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First Report of *Macrophomina phaseolina* Causing Dry Root Rot of Chickpea (*Cicer arietinum*) in Serbia

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Macrophomina phaseolina (Tassi) Goid has been reported as a severe pathogen of many plants worldwide (Mengistue et al. 2015), as well as in Serbia (Tančić Živanov et al. 2018). However, there has been no formal report of *M. phaseolina* causing dry root rot of chickpea (*Cicer arietinum* L.) in Serbia. In June 2018, the first symptoms were observed on affected field plants grouped in patches on approximately 10% of chickpea plants in a 3-acre area at Rimski Šančevi, Serbia. Withering and death of aerial parts and root rot were observed on chickpea plants at the V6 growth stage. Cuttings from symptomatic root tissues of five chickpea plants were surface disinfected with 1% NaOCl solution for 5 min, rinsed three times in sterile distilled water, air dried on sterilized filter paper, and plated on potato dextrose agar (PDA) and water agar amended with 100 mg/liter of streptomycin sulfate (Sigma-Aldrich). After 7 days of incubation at 28°C in the dark, isolates were preliminarily identified according to their morphological characters, as hyaline mycelia that turned from gray to dark with age and produced black pigmentation in agar caused by masses of dark oblong microsclerotia formed 3 days after incubation (Watanabe 2010). A representative isolate (K349) was purified by a hyphal-tip transfer technique for further analyses (Leslie and Summerell 2006). To confirm the morphological identification, the rRNA internal

transcribed spacer (ITS) region of the isolate K349 was amplified with universal primers ITS1 and ITS4. The resulting ITS sequence (GenBank no. MK418768.1) was used in a BLAST search of the NCBI nucleotide database and showed 100% identity with *M. phaseolina* isolates (e.g., GenBank nos. KJ744350.1, KP784426.1, KU831518.1, etc.). Translation elongation factor 1-alpha gene (TEF1-α) was amplified using primers EF1-728 and EF1-986, and the resulting sequence (GenBank no. MK430416) had 97.2 to 99.5% identity with *M. phaseolina* isolates (e.g., GenBank nos. MH000354.1, MG434668.1, KX400854.1, etc.). After the amplification with primers MpKF1 and MaKR1, specific for *M. phaseolina* (Babu et al. 2007), a 350-bp PCR fragment was obtained, indicating that the isolate K349 was indeed *M. phaseolina*. The pathogenicity was confirmed according to Koch's postulates. Chickpea seeds were surface disinfected with 1% NaOCl, air dried, and planted in 10-cm-diameter pots filled with sterilized potting mixture. In total, 10 plants (one per pot) were maintained in the growth chamber at a constant temperature of 25°C, 14-h photoperiod, with relative humidity 100%. Eight-day-old plants were inoculated with three PDA plugs of 5 mm diameter around the exposed stem base of each plant and covered with sterile soil mixture. PDA plugs without mycelia were used for control plants. After inoculation, plants were incubated under growth chamber conditions at 28°C with 14-h photoperiod and watered regularly. The first symptoms observed were wilting leaves and discoloration around the stem base 16 days after inoculation, whereas the control plants remained symptomless. The pathogen was successfully reisolated from the stem base and confirmed as *M. phaseolina.* To the best of our knowledge, this is the first report of *M. phaseolina* causing root rot on chickpea in Serbia.

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