotides long and shares 95.6 % identity with the corresponding nucleotide sequence of a BiMoV isolate from the United States. BiMoV-BR was mechanically transmitted and caused systemic infection on plants of *Zinnia* sp., hairy beggarstick, sunflower (*Helianthus annuus*), and lettuce (*Lactuca sativa*). *Myzus persicae* transmitted the virus to *Zinnia* sp. plants with efficacy of 8 % and 42 %, using one and ten aphids per plant, respectively. This is the first detection of BiMoV in Brazil. Further studies are necessary to evaluate the distribution of this potyvirus in the country.
Keywords: BiMoV, Potyvirus, high-throughput sequencing

Bidens mottle virus (BiMoV) is a member of the species *Bidens mottle virus*, genus *Potyvirus*, and the family *Potyviridae*. BiMoV was first identified causing mottling in plants of *Bidens* spp. and *Lepidium* spp. in the United States of America (USA) (Christie et al., 1968). In the early 1970s, this potyvirus was considered a limiting factor in the production of lettuce (*Lactuca sativa* L.) and endive (*Cichorium endivia* L.) (Purcifull and Zitter, 1973). Besides the United States, the virus has been reported only in Taiwan (Huang and Jan, 2011; Youssef et al., 2008).

Some BiMoV hosts include plants of *Ammi majus* L., *Calendula officinalis* L., *Chrysanthemum coronarium* L., *Helianthus annuus* L., *Lupinus angustifolius* L., *Solanum viarum* Dunal, *Vicia faba* L., and *Zinnia elegans* Jacq. (Baker et al., 2008, 2007, 2001; Chen and Lee, 2012; Edwardson et al., 1976; Huang and Jan, 2011; Liao et al., 2009; Logan and Zettler, 1984). BiMoV can be transmitted by the aphids *Acyrthosiphon pisum* Harris, *Aphis craccivora* Koch, *A. spiraecola* Patch, *Lipaphis pseudobrassicae* Davis, and *Myzus persicae* Sulzer in a non-persistent virus-vector relationship (Christie et al., 1968; Sastry et al., 2019). All these aphid species occur in Brazil (Cunha et al., 2016; Garcêz et al., 2015; Resende et al., 2006). Seeds do not transmit BiMoV (Christie et al., 1968; Sastry et al., 2019).

Zinnia sp. and hairy beggarticks (*Bidens pilosa* L.) are species of the family Asteraceae and their center of diversification is Mexico (Ballard, 1986; Stimart and Boyle, 2006). *Zinnia* plants are globally cultivated as ornamentals due to the diversity and color of their flowers (Stimart and Boyle, 2006). Hairy beggartick is a weed that colonizes different ecosystems worldwide and can negatively affect agricultural production. It is commonly found infesting crops, such as maize (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), sorghum (*Sorghum bicolor* (L.) Moench), rice (*Oryza sativa* L.), cotton (*Gossypium hirsutum* L.), vegetables, and pastures (Chauhan et al., 2019).

During the years 2020 and 2021, three plants of *Zinnia* sp. exhibiting mosaic, leaf deformation, and flower variegation (Figures 1A and 1B) and two plants of hairy beggarticks with symptoms of leaf mottle (Figures 1C and 1D) were found near a passion fruit orchard (22°51′26.7″ S 47°24′34.8″ W, altitude 588 m) in the municipality of Santa Bárbara d'Oeste, state of São Paulo, Brazil. Foliar samples of the symptomatic plants were collected and taken to the laboratory to identify the causal agent.

Transmission electron microscopy (TEM) was used to detect viral particles in symptomatic leaf extracts of both plants, negatively stained with 1 % uranyl acetate. Ultra-thin sections of symptomatic foliar tissues were prepared following procedures described by Kitajima and Nome (1999). Leaf fragments were fixed in modified Karnovsky's buffer (2.5 % glutaraldehyde, 2 % formaldehyde in 0.05 M cacodylate buffer, pH 7.2) for 2 h and post-fixed in 1 % osmium tetroxide solution for 1 h. The samples were dehydrated in an increasing series of acetone (30, 50, 70, 90, and 100 %). Subsequently, the samples were infiltrated and embedded in low-viscosity Spurr resin. The blocks were sectioned on a Leica UC6 ultramicrotome, and the ultra-thin sections obtained were contrasted with 3 % uranyl acetate and 0.5 % lead citrate. The analyses were performed on a JEOL JEM 1011 TEM.

Figure 1 – Plants of *Zinnia* sp. (A and B) infected with bidens mottle virus (BiMoV-BR) showing symptoms of mosaic, leaf deformation, and flower variegation. Plants of hairy beggarsticks (C and D) infected with BiMoV-BR showing symptoms of leaf mottle.

Total RNA was extracted separately from the leaf tissues of *Zinnia* sp. and hairy beggartick symptomatic plants using the Purelink Viral DNA/RNA kit (Thermo Fisher Scientific), according to the manufacturer's recommendations. The extracted RNA was used for virus detection by reverse-transcription polymerase chain reaction (RT-PCR) using the potyvirus universal primers WCIEN/PV1 (Maciel et al., 2011), which amplifies a fragment of 800 bp containing part of the coat protein (CP) gene and the 3′ non-translated region. An amplicon obtained from each host plant was sent to Macrogen Inc. for nucleotide sequencing. The nucleotide sequences obtained were compared to corresponding sequences deposited in the GenBank using the BLASTn algorithm, available at https://www.ncbi.nlm.nih.gov/blast.

For diagnostic purposes, a pair of primers was designed based on nucleotide sequences of BiMoV isolates deposited in GenBank using the Primer-Blast program, available at Primer designing tool – NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi). The primers BiMoV-CP F (GGAACAATGACAGTGCCACG) and BiMoV-CP R (CGGTTGGGTGTACGAGAAGT) amplify a fragment of 513 bp of the CP gene of BiMoV. The reactions were performed with 3 µL of total RNA, 12.5 µL of GoTaq Green Master Mix (Promega), 6.8 µL of nuclease-free water, 1.25 µL of each primer at a concentration of 20 µM, and 0.2 µL of the reverse transcription enzyme AMV (Promega). The amplification conditions were 42 °C for 50 min for reverse transcription, followed by 94 °C for 3 min for initial denaturation, 35 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. The RT-PCR product was subjected to agarose gel electrophoresis and the amplicons were visualized under a UV transilluminator. An amplicon obtained from each host was sent to Macrogen Inc. for nucleotide sequencing.

To obtain the complete genome sequence of the BiMoV isolate from Brazil, total RNA was extracted from an infected *Zinnia* sp. plant using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. The extracted RNA was sent to Macrogen Inc. for high throughput sequencing (HTS). Libraries of cDNA were prepared with TruSeq Stranded Total RNA with Ribo-Zero Plant Kit (Illumina). Transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform with 101-base paired-end reads mode. The raw reads obtained were trimmed and assembled *de novo* using CLC Genomics Workbench v. 9.0.3 software (QIAGEN). Contigs were extended and genome coverage depth was calculated using Geneious 11.1.6 (Biomatters). The nucleotide sequence of a BiMoV isolate (EU250214) was used as a reference for mapping HTS data.

The identity of the consensus nucleotide sequence obtained was determined using BLASTn. Polyprotein cleavage sites were identified by comparison with those described for a BiMoV isolate from Taiwan (GenBank EU250214) and deduced amino acid sequences were obtained using Geneious 11.1.6. A pairwise comparison of nucleotide and amino acid sequences of BiMoV isolates was performed using Muscle 5.1 alignment within Geneious 11.1.6. The sizes of the proteins were predicted using the Protein Molecular Weight program (https://www.bioinformatics.org/sms/prot_mw.html). Phylogenetic relationships were inferred using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993), implemented in MEGA 11 (Tamura et al., 2021), with 1000 bootstraps.

The isolate of BiMoV sequenced was used for biological studies. The leaf tissues of a symptomatic *Zinnia* sp. plant were macerated in 0.02 M phosphate buffer, pH 7. The extract obtained was used for mechanical inoculation of the following plants: hairy beggarstick, sweet pepper (*Capsicum annuum* L. cv. Dahra), *Chenopodium amaranticolor* Coste & Reyn, endive (*Cichorium endivia* L.), zucchini (*Cucurbita pepo* L. cv. Caserta), cucumber (*Cucumis sativus* L.), *Datura stramonium* L., sunflower (*Helianthus annuus* L.), lettuce (*Lactuca sativa* L. types 'americana', 'japonesa', 'lisa', and

'romana'), common bean (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L. cv. Compack), and *Zinnia* sp. One plant of each species was mock-inoculated as a negative control.

The aphid *Myzus persicae*, reared on plants of *D. stramonium*, was used on transmission assays of BiMoV to *Zinnia* sp. plants. Virus-free apterous adults were removed from the colony, fasted for 30 min, and transferred to BiMoV-infected *Zinnia* sp. leaves for a 10-min acquisition access period (AAP). After virus acquisition, the aphids were transferred to healthy *Zinnia* sp. plants for a 24-h inoculation access period (IAP). Twelve *Zinnia* sp. plants were inoculated using one aphid and the other 12 plants were inoculated using ten aphids.

Elongated and flexuous particles were seen by transmission electron microscopy (TEM) in the extracts obtained from the *Zinnia* sp. (Figure 2A) and hairy beggarticks symptomatic plants. Potyvirus-like particles and potyvirus-cytoplasmic inclusion bodies were observed in epidermal and mesophyll cells of ultra-thin sections of infected tissues (Figure 2B), typical of viruses of the family *Potyviridae* (Inoue-Nagata et al., 2022).

The infection of the three *Zinnia* sp. and two hairy beggartick plants with a potyvirus was confirmed by RT-PCR with the universal primers WCIEN/PV1. The nucleotide sequence of the amplicons obtained from hairy beggartick (785 bp) and *Zinnia* sp. plants (747 bp) showed 95.3 % and 95.7 % identity, respectively, with the corresponding nucleotide sequence of an isolate (GenBank AB601905) of BiMoV identified in infected *Eustoma grandiflorum* (Raf.) Shinn. plants in Taiwan (Chen et al., 2016).

All symptomatic *Zinnia* sp. and hairy beggartick plants tested positive for BiMoV when analyzed by RT-PCR using specific primers for this potyvirus. The nucleotide sequences of one amplicon obtained from total RNA extracted from plants of each species showed 96.3 % and 96.5 % identity, respectively, with the BiMoV isolate (GenBanK EU078960) identified in *Bidens alba* (L.) DC. in the United States (Youssef et al., 2008). The partial nucleotide sequences of BiMoV isolates from *Zinnia* sp. and infected hairy beggartick plants shared 100 % identity. The fact that the infected *Zinnia* sp. and hairy beggartick plants were within less than 2 m from each other suggests the potential transmission of the virus by a common aphid vector.

After the transcriptome sequencing was performed on the Illumina NovaSeq 6000 platform, a total of 47,862,488 readings were generated for the total RNA extracted from symptomatic leaves of *Zinnia* sp. plant. After trimming and adapter sequences removal, 45,618,008 (average length 145.9) paired readings were retained. After mapping the trimmed readings, the near complete nucleotide sequence of BiMoV was determined with a mean coverage depth of 31756.4. The near complete genome is 9700 nucleotides long and shares 95.6 % identity with the corresponding

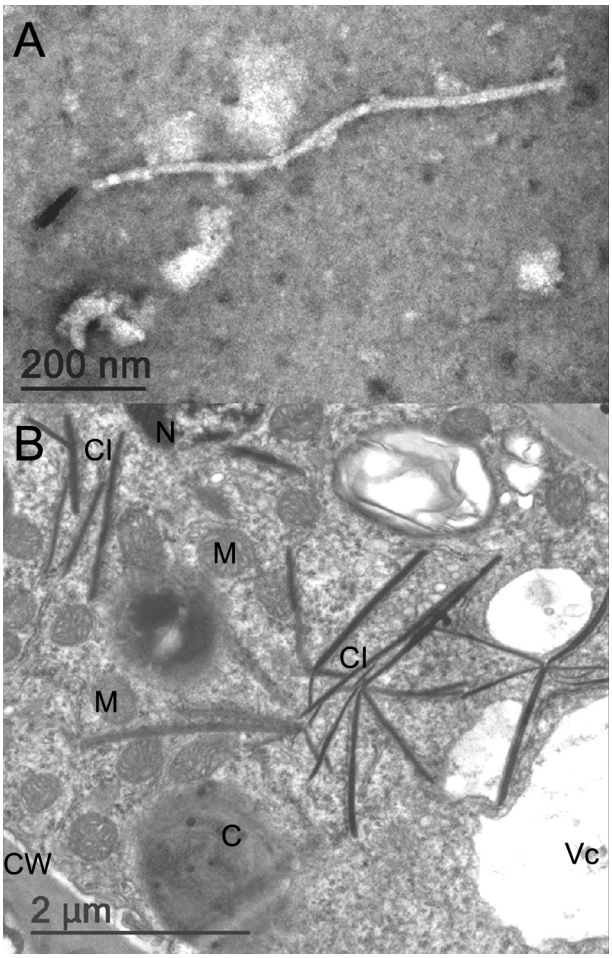

Figure 2 – Transmission electron micrograph of leaf samples of *Zinnia* sp. infected by bidens mottle virus (BiMoV). A) Negatively stained leaf extract shows an elongated, flexuous, potyvirus-like particle. B) Thin section of a leaf palisade parenchyma cell showing type 2 cylindrical inclusions (CI), forming a pinwheel configuration. C = chloroplast; CW = cell wall; M = mitochondrion; N = nucleus; Vc = vacuole.

nucleotide sequence of a BiMoV isolate (GenBank ON398504) identified in infected *Bidens* sp. plants in the United States (unpublished). Therefore, the potyvirus infecting *Zinnia* sp. corresponds to an isolate of bidens mottle virus named BiMoV-BR.

The nucleotide sequence of BiMoV-BR is nearly complete, with some missing nucleotides in the 5′ and 3′ untranslated regions and excluding the poly-A tail. The genome of the BiMoV-BR isolate (GenBank OQ656764) contains an open reading frame (ORF) with 9,216 nucleotides that encodes a putative polyprotein of 3,072 amino acids with a molecular weight of 349.16 kDa. The polyprotein is potentially cleaved into ten mature proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP (Figure 3). Nine putative cleavage sites were identified based on alignment of the BiMoV genome sequence with genome sequences of other

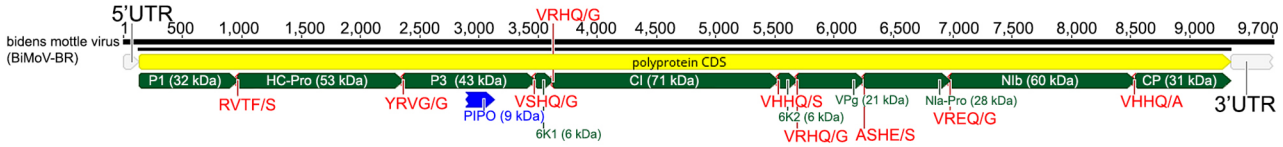

Figure 3 – Organization of the BiMoV-BR genome and predicted polyprotein cleavage sites. White boxes indicate the 5' and 3' untranslated regions and the yellow box shows the polyprotein open reading frame (ORF). Green boxes indicate the ten putative mature proteins and their sizes and the blue box indicates the putative PIPO protein. The location of each putative cleavage site in the polyprotein amino acid sequence is indicated in red. Figure created using Geneious v. 11.1.6.

Table 1 – Pairwise comparison of the polyprotein nucleotide (above diagonal) and deduced amino acid (below) sequences of bidens mottle virus (BiMoV) isolates.

Isolates	1	2	3	4	5	6	7	8
1-BiMoV-BR OQ656764		91.6	91.4	91.3	91.1	91.4	91.5	92.6
2-BiMoV-TW AF538686	92.5		97.7	97.6	97.3	97.6	97.7	97
3-BiMoV-TW EU250210	92.8	98.5		99.7	99.5	97.3	97.5	96.8
4-BiMoV-TW EU250211	92.7	98.3	99.7		99.7	97.2	97.4	96.7
5-BiMoV-TW EU250212	92.3	97.9	99.3	99.4		97	97.2	96.5
6-BiMoV-TW EU250213	92.5	98.4	98.6	98.4	98		97.4	96.6
7-BiMoV-TW EU250214	92.4	98.4	98.5	98.3	97.9	98.5		96.8
8-BiMoV-USA ON398504	93.3	97.9	98.1	97.9	97.5	98	97.9	

BiMoV isolates available in the GenBank (Figure 3). The small Pretty Interesting *Potyviridae* ORF (PIPO) was also found in the genome of the BiMoV-BR isolate. The PIPO is located inside the gene encoding the P3 protein and encodes a putative protein of 80 amino acids (aa) with 9 kDa (Figure 3).

The pairwise comparison of nucleotide and amino acid sequences of the polyprotein of the BiMoV-BR isolate with corresponding sequences from other BiMoV isolates available in the GenBank is presented in Table 1. The highest percentages of nucleotide and amino acid sequences identities of the BiMoV-BR isolate were 92.6 % and 93.3 %, respectively, with the BiMoV isolate from the United States. In the phylogenetic analysis, the nucleotide sequence of BiMoV-BR was distantly related to that of other BiMoV isolates (Figure 4). The BiMoV isolates from Taiwan formed a group that can be divided into two subgroups. The first subgroup consisted of BiMoV isolates (GenBank EU250212, EU250211, and EU250210) identified in infected *Bidens* sp. plants. The second subgroup was composed of isolates (GenBank EU250214, EU250213, and AF538686) identified in infected lettuce and sunflower plants. The BiMoV isolate from the United States (GenBanK ON398504), identified in *Bidens* sp. plants was more closely related to the BiMoV isolates from Taiwan than the BiMoV-BR isolate (Figure 4).

The partial host range of BiMoV-BR was evaluated by monitoring symptoms after sap inoculation to plants of different species, RT-PCR with specific primers, and nucleotide sequencing of the amplicons. BiMoV-BR isolate was transmitted and infected systemically only plants of the family Asteraceae (Table 2). Infected plants of *Zinnia* sp., hairy beggarticks, and sunflower showed symptoms of vein clearing and leaf mottle after 10-16 days post-inoculation (DPI), similar to descriptions

Table 2 – Reaction of plants of different species mechanically inoculated with bidens mottle virus (BiMoV-BR)

Family, species and cultivar	n° of inoculated plants /n° of infected plants	Symptoms
Amaranthaceae		
Gomphrena globosa	0/2	-
Asteraceae		
Bidens pilosa	7/7	VC, MT
Cichorium endivia	4/4	AS
Helianthus annuus	6/6	VC, MT
Lactuca sativa type 'americana'	0/4	-
L. sativa type 'japonesa'	0/4	-
L. sativa type 'lisa'	0/4	-
L. sativa type 'romana'	2/4	MT
Zinnia sp.	4/6	VC, MT
Chenopodiaceae		
Chenopodium amaranticolor	2/2	CLL
Cucurbitaceae		
Cucumis sativus	0/4	-
Cucurbita pepo cv. Caserta	0/2	-
Fabaceae		
Phaseolus vulgaris cv. Carioca	0/4	-
Solanaceae		
Capsicum annuum cv. Dahra	0/4	-
Datura stramonium	0/4	-
Solanum lycopersicum cv. Compack	0/4	-

CLL = chlorotic local lesions; MT = mottling; VC = vein clearing; AS = asymptomatic.

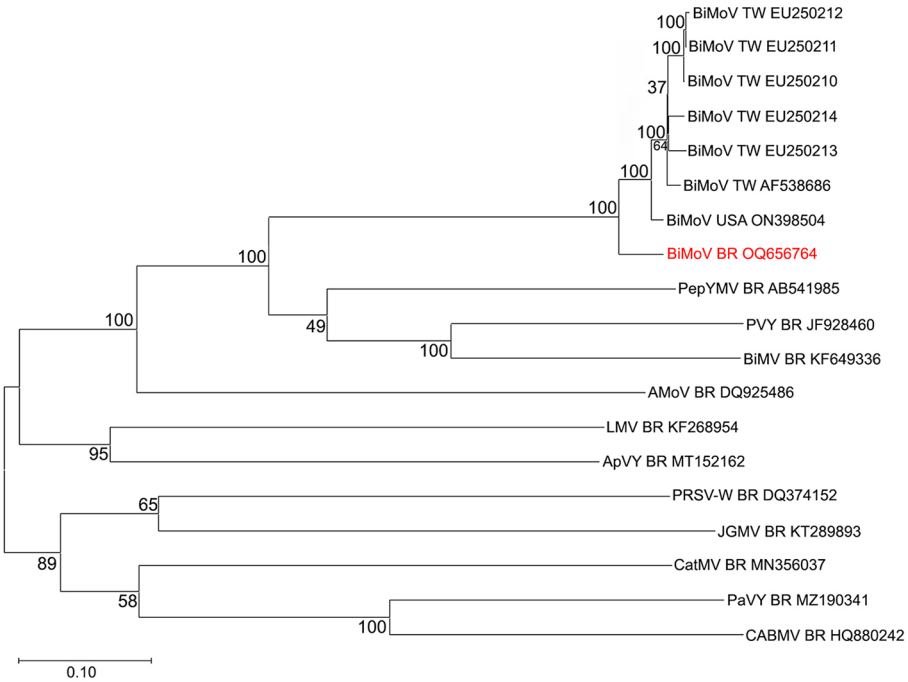

Figure 4 – Phylogenetic tree constructed with the nucleotide sequences of the polyprotein of different isolates of bidens mottle virus (BiMoV) and other potyviruses reported in Brazil. Bootstrap values in 1000 replicates are printed in the nodes. Potyviruses used: passiflora virus Y (PaVY); cowpea aphid-borne mosaic virus (CABMV); catharanthus mosaic virus (CatMV); johnsongrass mosaic virus (JGMV); papaya ringspot virus – type W (PRSV-W); apium virus Y (ApVY); lettuce mosaic virus (LMV); arracacha mottle virus (AMoV); pepper yellow mosaic virus (PepYMV); bidens mosaic virus (BiMV); potato virus Y (PVY). Country abbreviations: Brazil (BR); United States of America (USA); Taiwan (TW). The corresponding GenBank accession number of each virus sequence is given in the figure. Bar = number of substitutions per site. BiMoV BR is highlighted in red in the tree.

by Baker et al. (2007). Lettuce plants type 'Romana' showed leaf mottling 12 DPI, and endive-infected plants remained asymptomatic. *C. amaranticolor* plants reacted with chlorotic local lesions on the inoculated leaves eight DPI. The remaining mechanically inoculated plants tested negative in RT-PCR assays with the specific primer for BiMoV. Nucleotide sequences obtained from amplicons of experimentally infected *Zinnia* sp., hairy beggarticks, sunflower, lettuce type 'Romana', and endive plants shared 100 % identity with the nucleotide sequence of the BiMoV-BR isolate.

Myzus persicae transmitted BiMoV-BR to *Zinnia* sp. plants. Transmission efficacies were 8 % (1/12) and 42 % (5/12) using, respectively, one and ten aphids to inoculate each plant.

In Brazil, *Zinnia elegans* has already been identified as naturally infected with the potyviruses bidens mosaic virus (BiMV) and sunflower chlorotic mottle virus (SuCMoV), the tobamovirus tobacco mosaic virus (TMV), and the orthotospovirus groundnut ringspot virus (GRSV) (Oliveira et al., 2022; Kitajima, 2020). Hairy beggartick plants have been reported in the country infected with the nucleorhabdovirus sowthistle yellow vein virus (SYVV), the polerovirus potato leafroll virus (PLRV), and the potyviruses BiMV and potato virus Y (PVY) (Kitajima, 2020).

Here, we presented the first near-complete genome sequence of a Brazilian isolate of BiMoV. This is also the first detection of BiMoV in Brazil. Studies are needed to verify the occurrence of BiMoV in other crops, such as endive, lettuce, and sunflower, to better evaluate the distribution and importance of this potyvirus in the country.

Authors' Contributions

Conceptualization: Favara GM, Rezende JAM. **Data curation:** Favara GM, Rezende JAM. **Formal analysis:** Favara GM, Ferro CG, Bello VH, Oliveira FF, Kraide HD, Kitajima EW. **Funding acquisition:** Rezende JAM, Kitajima EW. **Investigation:** Favara GM, Ferro CG, Bello VH, Oliveira FF, Kraide HD, Kitajima EW, Rezende JAM. **Methodology:** Favara GM, Ferro CG, Bello VH, Oliveira FF, Kraide HD, Ribeiro-Junior MR, Kitajima EW, Rezende JAM. **Project administration:** Rezende JAM. **Resources:** Rezende JAM. **Software:** Favara GM, Ferro CG, Ribeiro-Junior MR. **Supervision:** Rezende JAM. **Validation:** Favara GM, Rezende JAM. **Writing – original draft:** Favara GM. **Writing – review & editing:** Favara GM, Ferro CG, Bello VH, Oliveira FF, Kraide HD, Ribeiro-Junior MR, Krause-Sakate R, Kitajima EW, Rezende JAM.

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