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First report of *Yambean mosaic virus* in Brazil

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Abstract
Potyvirus can cause losses in several important crops worldwide. This study describes the identification of a potyvirus infecting Jack bean plants and causing severe mosaic in Piauí state, Brazil. The virus reproduced original symptoms in mechanically inoculated Jack bean plants. Further molecular and transmission electron microscopy assays enabled the identification of the causal virus as *Yambean mosaic virus* (YBMV). To the best of our knowledge, this report is the first to describe YBMV in Brazil.

Keywords *Canavalia ensiformis* · Cylindrical inclusions · Jack bean · *Potyvirus*

The Jack bean (*Canavalia ensiformis*) is a legume with tropical and subtropical distribution that is used as a green manure for soil enrichment and is commonly used in cattle feed (Suzuki and Alves 2006). Among diseases that occur in Jack beans in Brazil, mosaic has been historically associated with potyviruses, although precise identification at the species level has not been possible because previous studies used less precise techniques and because data on the genomes of these viruses is lacking (Costa et al. 1989; Santos et al. 1990, 1991). In February 2018, Jack bean plants exhibiting mosaic, leaf deformation and blistering symptoms (Fig. 1) were found in the state of Piauí, Brazil. We investigated the aetiology of the disease by amplifying and sequencing two genomic regions (CI and NIb gene) complemented by TEM, identifying the causal agent of mosaic in Jack bean as an isolate of *Yambean mosaic virus* (YBMV).

The leaf samples of symptomatic Jack beans were collected at the experimental unit (S 05° 2′ 33.55″ and W 42° 47′ 3.08″) of the Department of Plant Science at the UFPI, Teresina, Piauí, Brazil. Symptomatic leaves were collected for further analysis by transmission electron microscopy (TEM) and for molecular diagnosis by amplification and sequencing of genome regions. Samples were identified and preserved at −80 °C. TEM was employed to examine negatively stained leaf extracts (Kitajima 1965) and to observe the cytopathic effects on leaf tissues exhibited by symptomatic Jack bean plants. For direct observation of leaf extract, symptomatic leaves were macerated in the presence of phosphate buffer (0.01 M, pH 7.0), transferred onto carbon-Formvar coated grids and negatively contrasted with uranyl acetate. For ultrastructural analysis by TEM, small fragments of symptomatic leaves of Jack bean were fixed in a glutaraldehyde (2.5%)/paraformaldehyde (2%) mixture in cacodylate buffer (0.05 M, pH 7.2) for several hours, post-fixed with 1% OsO_4, dehydrated in a graded ethanol series, and subjected to infiltration and embedding in the Spurr epoxy resin. The

Fig. 1 Symptoms of YBMV on the leaves of naturally infected Jack beans (*Canavalia ensiformis*)

The sequences reported in this paper have been deposited in the GenBank under accession numbers MK825543, MK825544 and MK829488.

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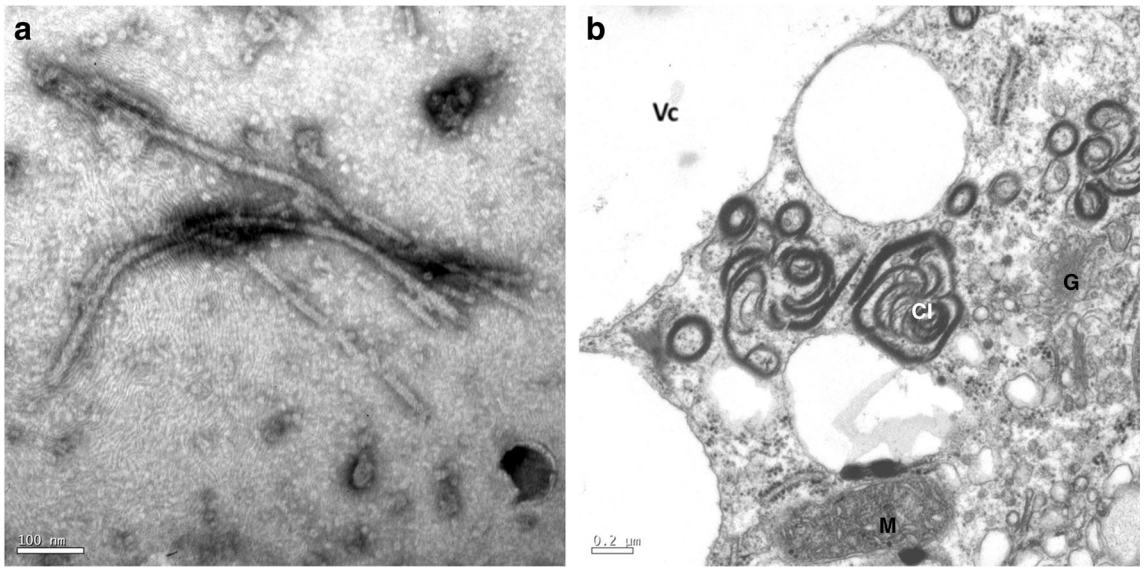

Fig. 2 Transmission electron micrograph of the isolate of YBMV infecting Jack bean (*Canavalia ensiformis*). **a** Elongated, flexuous particles present in a negative-stained extract from infected Jack bean leaf. **b** Typical potyvirus cylindrical inclusions (CI) of type II in Edwardson's classification formed by groups of scrolls joined in the centre and the absence of lamellar aggregates present in a section of leaf parenchyma of infected Jack bean. G- Golgi complex; M- mitochondria; Vc- Vacuole

blocks were sectioned in a Leica Ultracut UCT ultramicrotome equipped with a Diatome diamond knife, and 70–100-nm-thick sections were collected on 300 mesh copper grids. Sections were contrasted with uranyl acetate and lead citrate and examined in a TEM JEOL 1011. The images were digitally recorded (Kitajima and Nome 1999). Total tissue leaf RNA from symptomatic plants was extracted with the Trizol® reagent (Thermo Fisher Scientific, USA) following the manufacturer's recommendations. The pellets were resuspended in 50 µL of ultrapure distilled water free of nucleases. The RNA samples were analysed by 1% agarose gel electrophoresis in 0.5x TBE (Tris-Borate-EDTA) buffer, stained with ethidium bromide and visualized under UV light in the UVB transilluminator (Loccus, São Paulo, Brazil). The RNAs were stored at −20 °C. The reactions to amplify the CI and NIb genes were performed in two steps. First-strand cDNAs were synthesized using GoScript® reverse transcriptase enzyme (Promega, Madison, WI, USA) according to the manufacturer's recommendations and using CIRev and NIb3R as the initial primers. Polymerase chain reaction (PCR) assays were performed with the universal oligonucleotides CIFord and CIRev (Ha et al. 2008) and the NIb2F and

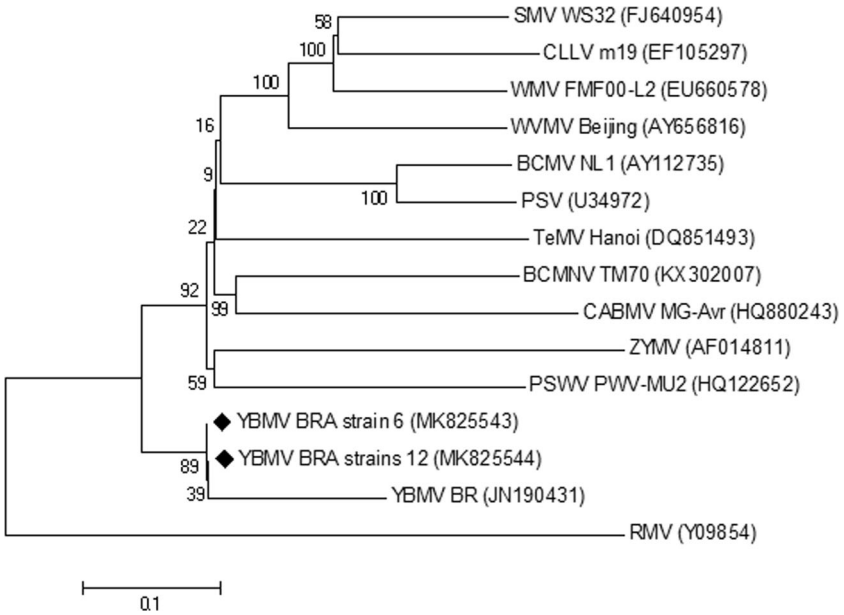

Fig. 3 Neighbour-joining phylogenetic tree of cylindrical inclusion (CI) protein gene sequences of YBMV isolates (indicated by black diamond) compared with previously reported YBMV and other potyviruses. Analysis was performed with MEGA8 with 1000 replicates of bootstrapping. The isolates from this study are marked (♦)

NIb3R (Zheng et al. 2010) of potyviruses. For synthesis of the PCR, the GoTaq® Hot Start Polymerase (Promega, Madison, WI, USA) was used according to the manufacturer's recommendations. Samples were also tested by reverse transcription PCR (RT-PCR) using specific or universal primers for different viruses that incite similar symptoms on legume plants: *Cucumber mosaic virus* (Kim et al. 2014), *Cowpea severe mosaic virus* (Brioso et al. 1996), and *Cowpea mild mottle virus* (Lamas et al. 2017). The reactions were performed in AmpliTherm® thermal cyclers (Axygen). The amplified fragments were purified and sequenced in both directions (forward and reverse) by Macrogen Inc. (Seoul, South Korea). The edited sequences were compared to sequences deposited in GenBank by the BLASTn tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were generated using the MUSCLE algorithm implemented by the MEGA v.8.0 program (Kumar et al. 2018). The alignments were corrected manually and analysed with the MEGA v.8.0 program using the neighbour-joining method.

Leaf samples from Jack beans plants that were previously confirmed to be infected with only one virus species were mechanically inoculated via buffered plant extract (0.01 M potassium phosphate, pH 7.5) and abrasive (carborundum) in five healthy Jack bean plants. The inoculation occurred 20 days after emergence of the seedlings. The plants were cultivated in 2.8 L pots containing sterile substrate (70% soil, 15% burned rice straw and 15% cattle manure) and kept in insect-free cages throughout the entire experiment period. Two plants were used as controls.

TEM examination of extracts from symptomatic Jack bean plants revealed the consistent presence of elongated and flexuous particles ca. 13 nm in diameter and 700–750 nm in length (Fig. 2a). The presence of cylindrical inclusions (Fig. 2b) in the cytoplasm of leaf cells of symptomatic plants suggested that the infection was caused by a member of the genus *Potyvirus*. Comparisons of the 700 bp and 350 bp CI and NIb gene amplicon sequences, respectively, with sequences available from GenBank revealed 88% and 88.26% identity with YBMV isolated from yam bean plants in Peru (accession number JN190431) (Fuentes et al. 2012) and Indonesia (accession number AB289438) (Damayanti et al. 2008), respectively. These values are above the limit of demarcation of species of potyviruses, that is, 78.3% and 76.6% for the genes CI and NIb, respectively (Addans et al. 2005), according to the International Committee on Taxonomy of Viruses (ICTV), indicating that the potyvirus infecting and causing mosaic in Jack bean in Brazil is an isolate of YBMV. All samples presented negative results for the presence of CMV, CPMMV and comoviruses (data not shown). Phylogenetic analysis using the CI region and including other related potyviruses showed that they formed a well-supported clade, with the YBMV isolate from Peru (Fig. 3). This report is the first to describe YBMV in natural infections in Brazil. YBMV has probably been detected in the country since the 1980s, but the identification techniques used at the time were not sufficiently accurate for the correct identification of the causal agent, which was tentatively identified as an isolate of a *Bean common mosaic virus* (BCMV), serologically close to *Cowpea aphid borne mosaic virus* (CABMV) (Costa et al. 1989), or even as an unidentified species but related to BCMV (Santos et al. 1990, 1991). Additional surveys investigating the natural occurrence of YBMV in Brazil are required to assess its dissemination, since it is known that this virus may cause diseases in cash crops, such as cowpea and tomato (Damayanti et al. 2008).

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Compliance with ethical standards

Disclosure of potential conflicts of interest We declare that the authors have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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