



Charcoal rot of *Stevia rebaudiana* caused by *Macrophomina phaseolina* in Brazil

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In January 2018, plants of *Stevia rebaudiana* with symptoms of leaf chlorosis, wilting, browning and the presence of microsclerotia in the stem and root, which progressed to premature death, with an incidence of 10% were observed and collected in a production field near Uberlândia-MG, Brazil. The isolations on PDA medium of the lesions allowed to obtain three isolates. After 15 days of incubation at 25 °C/12 h light on PDA, colonies showed an initial dark gray color, then turning black and smooth. Mycelium was septate and branched. The presence of microsclerotia (n = 100, 47–164 µm diameter) was observed. These characteristics were similar to those described in the literature for *Macrophomina phaseolina* (Barnett and Hunter 1998). DNA from the three isolates was extracted and amplified using specific MpKF1/MpKR1 primers (Babu et al. 2007), generating a 350 bp fragment. Furthermore, the ITS region of rDNA was sequenced using ITS1/ITS4 primers. Obtained sequences were deposited in GenBank (MN096209; ON286930; ON306395) and compared to other sequences using BLAST. The isolates showed more than 99% identity with other *M. phaseolina* sequences (MT102264; MW585378; MH141281), confirming the morphological identification.

To fulfill Koch's postulates, the inoculation of 8-week-old plants grown was performed in a greenhouse by ten mycelium plugs (5 mm) located at 1 cm from the base of the plant roots (buried 1 cm deep), while controls were inoculated with PDA plugs (Koehler and Shew 2018). The test was performed with five repetitions and repeated twice.

After three weeks, the inoculated plants showed leaf wilt and black-gray necrosis in the roots (presence of microsclerotia). While the same pathogen was re-isolated from inoculated plants, the controls remained healthy. It was concluded that the root rot observed in *S. rebaudiana* plants was caused by *M. phaseolina*, being the first report of this pathogen causing damage to the crop in Brazil.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42161-022-01193-0>.

Data Availability The data that support the findings of this study are openly available in NCBI at <https://www.ncbi.nlm.nih.gov/nucleotide/MN096209.1>, <https://www.ncbi.nlm.nih.gov/nucleotide/ON286930.1>, <https://www.ncbi.nlm.nih.gov/nucleotide/ON306395.1>, reference numbers GenBank: MN096209.1; ON286930.1; ON306395.1.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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