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# Evidence of infection of cassava plants with the begomovirus passionfruit severe leaf distortion virus in Brazil

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**ABSTRACT**: Symptoms of severe vein clearing, leaf rolling, and blistering were observed in cassava plants in southwestern Bahia State, Brazil. The plants were collected, vegetatively propagated, and kept in a greenhouse. Leaf samples tested positive for begomovirus infection by Polymerase chain reaction (PCR). Complete nucleotide sequences of DNA-A and DNA-B of isolate PSLDV-Man04 revealed 99.89 % and 96.2 % identity, respectively, with the bipartite begomovirus passionfruit severe leaf distortion virus (PSLDV) genome. This PSLDV isolate was experimentally transmitted to two and four of the five cassava plants and ten passion fruit plants, respectively, inoculated with *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1). This is the first report of the natural infection of cassava plants by the PSLDV begomovirus in Brazil.

**Keywords**: PSLDV, first report, complete genome, root crop, natural infection

Cassava (*Manihot esculenta* Crantz) belongs to the Euphorbiaceae family which originated in South America and is one of the world's most important tropical root crops. This perennial shrub is widely cultivated for food and industrial applications (Nassar, 2007). Although cassava is primarily grown in family farming systems, it is an essential income source, and production efficiency is extremely high (Ceballos et al., 2004). Brazil is the fourth largest producer of cassava in the world, with a production of 18 million tons harvested from 1.2 million hectares in 2021 (FAO, 2023). The following viruses have been reported in Brazilian's cassava crops: cassava common mosaic virus (CsCMV) (Costa, 1940; Kitajima, 2020), cassava frogskin disease associated virus (CaFDaV) (Kitajima, 2020; Poltronieri et al., 1998), an unidentified nucleorhabdovirus, tobacco necrosis virus (TNV) (Kitajima, 2020), and cassava vein mosaic virus (CaVMV) (Costa, 1940; Kitajima, 2020); however, occurrences are of low economic importance, in most cases.

In 2016, around 100 cassava plants in a single field adjacent to passion fruit plants infected by passion fruit severe leaf distortion virus (PSLDV) were found and showed severe vein clearing, leaf rolling, and blistering symptoms in the municipality of Anagé (14°37′00″ S, 41°10′18″ W, 416.58 m), Bahia State, Brazil. *Bemisia tabaci* was found colonizing the plants. Eight cassava plants were collected, vegetatively propagated, and kept in a greenhouse in the municipality of Vitória da Conquista, Bahia State. Over time, severe mosaic symptoms alternately appeared and disappeared among the cassava plants (Figures 1A-C). The plants exhibited intermittent symptoms with periods of symptom absence and mild or severe symptoms in some leaves at different times. In 2021, symptomatic leaves of two of the eight field-cassava plants kept in the greenhouse were received for diagnostic testing at the Laboratory of Plant Virology, ESALQ/Universidade de São Paulo, municipality of Piracicaba, São Paulo State, Brazil.

Total RNA/DNA was extracted from each symptomatic leaf sample using the Purelink viral RNA/DNA kit as recommended by the manufacturer (Thermo Fisher Scientific). The polymerase chain reaction (PCR) was performed with 12.5 µL of 2 × PCR Master Mix (Promega), 10.0 µL of DNAse-free water, 2.0 µL of total DNA, and 0.25 µL of 20 mM of each begomovirus degenerate primers PAL1v1978/PAR1c496 (Rojas et al., 1993). The thermal cycler procedure was 2 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 2 min at 58 °C, 1 min at 72 °C, and a final extension at 72 °C for 10 min. The expected fragment of 1.2 kb corresponding to part of replication-associated protein (Rep) gene, part of the coat protein (CP) gene, and the entire intergenic region (IR) of begomoviruses was analyzed by 1 % agarose gel electrophoresis, containing 0.5 × TBE buffer (45 mM Tris base, 45 mM boric acid, 0.05 mM EDTA, pH 8.0) and visualized in a UV light transilluminator after staining with SYBR® Safe (Life Technologies). The two analyzed samples tested positive in the PCR reaction. One amplicon from each sample was purified using the Wizard SV Gel and PCR Clean-Up System kit (Promega) and directly sequenced in both directions at Macrogen Inc. The nucleotide sequences of both fragments shared 99.7 % identity (Genbank Accessions OQ789384 and OQ789385), and the consensus sequence shared 99.1 % identity with the DNA-A sequence of PSLDV from Bahia State (MT103972). The two samples were also tested by RT-PCR for the presence of the potexvirus cassava common mosaic virus (CaCMV), using the potexvirus primers 2RC and Potex5 (van der Vlugt and Berendsen,

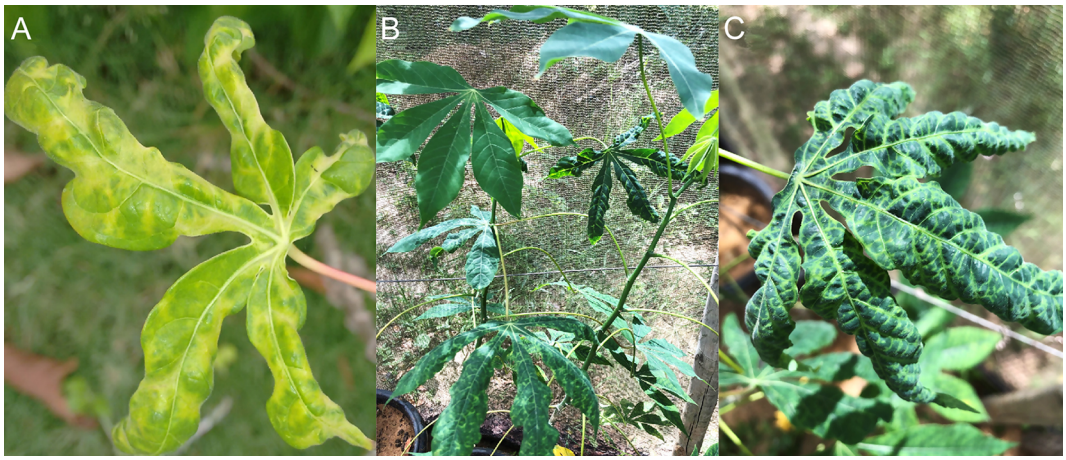


**Figure 1** – A) Leaf of field-infected *Manihot esculenta* in Bahia State (Brazil); B) Cassava plant in the greenhouse in Bahia State with some leaves exhibiting passionfruit severe leaf distortion virus (PSLDV) infection; C) Cassava leaf showing severe vein clearing, leaf rolling, and blistering.

2002). Cassava common mosaic is the predominant virus disease in cassava crops in Brazil (Venturini et al., 2016). Both samples tested negative.

Total DNA was extracted from one symptomatic leaf sample (Man04) and used as a template for rolling-circle amplification (RCA) of begomovirus genomes (Inoue-Nagata et al., 2004). The RCA products were cleaved into DNA-A and B components with ApaI and ClaI, respectively. They were ligated into the pBLUESCRIPT-KS + (pKS + ) plasmid vector (Stratagene), previously cleaved with the same enzymes, resulting in clones with the complete genome component of the begomovirus. One clone of each genomic component was sequenced by primer walking at Macrogen. The nucleotide sequences were analyzed using BLASTn, and ORF finder was used to determine amino acid sequences (Wheeler et al., 2003). The DNA-A and B sequences of isolate PSLDV-Man04 were determined at 2,672 and 2,616 nucleotides, respectively (GenBank Accessions No. OQ789382, and OQ789383, respectively). The DNA-A and DNA-B of PSLDV-Man04 shared 99.89 % and 96.2 % nucleotide sequence identity, respectively, with those of PSLDV isolates from Dom Basilio (GenBank Accession N° OR608383) and Seabra (GenBank Accession N° MT104028), from Bahia State.

Symptomatic cassava leaves from a PSLDV-infected plant were used for virus transmission by *B. tabaci* MEAM1 to healthy *M. esculenta* and *Passiflora edulis* Sims plants. The virus acquisition access period was 72 h. Five and ten healthy plants of cassava and passion fruit, respectively, were inoculated with an average of 100 potentially PSLDV-viruliferous adult whiteflies. The inoculation access period (IAP) was 72 h. Approximately 55 days after inoculation, two of the five cassava plants showed mild symptoms on some leaves. Four of the ten inoculated passion fruit plants showed mosaic on some leaves 40 days after inoculation. All plants inoculated with PSLDV were tested by PCR to confirm the infection using the specific primers PSLDV-254F and PSLDV-1180R (Rodrigues et al., 2019), which amplify a 926 bp fragment of DNA-A corresponding to part of the coat protein (CP) and replication enhancer (Ren) genes of the virus. The PCR reaction was performed with 12.5 µL of 2 × PCR Master Mix (Promega), 10.0 µL of DNAse-free water, 100 ng of total DNA, and 0.25 µL of each PSLDV-specific primer. The thermocycler condition was 2 min at 94 °C, 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min 20 s, and final extension of 10 min at 72 °C. The amplified DNA fragments were analyzed by 1 % agarose gel electrophoresis, containing 0.5 × TBE buffer, stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific), and observed in a transilluminator. Only symptomatic cassava and passion fruit plants were positive for PSLDV by PCR, and two randomly chosen amplicons, one of each host plant, respectively, were purified and directly sequenced to confirm PSLDV infection. Three healthy cassava and passion fruit plants used as negative controls remained asymptomatic and tested molecularly negative for virus infection at the end of the experiment.

The results above show that the severe vein clearing, leaf rolling, and blistering in Bahia State cassava plants were apparently associated to a PSLDV isolate. To date, natural infections of PSLDV have only been identified in passion fruit orchards in Bahia State, but they have occurred intermittently. The first begomovirus epidemic in passion fruit plants, associated with a large population of *B. tabaci* colonizing these plants, was observed in 2001 in the municipality of Livramento de Nossa Senhora, Bahia State (Novaes et al., 2003), which was associated with a begomovirus tentatively identified as passionflower little leaf mosaic virus (PFLLMV) (Novaes et al., 2003) and later biologically and molecularly characterized as PSLDV (Ferreira et al.,

2010). A second PSLDV outbreak, also associated with a high population of *B. tabaci* MEAM1, was observed during 2012-2014 in passionflower orchards in ten municipalities in Bahia State, including Livramento de Nossa Senhora (Rodrigues et al., 2019).

This is the first report of natural infection of cassava plants with the PSLDV begomovirus in Brazil, representing an alert to encourage frequent monitoring to prevent begomovirus from becoming a threat to the cassava crops in that region and potentially spreading to other cassava-producing areas in the country. In the African continent, the cassava mosaic disease caused by a complex of begomoviruses is one of the significant constraints to cassava production (Chikoti and Tembo, 2022). *B. tabaci* is reported to transmit all begomoviruses (Navas-Castillo et al., 2011). High species richness of whiteflies was observed in cassava crops in Brazil; however, it appears that begomovirus epidemics did not occur in these crops due to the absence of competent vector populations. However, a continuous adaptation process of *B. tabaci* MEAM1 to cassava is occurring, which may increase the emergence of begomoviruses in this crop (Xavier et al., 2021), including the PSLDV. Furthermore, due to predominant vegetative propagation, cassava plants could be a long-lasting PSLDV reservoir host, sustaining the viral population from one cropping cycle to the next (Legg et al., 2015). Further information is needed to determine the frequency of infection in the field and the role of PSLDV-infected passion fruit and cassava on the epidemiology of the disease in both crops.

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## Authors' Contributions

Conceptualization: Ferro CG, Rezende JAM. Data curation: Ferro CG. Formal analysis: Ferro CG. Funding acquisition: Rezende JAM. Investigation: Ferro CG, Favara GM, Kraide HD, Mesquita NLS, Novaes QS. Methodology: Ferro CG, Favara GM, Kraide HD. Project administration: Ferro CG. Resources: Rezende JAM. Supervision: Rezende JAM. Writing-original draft: Ferro CG. Writing-review & editing: Ferro CG, Favara GM, Kraide HD, Mesquita NLS, Novaes QS, Rezende JAM.

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