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Cauliflower mosaic virus naturally infects wild radish (*Raphanus raphanistrum*) in Brazil

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Abstract
Cauliflower mosaic virus (CaMV) infects species of Brassicaceae worldwide, including weeds that can act as virus reservoirs. In a survey carried out in brassica fields, we detected CaMV infecting wild radish (*Raphanus raphanistrum*) from Southeast and Midwest of Brazil. To our knowledge, this is the first report of CaMV infecting this weed in Brazil.

Keywords Brassicaceae · CaMV · *Caulimovirus*

Currently, there are 13 species of viruses belonging to the genus *Caulimovirus*, and *Cauliflower mosaic virus* is the type species of this genus in the family *Caulimoviridae*, order *Ortervirales* (ICTV 2019 - https://talk.ictvonline.org/). Cauliflower mosaic virus (CaMV) has isometric particles 50 nm in diameter and genome consisting of a double-stranded circular DNA of approximately 8,000 bp in size. CaMV genome has seven open reading frames, which encode six well-characterized viral proteins (P1-P6): P1 (40 kDa), a cell-to-cell movement protein; P2 (18 kDa), a protein associated to aphid transmission; P3 (15 kDa), a protein with a dual role in cell-to-cell movement and aphid transmission; P4 (56 kDa), the capsid protein; P5 (78 kDa), a polyprotein precursor of proteinase, reverse transcriptase and ribonuclease H; and P6 (62 kDa), a multifunctional protein, the major component of cytoplasmic inclusion bodies (viroplasm or viral factories) associated to virus intracellular movement, symptoms development and host defences. As CaMV replicates by the reverse transcription of an RNA intermediate, it is also classified in the pararetrovirus supergroup (Hohn 2013; Schoelz et al. 2016).

CaMV infects brassicas worldwide and it is transmitted either mechanically or by aphids in a non-circulative manner. Its narrow host range is restricted to plants of the Brassicaceae, but some CaMV strains can also infect species of Solanaceae (Haas et al. 2002). In Brazil, wild radish (*Raphanus raphanistrum*, Brassicaceae), a weed commonly found in brassica fields, has been described as host only of the potyvirus turnip mosaic virus (TuMV) and the polerovirus beet western yellows virus (compiled by Kitajima 2015).

In a survey carried out in Brazilian brassica fields, in 2018, wild radish plants showing leaf mosaic, vein clearing and necrosis were collected in the municipalities of Pinhais (25°26′41”S, 49°11′33”W, 893 m), State of Paraná (sample T106), and Pirenópolis (15°51′09”S, 48°57′33”W, 770 m), State of Goiás (sample T109) (Fig. 1). Both samples were submitted to biological, molecular and electron microscopy analysis. For transmission electron microscopy, small fragments of symptomatic leaves were fixed in a mixture of paraformaldehyde (2%) and glutaraldehyde (2.5%) in 0.05 M cacodylate buffer, posfixed in 1% OsO_4, dehydrated in ethanol, infiltrated and embedded in epoxy Spurr medium. Sections were cut with Diatome diamond knife in a Leica UTC

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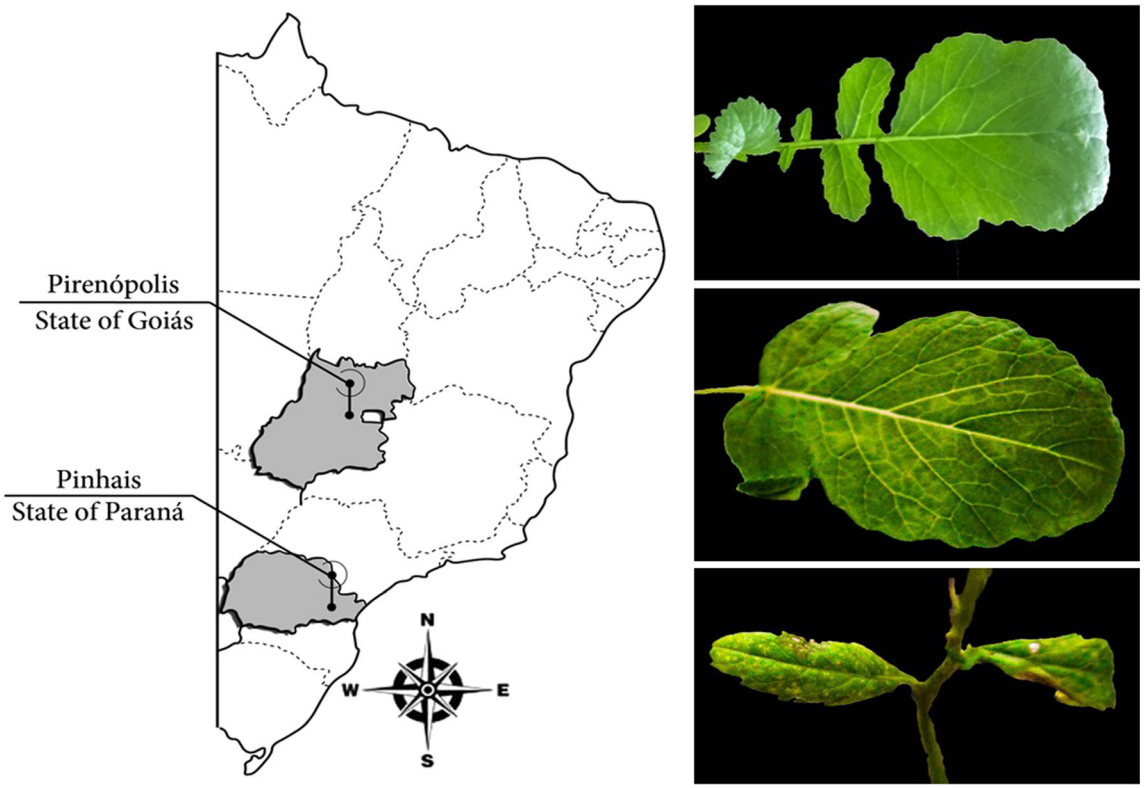

Fig. 1 On the left, a map of Brazil with the indications of the municipalities of Pinhais (State of Paraná) and Pirenópolis (State of Goiás) where wild radish (*Raphanus raphanistrum*) weeds were collected. On the right, healthy asymptomatic wild radish leaf (above), and mosaic, vein clearing and necrosis symptoms induced by cauliflower mosaic virus (CaMV) in wild radish plants

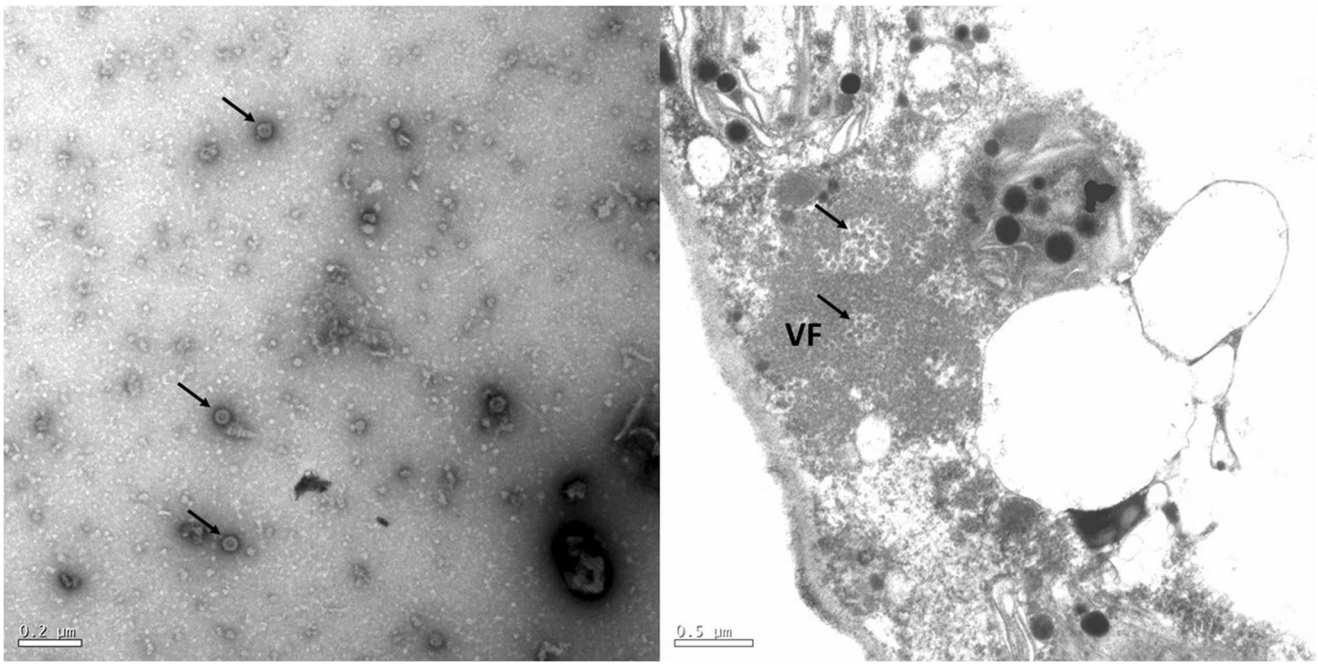

Fig. 2 Transmission electron micrographs of cauliflower mosaic virus (CaMV) in infected wild radish (*Raphanus raphanistrum*) leaves. On the left, isometric particles of ca. 50 nm in diameter (arrows) can be observed in negatively stained (leaf-dip) of leaf extract. On the right, viral factories (VF), typical caulimoviruses inclusions, and virions (arrows) can be observed in the cytoplasm of the mesophyll parenchymal infected leaf cell

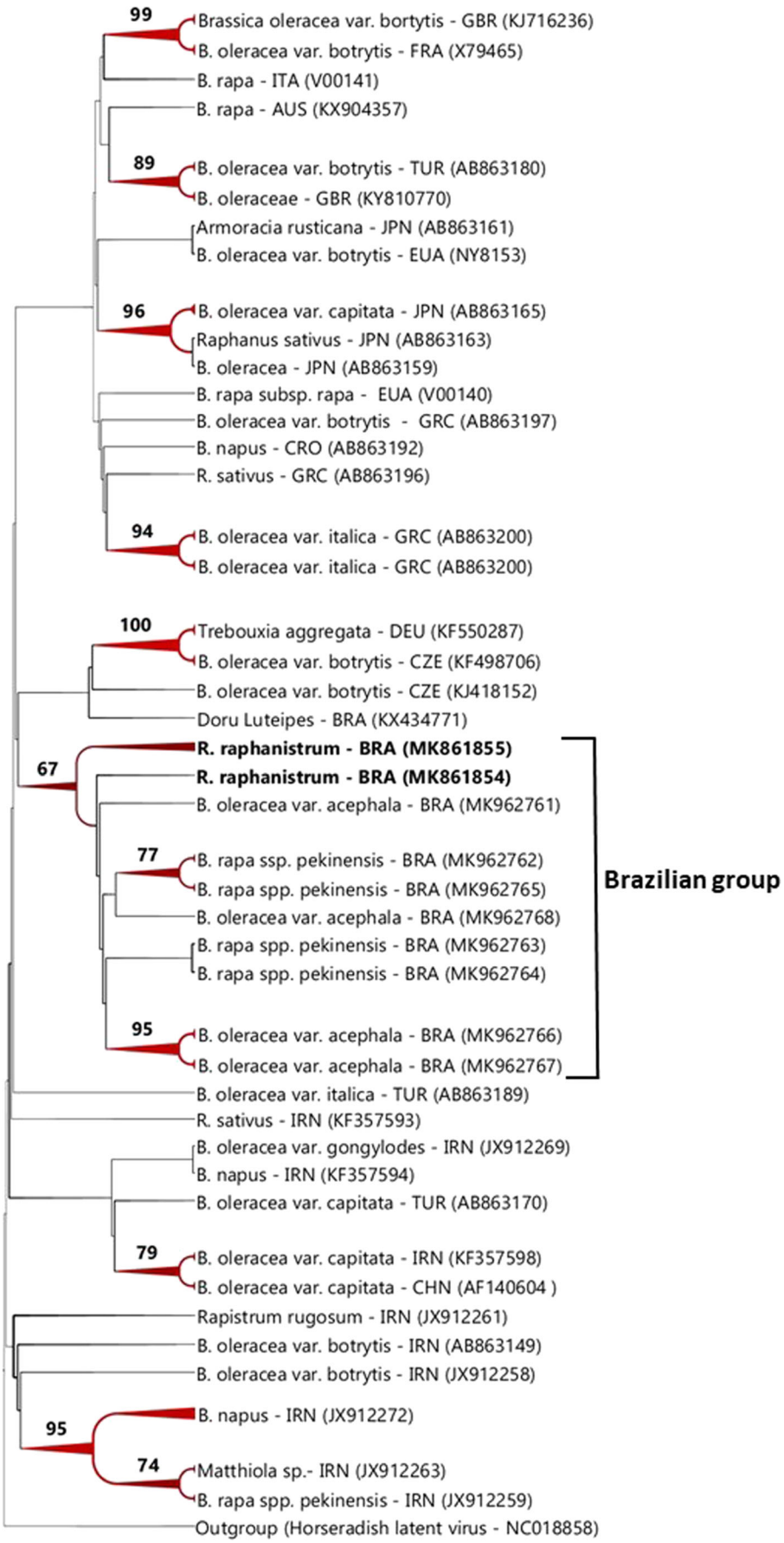

Fig. 3 Phylogenetic tree based on the multiple sequence alignment of the 361 nucleotides of part of the movement protein gene (ORF 1) of Brazilian cauliflower mosaic virus (CaMV) wild radish (*Raphanus raphanistrum*) isolates (sequenced in this work) and other sequence variants obtained from Genbank. Searches were done using maximum likelihood method and the tree was obtained using MEGA software (version 7.0). Bootstrap (analyses for 1,500 replications) percentage values are shown above the branches

ultramicrotome, stained with uranyl acetate and Reynold's lead citrate, and examined in a JEOL JEM 1011 transmission electron microscope. Images were recorded digitally. Naturally-diseased wild radish leaf fragments were ground in 0.05 M potassium phosphate buffer, pH 8.0, plus 0.5% sodium sulphite, and the inoculum rubbed on previously carborundum-dusted leaves of the healthy indicator host plants. For total DNA extraction, young symptomatic wild radish leaves (0.2 g) were ground in liquid nitrogen and immediately transferred to 1.5 mL microfuge tubes, following protocol described by Lopez-Moya et al. (1992). DNA was employed as template for PCR amplifications, using *Go Taq Flexi DNA polymerase* kit (Promega), according to the manufacturer's instructions, with CaMV-specific primers from part of the movement protein gene, ORF 1 (Lopez-Moya et al. 1992). Total RNA was also extracted from young symptomatic wild radish leaves (0.1 g), using *TRIzol® Reagent* (Invitrogen) according to the manufacturer's instructions. RT-PCR was carried out by mixing approximately 0.5 μg of total RNA with M-MLV *reverse transcriptase* kit (Promega), according to the manufacturer's instructions, with the reverse TuMV-specific primer. PCR was done with 1 μL of the complementary DNA, using *Go Taq Flexi DNA polymerase* kit (Promega), according to the manufacturer's instructions, with TuMV-specific primers from the portion of the virus genome correspondent to the cytoplasmic inclusion, CI (Ha et al. 2008).

Isometric particles of 50 nm in diameter were observed in negatively stained of leaf extract preparations (leaf-dip) and typical caulimoviruses inclusions (viral factories) and virions were found in the cytoplasm of the mesophyll parenchymal infected leaf cells in both samples (Fig. 2). Both isolates systemically infected cauliflower (*Brassica oleracea*), mustard (*B. rapa*), rocket plants (*Eruca sativa*) and wild radish (*R. raphanistrum*). PCR results obtained for CaMV detection and RT-PCR for TuMV showed that sample T106 was co-infected with CaMV and TuMV (data not shown), and sample T109 was only infected with CaMV. Amplified CaMV DNA fragments were purified and sequenced. Sequences obtained [accession numbers: MK861854 (T106) and MK861855 (T109)] were aligned and compared with other sequences from Genbank. They shared more than 96% nucleotide and amino acid sequences identity with known CaMV isolates. In the phylogenetic analyses, both CaMV isolates clustered in a clade with other Brazilian CaMV isolates (Fig. 3).

In Brazil, CaMV has been described infecting vegetables such as broccoli, cauliflower, kale (*B. oleracea*), canola (*B. napus*), Chinese cabbage (*B. rapa* ssp. *Pekinensis*), hoary-stock (*Matthiola incana*), watercress (*Nasturtium officinale*) and *Sinapsis alba* (white-mustard) (compiled by Kitajima 2015). To our knowledge, this is the first report of CaMV infecting wild radish in Brazil. This weed is often associated with aphids and cultivated brassicas in the producing vegetables fields in Brazil, providing reservoirs and source of inoculum of TuMV. In addition, our results confirmed that wild radish can be, simultaneously, reservoir of TuMV and CaMV, playing an important role in these viruses epidemiology in cultivated brassicas areas in subtropical and tropical conditions.

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