First Report of Fusarium oxysporum f. sp. ciceris on chickpea (Cicer arietinum L.) in Serbia

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Chickpea (Cicer arietinum L.) is one of the most commonly consumed legume crops worldwide, cultivated in more than 55 countries (FAOSTAT, 2017). However, in Serbia, it is a novel crop grown on approximately 120 acres, but the areas under this crop slightly increase each year. Fusarium wilt caused by F. oxysporum f. sp. ciceris is one of the most economically important disease in the most chickpea-growing areas (Jimenez-Diaz et al., 2015), but yet there has been no formal report of the Fusarium wilt of chickpea in Serbia. In June 2018, the first symptoms of Fusarium wilt were registered at Rimski Šančevi (Vojvodina Province), Serbia (N 45019.311' E 019049.933') as wilted chickpea plants grouped in patches on approximately 5% plants in the area of 3 acres. Symptoms as yellowing and necrosis of foliage appeared as late wilt in the podding stage. Roots of affected plants showed no external discoloration, but cross-sections showed darkbrown discoloration of xylem tissue. To isolate the causal agent, symptomatic plants were collected, and ten cuttings of symptomatic root tissue were surface disinfected with 2% sodium hypochlorite 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 (WA) amended with streptomycin sulfate. After seven days of incubation at 25°C in the dark, isolates were preliminarily identified according to their morphological characters and Fusarium Laboratory Manual (Leslie and Summerell, 2006). For each isolate 100, micro/macroconidia and chlamydospores were measured. Conidia were hyaline; macroconidia sickle-shaped, with blunt ends, 2-4 septa (10.1-17.7 x 3.1-5.8 µm); microconidia ellipsoidal, 0-1 septa (4.9-8.6-2.7-3.5 µm). Chlamydospores were globose (4.3-8.8 µm). Representative isolates (K343, K375 and K378) were purified by a single-spore technique for further analyses (Leslie and Summerell, 2006). Molecular identification of three representative isolates (K343, K375 and K378) was made by sequencing the rRNA internal transcribed spacer (ITS) region and translation elongation factor 1a (TEF1) gen. For all isolates, the ITS and TEF1 genes were amplified and sequenced with primers ITS1/4 (White et al. 1990), EF1-728 and EF1-986 (Rehner and Buckley 2005). Based on a BLAST search of the NCBI nucleotide database, the ITS sequences (GenBank MK920204.1, MK928423.1 and MK928424.1) had 99.8% identity with F. oxysporum f. sp. ciceris isolate (MK074845.1). The TEF1 (GenBank MN788462.1, MN788463.1 and MN788464.1) had 96.3 -100% identities with F. oxysporum f. sp. ciceris isolate (FJ538245.1).

The pathogenicity test was conducted on 7-day old plants according to the drench method described by Maitlo et al. (2016). The concentration of inoculation suspensions was adjusted on 1×10^6 , and ten plants per isolate were tested and inoculated with 10 ml of suspension. Control plants were drenched with 10 ml of sterilized distilled water. Nine days after inoculation, first symptoms as leaves wilting and white mycelia presence around stem base occurred on plants inoculated with isolate K378. On day 11, the first symptoms occurred on plants inoculated with isolates K343 and K375 as well. Up to the 13th day after inoculation, all plants were wilted, and the pathogen was successfully reisolated and confirmed as *F. oxysporum* f.sp. *ciceris*.

To the best of our knowledge, this is the first report of *F. oxysporum* f.sp. *ciceris* causing Fusarium wilt on chickpea in Serbia.

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