



First report of *Groundnut ringspot virus* in cucumber fruits in Brazil

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Received: 28 Mar 2014. Published: 08 Jun 2014. Keywords: *Cucumis sativus*, *Capsicum annuum*, virus plant disease

Cucumber (*Cucumis sativus*) fruits and pepper plants (*Capsicum annuum*) cultivated in the same commercial greenhouse in Vitoriana, São Paulo, Brazil, in December 2012 and March 2013, respectively, were found with necrotic concentric rings, typical of tospovirus infection (Fig. 1). In addition, thrips were observed in cucumber, pepper and weeds flowers. To confirm the presence of virus, cucumber fruits and pepper leaves with symptoms were collected and tested by plate-trapped antigen (PTA) enzyme linked immunosorbent assay (ELISA) (Mowat & Dawson, 1987) using polyclonal antisera against *Groundnut ringspot virus* (GRSV), *Tomato spotted wilt virus* (TSWV), *Tomato chlorotic spot virus* (TCSV) and *Zucchini lethal chlorosis virus* (ZLCV). The samples reacted only with the antisera against GRSV. Total RNA was extracted, using Norgen total RNA purification kit (Norgen Biotek Corporation, Canada), according to the manufacturer's instructions and tested by reverse transcription-polymerase chain reaction (RT-PCR). Fragments of expected size (453 bp) were amplified using primers BR 60 (5' CCCGGATCCTGCAGAGCAATTGTGTCA 3') and BR 65 (5' ATCAAGCCTTCTGAAAGTCAT 3') corresponding to the part of the nucleocapsid (N) gene as described by Eiras *et al.* (2002). The RT-PCR amplicons were purified with the Qiagen PCR purification kit (Qiagen, Inc. Valencia, CA, USA), and then directly sequenced in both directions at Macrogen Inc., Seoul, Korea. BLAST analysis of the sequenced fragments from cucumber and pepper, deposited in GenBank (Accession Nos. KJ605652 and KJ782432, respectively), revealed 98% nucleotide sequence identity with an isolate of *Groundnut ringspot virus* (GRSV; GRU49702). Total genomic DNA was extracted from a single female thrips using the Chelex method (Boonham *et al.*, 2002). A portion of the mitochondrial cytochrome oxidase I gene (COI) was amplified via standard PCR reaction using the primers mtD-7.2F and mtD-9.2R (Brunner *et al.*, 2002). The 450 pb fragment amplified showed 98% identity with *Frankliniella schultzei* (KF560548).

Cucumber fruits and pepper leaves were used for separate sap inoculations

onto different species of plants. GRSV from cucumber did not induce symptoms and was not detected in any of the species tested. Using pepper leaves chlorotic local lesions were observed on *Chenopodium quinoa*, *Cucumis sativus* cvs. Caipira (Fig. 2), Salada and Aodai; additionally necrotic local lesions on *Citrullus lanatus* cv. Crimson Sweet (Fig. 3) and initial concentric rings that evolved into systemic necrosis on *Datura stramonium*, *Nicotiana tabacum* cv. TNN, *N. rustica*, *N. occidentalis* and *Capsicum annuum* cv. Magali. *Gomphrena globosa* showed chlorotic local lesions, leaf deformation and mottling. The presence or absence of GRSV in plants was confirmed by PTA-ELISA, RT-PCR and viral sequencing. To our knowledge, this is the first report of natural infection of GRSV in cucumber and causing symptoms on fruits.

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Figure 1



Figure 2



Figure 3

To cite this report: Spadotti DMA, Leão EU, Rocha KCG, Pavan MA, Krause-Sakate R, 2014. First report of *Groundnut ringspot virus* in cucumber fruits in Brazil. *New Disease Reports* **29**, 25. <http://dx.doi.org/10.5197/j.2044-0588.2014.029.025>

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