



Tobacco mild green mosaic virus found naturally infecting *Nicotiana glauca* in Brazil

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Abstract

Nicotiana glauca exhibiting very mild mottling on the leaves was found in the municipality of Piracicaba, state of São Paulo, Brazil. Transmission electron microscope analysis of symptomatic tissue, biological assay on virus indicator hosts, and near complete genome sequence indicated infection with the tobamovirus Tobacco mild green mosaic virus. To our knowledge this is the first report of the presence of this tobamovirus in Brazil.

Keywords Occurrence · Identification · Tobamovirus · Complete genome

The tobacco tree (*Nicotiana glauca*) is an herbaceous plant, native to South America, with some medical properties, used also as ornamental, but considered in some circumstances as invasive. A plant of *N. glauca* showing very mild mottling was found in a residential garden in Piracicaba municipality, state of São Paulo, Brazil (Fig. 1a). Suspecting a possible viral etiology, some leaves were collected and subjected to further analysis. Preliminary transmission electron microscopic examination of negatively stained leaf extract revealed abundant rod-shaped particles, 18 nm wide and ca. 300 nm long (Fig. 1b), suggesting infection by a tobamovirus. Examination of thin sections of symptomatic *N. glauca* leaf tissues confirmed the presence of large, parallel aggregates of rigid, elongated particles (Fig. 1c). Mechanical transmission assays of phosphate-buffered extracts of symptomatic *N. glauca* leaves resulted in local lesions in *Chenopodium amaranticolor*, *C. quinoa*, *Datura stramonium*, *Gomphrena globosa*, *Nicotiana glutinosa*

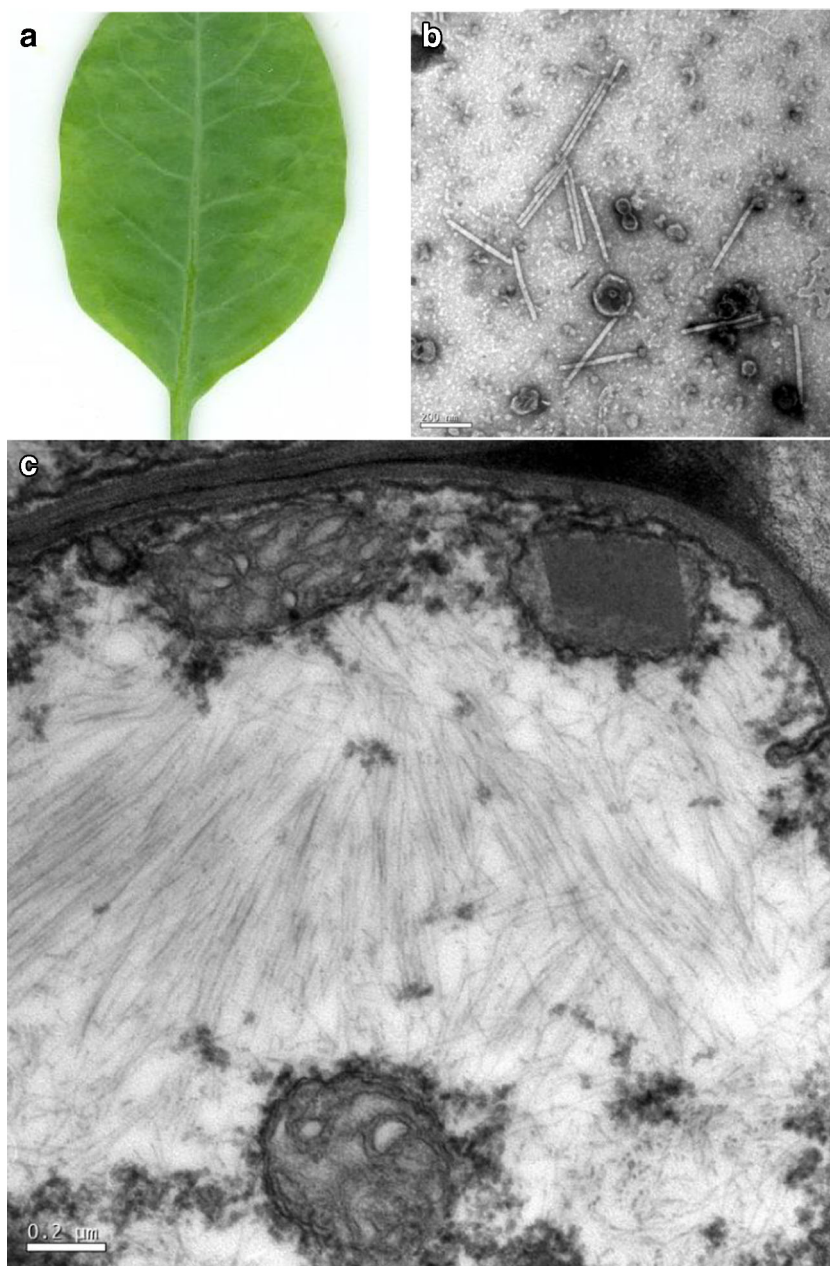
and *N. tabacum* cv. TNN. Inoculated *N. glauca* reproduced the systemic mild mottling, whereas systemic infection of *N. tabacum* cvs. Havana and Turkish caused severe mosaic. Inoculated plants of *N. benthamiana* resulted in initially symptomless infection, but the plant wilted and died two weeks after inoculation. Negatively stained extracts of all these symptomatic plants exhibited rod shaped particles under electron microscopic examination and sectioned leaf tissues exhibited aggregates of rod-shaped particles in the cytoplasm. The tobamovirus was partially purified by ammonium sulfate precipitation and the virus RNA isolated using the Purelink® viral RNA/DNA kit (Thermo Fisher Scientific, Waltham, USA). A cDNA library was prepared with the Illumina TruSeq Stranded mRNA Sample Prep LT Protocol (Illumina, San Diego, USA). The nucleotide sequencing was done in an Illumina HiSeq 2500 System at the Center of Functional Genomics (ESALQ/USP, Piracicaba, Brazil), using a HiSeq Flow Cell v4 and the HiSeq SBS v4 kit (Illumina) in paired-end mode, producing reads of 100 bp (2x100bp). The near-complete genome has 6.352 nt (GenBank MK005155) and showed 96.74% to 97.67% identities with corresponding nucleotide sequences from different isolates of Tobacco mild green mosaic virus (TMGMV) deposited on GenBank. RT-PCR assays on experimentally inoculated test plants, as well as the original sample of symptomatic *N. glauca*, using specific pair of primers for coat protein gene of TMGMV (Cohen et al. 2001) amplified fragments of expected size (455 bp), confirming the infection by this tobamovirus. TMGMV, originally described by McKinney (1929) in

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Fig. 1 Mild mottling on *Nicotiana glauca* (a), rod-shaped particles, 18 nm wide and ca. 300 nm long, in negatively stained leaf extract of mechanically inoculated *Chenopodium quinoa* (b), large, parallel aggregates of rigid, elongated particles in thin section of leaf tissue of symptomatic *Nicotiana glauca* (c)



N. glauca in the Canary Island, has been detected infecting *N. glauca* in several parts of the world, as well as some cultivated plants, and considered of medium risk of potential economic losses (Skelton and Sansford 2012). This is the first report of the presence of TMGMV in Brazil.

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