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First Report of Groundnut Ringspot Orthotospovirus Infecting Soybeans in Brazil

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Soybean (*Glycine max* [L.] Merrill) is a major fabaceous crop in Brazil. In 2017, plants of soybean cultivar BRS 8180 RR were found exhibiting virus-like symptoms including apical mottling, mosaic, and chlorotic ringspots in three fields in Brasília–DF, Brazil. The overall disease incidence across the three fields was ≈5%. Seventy-two leaf samples (with and without virus-like symptoms) were collected in all three fields (24 samples per field). Samples were analyzed using nitrocellulose membrane enzyme linked immunosorbent assay with antisera (produced at CNPH) specific to the nucleocapsid (N) protein of three orthotospovirus species: tomato spotted wilt orthotospovirus (TSWV), tomato chlorotic spot orthotospovirus (TCSV), and groundnut ringspot orthotospovirus (GRSV). Twelve out of the 72 samples reacted positively only to GRSV-specific antiserum. To confirm the identity of the virus, total RNA was isolated from leaf samples of the 12 GRSV-positive plants using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA). Purified nucleic acids were used as template for the reverse transcription step. For cDNA synthesis, the J13 primer (5′-CCCGGATCCAGAGCAAT-3′), which encompasses eight conserved nucleotides at the 3′ termini of the three genomic RNAs (small [S], medium [M], and large [L]) of orthotospoviruses, was used. Polymerase chain reaction assays were then conducted with a set of virus-specific primers targeting the N gene of TSWV (5′-GCTGGAGCTAAGTATAGC-3′ and 5′-CACAAGGCAAAGACCTTGAG-3′) (Adkins and Rosskopf 2002), GRSV (5′-AGAGCTTCCTTAGTGTTGTACTTAG-3′ and 5′-GAAAGGTCTAGATCTAAACTGCCAC-3′) (Webster et al. 2011), and TCSV (5′-CTCGGTTTTCTGCTTTTC-3′ and 5′-CGGACAGGCTGGAGAAATCG-3′) (Baysal Gurel et al. 2015). Only GRSV-specific amplicons (≈600 bp) were obtained from all 12 GRSV-positive soybean plants. Amplicons of the three GRSV isolates S4 (MH388802), S8 (MH388803), and S30 (MG029625) were Sanger sequenced at CNPH. The consensus sequences were aligned and compared with other GRSV sequences from the database, using SDT version 1.2 (Muhire et al. 2014). Alignments of the N gene sequences of these GRSV isolates displayed nucleotide sequence identity above 95% with other GRSV isolates available at the GenBank database. In order to expand the genomic analysis of these isolates, we designed primers targeting the NSm gene (5′-TGACACTTTTCGGCAGCAA-3′ and 5′-CAAACACCTTCTTCTTCTTCC-3′) and the NSs gene (5′-ATAAGCACAAGAGCACAAG-3′ and 5′-CTGTAGCCATGAGCAAAGA-3′). The sequence alignments of the NSm (≈800 bp) (MH388796 to MH388798) and the NSs (≈702 bp) (MH388799 to MH388801) genes from the three soybean isolates displayed nucleotide identity above 95% with other GRSV isolates of a wide range of hosts. So far, only field peas and peanuts (Camelo-García et al. 2014) have been reported as fabaceous hosts of GRSV in Brazil. The persistent presence of viruliferous thrips (mainly *Frankliniella schultzei* [Trybom]; Thysanoptera: Thripidae) and alternative hosts belonging to native flora, solanaceous weeds, and cultivated plant species around/within soybean-planting areas in central Brazil might increase the importance of orthotospovirus to the crop. In addition, the identification of soybean as a new host of GRSV may impact crop rotation strategies, because this legume is often cultivated after processing tomatoes in this region.

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