Disease Note

Diseases Caused by Viruses

First Report of Groundnut Ringspot Virus Infecting *Zinnia* sp. in Brazil

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Zinnia sp. is a genus belonging to Asteraceae family that originated in Mexico and is adapted to a warm-hot climate (Hemmati and Nikooei 2017). Several types of zinnias with different flower color and forms are cultivated in Brazil (Min et al. 2020; Souza Junior and Assunção 2020). Characteristic symptoms of infection caused by orthotospovirus, including chlorotic spots and concentric rings on the leaves, were observed in two plants of *Zinnia* sp. of a florist located in the city of Piracicaba, State of São Paulo, Brazil. Orthotospovirus-like particles were observed by transmission electron microscopy in leaf extracts from both plants, stained negatively with 1% uranyl acetate. By analyzing ultrathin sections of infected leaf tissues, particles of 80 to 100 nm in diameter were found in the lumen of the endoplasmic reticulum and nucleocapsid aggregates in the cytoplasm. Total RNA extracted separately from the leaves of both samples, using the PureLink Viral DNA/RNA kit (Thermo Fisher Scientific), was used to detect the virus by reverse transcription polymerase chain reaction (RT-PCR), using the universal primers for orthotospovirus BR60, complementary to the 3′ end of the nontranslated region of the S RNA (position 1 to 15 nt), and BR65, matching the nucleocapsid gene (N) (position 433 to 453 nt), generating an amplicon of 453 nt (Eiras et al. 2001). Amplicons of the expected size were obtained for the two samples. An amplicon was purified with the Wizard SV Gel and PCR Clean-Up System kit (Promega) and sequenced in both directions at Macrogen (South Korea). The nucleotide sequence (GenBank MW629018) showed 99.29 to 99.76% identity with nucleotide sequences of the orthotospovirus groundnut ringspot virus (GRSV) isolates (GenBank MH686229 and KY400110). Leaf extracts from symptomatic plants were also analyzed by plate-trapped antigen-enzyme-linked immunosorbent assay (PTA-ELISA), using polyclonal antiserum produced against the GRSV nucleocapsid protein (Esquivel et al. 2019). The absorbance values obtained for the extracts of the two symptomatic plants of *Zinnia* sp. (1.3 and 1.7) were twice as high as the value obtained for the healthy plant extract (0.5). Leaf extract of symptomatic *Zinnia* sp. was inoculated mechanically onto leaves of healthy plants of *Zinnia* sp., *Capsicum annuum* cv. Darha, *Cucumis sativus*, *Cucurbita pepo* cv. Caserta, *Chenopodium amaranticolor*, *Datura stramonium*, *Nicotiana tabacum* cv. Turkish, and *Solanum lycopersicum* cv. Compack. At 5 days postinoculation (dpi), inoculated leaves of *D. stramonium* reacted with local lesions, and at 9 dpi, newly developed leaves of inoculated *S. lycopersicum* plants showed necrotic spot and concentric ring symptoms, whereas *C. annuum* exhibited concentric rings at 10 dpi. Inoculated zinnia plants showed systemic chlorotic spot and concentric ring symptoms at 20 dpi, indistinguishable from those observed under natural infection. The other inoculated plant species were not symptomatic, nor was the virus detected. PTA-ELISA and RT-PCR confirmed infection with GRSV in symptomatic plants. The amplicons generated by RT-PCR of total RNA extracted from an experimentally infected plant of *C. annuum* and *D. stramonium* and from two plants of *Zinnia* sp. were sent for nucleotide sequencing. The obtained nucleotide sequences (MW629019, MW629020, MW629021, and MW629022) share 100% identity with the nucleotide sequence corresponding to the original GRSV isolate (MW629018) identified in *Zinnia* sp. This is the first report of the natural occurrence of GRSV in *Zinnia* sp. in Brazil. Studies on incidence and damage are needed to recommend alternatives for management.

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