Diseases Caused by Viruses

Natural Infection of *Cichorium intybus* (Asteraceae) by Groundnut Ringspot Virus (Genus *Orthotospovirus*) Isolates in Brazil

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Funding: Funding was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico. Plant Dis. 106:2005, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1184-PDN>. Accepted for publication 17 December 2021.

Leaf chicory (*Cichorium intybus* L.) is a nutritionally rich vegetable used in regional cuisine in Brazil. Plants of *C. intybus* displaying symptoms (viz., chlorotic and necrotic ringspots, mosaic, and leaf deformation) similar to those induced by orthotospoviruses (genus *Orthotospovirus*, family *Tospoviridae*) were observed in three fields (approximately 0.2 ha each) in Gama County in the Federal District, Brazil, from September 2016 to January 2020 in plants of the cultivars Folha-Larga and Spadona. Incidence of symptomatic plants was nearly 10% in each field. Transmission electron microscopic examination of thin sections from symptomatic leaf samples showed typical membrane-bounded orthotospovirus particles within cisternae of spongy parenchymal cells. Two individual leaf samples per field were collected and submitted to dot enzyme-linked immunosorbent assay with polyclonal antisera against the N protein of tomato spotted wilt virus (TSWV), groundnut ringspot virus (GRSV), and tomato chlorotic spot virus (TCSV). Symptomatic samples strongly reacted only against GRSV antibodies. Total RNA was extracted (Trizol, Sigma) from all six samples and used as a template in RT-PCR assays. The primer J13 (5'-CCCGGATCCAGCAAT-3') was employed for cDNA synthesis using M-MLV reverse transcription. PCR

assays were done with the primer pair BR60/BR65 (Eiras et al. 2001) to obtain an approximately 500-bp fragment of untranslated region and partial N gene in the S RNA segment from each sample. Purified RT-PCR products of two randomly selected individual samples were directly sequenced (GenBank MW467981 and MZ126602) and their BLASTn analyses displayed 99 to 100% nucleotide identity to GRSV isolates previously reported infecting *C. endivia* in Brazil (Jorge et al. 2021). Our analyses combining N protein serology and N-gene sequencing (both directed to the S RNA segment) allowed us to confirm the GRSV infection of *C. intybus*, but the potentially reassortant nature of these isolates (Silva et al. 2019; Webster et al. 2015) is unknown since their M RNA segments were not characterized. Individual leaf extracts (in phosphate buffer, pH 7.0) of the sequenced isolates were mechanically inoculated onto 10 seedlings of two *C. intybus* cultivars (Folha Larga and Pão-de-Açúcar) and three plants each of the indicator hosts *Capsicum chinense* PI 159236, *Nicandra physalodes*, *Nicotiana rustica*, *Datura stramonium*, and tomato cultivar Santa Clara. Systemic chlorotic and necrotic ringspots, mosaic, and leaf deformation developed in the indicator hosts, and infection by GRSV was confirmed via serological assays 20 days after inoculation. However, no symptoms and no serological reaction to GRSV antibodies were observed on the *C. intybus* cultivars even after two successive mechanical inoculations. This transmission failure might be due to factors such as the requirement of the thrips vector(s), physicochemical barriers in the foliage, or the presence of nonmechanically transmissible helper agent(s) necessary to ensure GRSV infection of *C. intybus*. The natural infection of *C. intybus* by a not fully characterized orthotospovirus (mostly likely TSWV) has been observed since 1938 in Brazil (Kitajima 2020). Our report of GRSV infecting *C. intybus* thus confirms previous speculation that similar symptoms in this vegetable crop were induced by orthotospovirus infection in Brazil.

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The author(s) declare no conflict of interest.

Keywords: etiology, pathogen detection, viruses and viroids

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