Diseases Caused by Fungi and Fungus-Like Organisms

Fusarium incarnatum-equiseti Species Complex Causing Root Rot Disease on *Leymus chinensis* in China

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Leymus chinensis (Trin.) Tzvel. is a rhizomatous grass widely grown in the grasslands of Eurasia. With strong fertility and stress resistance, *L. chinensis* makes an excellent pasture and mowing grass, contributing to animal husbandry and thus playing an important role in the local economy of the northern grassland area in China (Baoyin et al. 2014). During August to September 2019, diseased roots of *L. chinensis* were collected from an artificially planted grassland (40°47'44" N, 111°43'58" E, alt. 1,049 m) in Shaerqin County, Hohhot, China. Infected plants were scattered across the field with disease incidence up to 2%. Symptoms observed were wilted plants and rotten roots. In order to identify the causal pathogen of root rot on *L. chinensis*, symptomatic pieces (5 × 5 mm) of grass roots were excised and surface sterilized with 75% ethanol for 3 to 5 s followed by 1% NaClO for 2 to 3 min, rinsed three times with sterile distilled water, placed on water agar, and incubated at 25°C for 3 days. The mycelia were cut and transferred onto potato dextrose agar (PDA) for subculture. A fungus was consistently isolated, and a strain (LCH054) was obtained by hyphal tip culture. Culture developed as white and fluffy aerial mycelia, with diffused pink pigment on the reverse side of PDA after culturing at 25°C for 7 days. A culture of LCH054 was transferred to carnation leaf agar (CLA) (Li et al. 2014) and incubated at 25°C for 10 days. Microconidia were absent but macroconidia were produced. Macroconidia were hyaline, sickle-shaped, and had 4 to 7 septa, 19.8 to 63.6 (mean 43.8) × 1.8 to 5.7 (mean 3.2) μm ($n = 100$). Chlamydospores were ellipsoidal or subglobose, with thick walls in clumps or chains. All morphological characteristics of LCH054 resembled *Fusarium equiseti*

(Leslie and Summerell 2006). The primers of the internal transcribed spacer (ITS) region (White et al. 1990) and translation elongation factor 1 α gene (*TEF-1 α*) (O'Donnell et al. 1998) were used to amplify the isolate, and the fragments were sequenced. BLASTn search in the NCBI database using the ITS and *TEF-1 α* sequences revealed 99 to 100% similarities with *F. equiseti*. BLAST analysis of the ITS and *TEF-1 α* sequences in the *FUSARIUM-ID* database showed them to have 99.21% (500/504 bp) and 99.52% (622/625 bp) similarities with the *F. incarnatum-equiseti* species complex (FIESC) (strain NRRL 45997) (O'Donnell et al. 2009), respectively. The ITS and *TEF-1 α* sequences were deposited in GenBank as accession numbers MT937067 and MT947530, respectively. The strain LCH054 was identified as a member of the FIESC based on morphological and molecular characteristics. For the pathogenicity test, 100 *L. chinensis* seeds were planted into five pots (12 cm diam. × 15 cm high) and kept in a greenhouse under a 16-h photoperiod at 20 to 25°C and 40% relative humidity. The conidial suspension of LCH054 was prepared by washing 7-day-old fungal culture grown on CLA using sterile deionized water. Conidia were filtered through three layers of sterile cheesecloth, counted, and adjusted to 1 × 10⁵ conidia/ml with a hemocytometer. Forty 1-month-old healthy plants (four pots) were inoculated with 400 ml of conidia suspension using the root drenching method, whereas the inoculum was replaced with 100 ml sterile water on control plants (one pot). Fourteen days after inoculation, all inoculated plants showed the typical symptoms of root rot identical to those observed in the field, whereas control plants remained healthy. LCH054 was reisolated from the inoculated plants and identified by the morphological and molecular approaches as described above. To our knowledge, this is the first report of root rot caused by *F. incarnatum-equiseti* on *L. chinensis* in China as well as worldwide. The presence of the pathogen could cause significant economic losses in *L. chinensis* production. For this reason, strategies for the management and control of this disease should be developed and implemented.

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