Diseases Caused by Viruses

First Report of *Cichorium endivia* (Asteraceae) as a Natural Host of Groundnut Ringspot Orthotospovirus in Brazil

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Endive (*Cichorium endivia* L.) is a very important cash crop for small farmers in Brazil. During inspections conducted in the summer season of 2019 to 2020, leaf samples of *C. endivia* 'La Spezia' seedlings exhibiting typical symptoms of orthotospoviruses infection (namely, concentric chlorotic spots and apical leaf deformation; ≈10%) were collected in commercial greenhouses in Brasília-DF, Central Brazil. Leaves of one healthy and three symptomatic plants were initially evaluated via double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with polyclonal antibodies (produced at the National Center for Vegetable Crops Research [CNPV]) raised against the nucleoprotein of the three major orthotospoviruses: tomato spotted wilt orthotospovirus (TSWV), groundnut ringspot orthotospovirus (GRSV), and tomato chlorotic spot orthotospovirus (TCSV). Strong serological reactions were observed only against GRSV antibodies exclusively in extracts from symptomatic samples. To confirm the causal agent of those symptoms, total RNA was extracted (TRIzol; Sigma) from infected leaf samples and used in a two-step reverse transcription polymerase chain reaction (RT-PCR) approach. Synthesis of the cDNA was carried out with the J13 primer (5'-CCCGATCCAGAGCAAT-3') (Cortez et al. 2001) followed by PCR assays with the primer pair BR60 (5'-AGAGCAATCGTGTC-3') and BR65 (5'-ATCAAGCCTTCTGAAAGTCAT-3') (Eiras et al. 2001). This primer set amplifies a fragment of 453 bp including the untranslated region at the 3' terminus of the small RNA and the protein N-coding gene of at

least five orthotospoviruses: TSWV, GRSV, TCSV, chrysanthemum stem necrosis orthotospovirus, and zucchini lethal chlorosis orthotospovirus (Eiras et al. 2001). The obtained amplicons (≈432 bp) were subsequently subjected to Sanger dideoxy nucleotide sequencing at CNPH. BLASTn analysis showed >99% identity with a wide array of GRSV isolates available in GenBank. The nucleotide sequence of Tospo #1 (MT215222) and Tospo #3 (MT215224) isolates displayed 100% identity between them, whereas the Tospo #2 (MT215223) isolate displayed one nonsynonymous point mutation in the 3' untranslated region in comparison with the former two isolates. Three plants of *C. endivia*, *Capsicum annuum* L. cv. Ikeda, tomato (*Solanum lycopersicum* L.) cv. Santa Clara and its isoline 'LAM-147' (with the Sw-5 resistance gene), *Nicotiana rustica* L., *Lactuca sativa* L. ('Vanda' and 'PI-342444'), and *Gomphrena globosa* L. were mechanically inoculated individually with each GRSV isolate in order to confirm their pathogenicity. Chlorotic lesions and mosaic were observed 7 days after inoculation of all plant materials, except the tomato inbred line LAM-147, which has the Sw-5 gene that confers broad-spectrum resistance to all Brazilian orthotospoviruses (Boiteux and Giordano 1993). The GRSV infection was confirmed via DAS-ELISA and RT-PCR 15 days after inoculation, using the same set of antibodies and the primer pair BR60/BR65. Transmission electron microscopy of ultrathin sections from symptomatic leaf tissues, from both field-infected and experimentally inoculated endive, revealed the presence of typical orthotospovirus particles, within endoplasmic reticulum cisternae. Natural infection of endive by TSWV has been reported in Greece (Chatzivassiliou et al. 2000) and by TCSV in São Paulo State, Brazil, and in Florida, U.S.A. (Subramanya Sastry et al. 2019). To our knowledge, it is the first report of GRSV naturally infecting this Asteraceae species in Brazil. Confirmation of GRSV infection of *C. endivia* plants is a relevant piece of information aiming to design effective disease management strategies.

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