

## SHORT COMMUNICATION

# Ocurrence of pepper yellow mosaic virus and cucumber mosaic virus on *Capsicum chinense* in the state of Amazonas, Brazil

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## ABSTRACT

The habanero chilli pepper, *Capsicum chinense* is an important crop in the Amazon Basin, mainly grown by small-scale producers. *Capsicum chinense* plants in an experimental field in the northern Brazilian state of Amazonas were found exhibiting characteristic symptoms of viral infection. Leaf sap from symptomatic plants examined under a transmission electron microscope revealed the presence of elongated flexuous particles and isometric particles. Using molecular assays, the viruses were identified as pepper yellow mosaic virus (PepYMV) and cucumber mosaic virus (CMV). Aphids, identified as *Aphis gossypii*, were found colonizing the *C. chinense* plants in the field and may be the vector for both PepYMV and CMV. We report the first occurrence of these viruses infecting *C. chinense* in the state of Amazonas.

**KEYWORDS:** *Cucumovirus*, *Potyvirus*, transmission electron microscopy, RT-PCR, *Aphis gossypii*

## Ocorrência do pepper yellow mosaic virus e cucumber mosaic virus em *Capsicum chinense* no estado do Amazonas, Brasil

### RESUMO

A pimenta-de-cheiro, *Capsicum chinense* é uma cultura importante na Bacia Amazônica, cultivada principalmente por pequenos produtores. Plantas de *C. chinense* em um campo experimental localizado no norte do estado brasileiro do Amazonas, foram encontradas apresentando sintomas característicos de infecção viral. Extratos de amostras de folhas sintomáticas examinados ao microscópio eletrônico de transmissão revelaram a presença de partículas alongadas e flexuosas e de partículas isométricas. Análises moleculares permitiram identificar a presença do pepper yellow mosaic virus (PepYMV) e do cucumber mosaic virus (CMV). Pulgões, identificados como *Aphis gossypii* foram encontrados colonizando pimenteiros-de-cheiro neste campo experimental e podem representar o provável vetor de PepYMV e CMV. Este trabalho relata a primeira ocorrência desses vírus infectando *C. chinense* no estado do Amazonas.

**PALAVRAS-CHAVE:** *Cucumovirus*, *Potyvirus*, microscopia eletrônica de transmissão, RT-PCR, *Aphis gossypii*

*Capsicum chinense* Jacq. (Solanaceae), known in northern Brazil as *pimenta-de-cheiro*, is a genuinely Brazilian chili pepper, because its greatest genetic diversity is found in the Amazon Basin (Carvalho *et al.* 2006; Moreira *et al.* 2006). In the state of Amazonas, *C. chinense* is cultivated mostly by small-scale producers, and in recent years the area planted has increased (IDAM 2016).

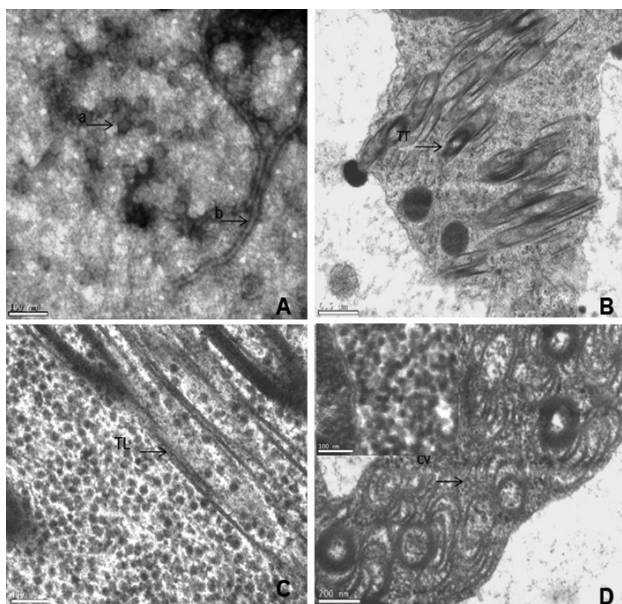
Plants of *C. chinense* showing foliar symptoms of mosaic, malformation and blistering, accompanied by stunting (Figure 1), suggesting possible viral infection, were observed in an experimental field at Estação Experimental de Hortaliças Dr. Alejo Von der Pahlen (02°59'45.7"S, 60°01'22.3"W), of Instituto Nacional de Pesquisas da Amazônia (INPA), in Manaus, Amazonas state, Brazil. Symptomatic leaf samples were collected for further analysis.

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Negatively stained leaf extracts from symptomatic samples of *C. chinense*, examined under a JEOL JEM 1011 transmission electron microscope (TEM) revealed the presence of elongated flexuous particles of approximately 13 nm in width and 700-800 nm in length, suggesting infection by potyviruses. Many isometric particles 25-30 nm in diameter, resembling cucumoviruses, were also observed (Figure 2a). For cytopathological studies, infected leaf tissues were



**Figure 1.** Symptoms exhibited by *Capsicum chinense* plants in an experimental field in Manaus, Amazonas state, Brazil. (A-B) mosaic and blistering; (C) stunting; (D) mosaic. This figure is in color in the electronic version.



**Figure 2.** (A) Negatively stained leaf extract exhibiting the presence of isometric particles (arrow a), and elongated flexuous virus-like particles (arrow b). (B-D) Transmission electron micrographs of symptomatic *Capsicum chinense* leaves showing lamellar inclusions forming cylindrical scrolls characteristic of potyvirus infection. TL – cylindrical scrolls cut longitudinally; TT – cross section of cylindrical scroll which, in favorable sections, show pinwheel-like configuration (CV).

processed following Kitajima and Nome (1999). Observation of ultrathin sections in the TEM indicated the presence of cytoplasmic lamellar inclusions forming long scrolls (Figure 2b-d), which, in cross section, formed a typical configuration referred to as a pinwheel, characteristic of potyvirus infection (Edwardson 1974). Isometric particles were occasionally seen forming small aggregates in the cytoplasm, which can be distinguished from ribosomal particles due to their smooth profile and slightly larger diameter.

Total RNA was extracted from eight symptomatic *C. chinense* plants using a PureLink Viral RNA/DNA kit (Thermo Fisher Scientific, Waltham, USA) following the manufacturer's recommendations. One-step RT-PCR was performed using universal primers PV1/SP6 and WCIEI-sense, for detection of potyviruses (Mackenzie *et al.* 1998; Maciel *et al.* 2011), and specific primers for the coat protein gene of cucumber mosaic virus (CMV) (Wylie *et al.* 1993), which amplify fragments of 800 and 480 bp, respectively. Amplicons of the expected size for each virus were purified and directly sequenced in both directions at Macrogen Inc. (Seoul, South Korea). The consensus nucleotide sequences were obtained using Electropherogram quality analysis (<http://bioinformatica.cenargen.embrapa.br/phph/>). The identity of the nucleotide sequences was defined using BLASTn (Altschul *et al.* 1990; Camacho *et al.* 2009). Nucleotide sequences (GenBank accession numbers MK512671, MK512672 and MK512673) obtained using the universal primers for potyviruses showed 96% to 98% identity with corresponding nucleotide sequences from pepper yellow mosaic virus (PepYMV) isolates from Brazil deposited in GenBank (AB541985, AF348610). Likewise, nucleotide sequences of two CMV amplicons (MK512669 and MK512670) showed 97% to 99% identity with corresponding nucleotide sequences of different CMV isolates (KX014666, KM091956, KM272276).

Immature and adult aphids were collected from some symptomatic plants and stored in tubes containing 70% alcohol for further identification. Aphid samples were mounted on slides, and examined under a light microscope for species identification. *Aphis gossypii* Glover, 1877 (Auchenorrhynca: Aphididae) was the only species found in the collected samples, and possibly represents the vector for PepYMV and CMV, both of which are stylet borne (non-persistent) (Whitfield *et al.* 2015). Identification of *Aphis gossypii* followed Blackman and Eastop (1984), using characteristics such as dark siphunculi uniformly sclerotized from tip to base, longer than the tail, and uniformly sclerotized whitish dorsal abdominal segments.

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