Disease Notes

Diseases Caused by Viruses

First Report of Bidens Mosaic Virus Infecting *Centella asiatica* in Brazil

Viviana Marcela Camelo-Garcia, Gabriel Madoglio Favara, Elliot Watanabe Kitajima,† and Jorge Alberto Marques Rezende

Department of Plant Pathology and Nematology, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP 13418-900, Brazil

Funding: Funding was provided by Convênio USP/ESALQ-Inst. Plantarum (USP 6159) and Fundação de Amparo à Pesquisado Estado de São Paulo (2017/18910-4). Plant Dis. 105:517, 2021; published online as https://doi.org/10.1094/PDIS-07-20-1437-PDN. Accepted for publication 1 September 2020.

Centella asiatica (Apiaceae) is a perennial herbaceous creeper long used for therapeutic and cosmetic purposes (Gohil et al. 2010; James and Dubery 2009) and easily propagated vegetatively. In 2018, plants exhibiting foliar mosaic and malformation were found in the Plantarum Institute botanical garden (PI, 22°46′45.8″S, 47°18′47.5″W) and an experimental area at Escola Superior de Agricultura Luiz de Queiroz (ESALQ) (22°42′26.0″S, 47°37′48.6″W). Plants were grown in ~4-m^2 beds, and all were symptomatic. Leaf extract from symptomatic plants was examined by TEM after negative staining with 1% uranyl acetate. Potyvirus-like flexuous filamentous particles were observed in leaf samples from both locations. TEM of thin sections of leaf tissues revealed cylindrical inclusions, characteristic of potyvirus infection, in the cytoplasm of epidermal, parenchymal, and vascular cells. Total RNA was extracted from symptomatic leaves collected at the PI (three) and ESALQ (one) using the Purelink viral RNA/DNA kit (Thermo Fisher Scientific). RT-PCR was performed using degenerate primers CIFor (5′-GGIVVIGTIGGIWSIGG IAARTCIAC-3′) and CIRev (5′-ACICCRTTYTCDATDATRTTIGTIGC-3′), amplifying a ~700-bp fragment in the cylindrical inclusion protein gene of potyviruses (Ha et al. 2008). Expected-size amplicons were obtained for all four samples. One amplicon per location was purified with a Wizard SV Gel and PCR Clean-Up System kit (Promega) and directly sequenced in both directions at Macrogen. Nucleotide sequences from symptomatic plants from PI (GenBank MT668627) and ESALQ (MT668626) showed 97.1 and 96.2% identity, respectively, with the sequence of a Brazilian isolate of Bidens mosaic virus (BiMV) (*Potyviridae*, *Potyvirus*) (KF649336). To confirm, previously extracted RNA was analyzed by RT-PCR with specific primers 8331 (5′-CGTGGGGCTATCCTGAATTG-3′) and 9046 (5′-CCACATCAGAGAAGTGTGCC-3′), amplifying a 715-bp fragment corresponding to the BiMV coat protein gene (Suzuki et al. 2009). Expected-size amplicons were obtained for all four samples. Nucleotide sequences of two amplicons (MT668628, MT668629), representing plants from each location, showed 94.6 to 95.6% identity with corresponding sequences of BiMV coat protein gene from Brazil (KF649336, AY960150, AY960151). A leaf extract of a symptomatic *C. asiatica* plant was mechanically inoculated to healthy plants of *Apium graveolens*, *Bidens pilosa*, *C. asiatica*, *Chenopodium amaranticolor*, *C. quinoa*, *Coriander sativum*, *Nicotiana benthamiana*, *N. tabacum*, and *Petroselinum crispum.* *C. asiatica* became systemically infected, reproducing the original symptoms. *N. benthamiana* developed severe mosaic symptoms; *C. amaranticolor* and *C. quinoa* reacted only with necrotic and chlorotic local lesions, respectively. Other plants were not infected. Potyvirus-like particles were observed by TEM in the infected plants, and BiMV was confirmed by RT-PCR. Transmission assays of the BiMV isolate by aphids *Myzus persicae* and *Aphis gossypii* to healthy *C. asiatica* plants were performed. Virus-free aphids reared on *Capsicum annuum* and *Gossypium hirsutum*, respectively, were fasted for 30 min and then placed, separately, on symptomatic *C. asiatica* leaves for a 10-min acquisition access period. Groups of six insects were transferred, separately, to four healthy *C. asiatica* plants for a 24-h inoculation access period. After inoculation the insects were killed manually. Roughly 30 days later, one plant inoculated with each aphid species exhibited symptoms; infection was confirmed by RT-PCR and nucleotide sequencing of the amplicons. BiMV was absent in noninoculated control plants in both assays. Infection of spontaneously growing *C. asiatica* plants by potyvirus, determined by TEM, was previously reported in Curitiba and Colombo, Paraná, Brazil (Lima Neto and Souza 1981), but the virus was not fully characterized and identified. *C. asiatica* plants are susceptible to infection with cucumber mosaic virus, as reported by Cardin and Moury (2010) in Madagascar. This is the first identification of BiMV naturally infecting *C. asiatica.* Additional work on effects of BiMV infection of *C. asiatica* on commercial production and pharmaceutical properties is required.

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The author(s) declare no conflict of interest.

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Keywords: *Potyvirus*, BiMV, occurrence, identification

†Indicates the corresponding author.
E-mail: E. W. Kitajima; ewkitaji@usp.br