Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on Kidney Bean Caused by *Nigrospora oryzae* in China

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Kidney bean (*Phaseolus vulgaris* L.) is a legume with high nutritional and economic value. This vegetable crop is widely cultivated in China, providing a year-round supply of young edible pods. In July 2020, a leaf spot disease on kidney bean cultivar ‘Dabailong’ was observed on a 2-ha field in Longli County (26°16′15.66″ N, 106°48′12″ E), Guizhou Province, China. Disease incidence was estimated to be nearly 50%. Foliar symptoms manifested as black circular spots, surrounded by a yellow halo and accompanied by white mycelium. To identify the pathogen, small portions of tissue (5 by 5 mm) from margins of leaf spots were cut from 20 symptomatic leaves, surface disinfected with 75% ethanol for 30 s, rinsed two times with sterile distilled water, dried on a sterile filter paper, and incubated on potato dextrose agar (PDA) at 28°C for 3 days. In total, 39 single-spore isolates were obtained. The colonies on PDA were fluffy, changing from white to gray or black with age, and reaching 7 cm in diameter in 5 days at 28°C. Conidia were black, globose to subglobose, smooth, solitary, measuring 13.0 to 16.0 by 10.5 to 16.0 µm ($n = 30$). Morphological characteristics were consistent with *Nigrospora oryzae*. In addition, the rDNA internal transcribed spacer (ITS), large subunit (LSU), β -tubulin (*TUB*), and translation elongation factor 1- α (*TEF1*) loci were amplified by PCR and sequenced (Carbone and Kohn 1999; Glass and Donaldson 1995; O'Donnell et al. 1998; White et al. 1990). The ITS, LSU, *TUB*, and *TEF1* sequences of two isolates, GUCC19-5105 and GUCC19-5192, were submitted to GenBank. BLASTn analysis of these sequences showed >98% homology with those of *N. oryzae* strain LC 7293 in GenBank, as follows: ITS, 99.80% (MZ145361 versus

KX985931 = 498/499 bp) and 99.62% (MZ148445 versus KX985931 = 525/527 bp); LSU, 100% (MZ146317 versus KY806236 = 837/837 bp) and 99.76% (MZ148446 versus KY806236 = 847/849 bp); *TUB*, 98.72% (MZ329335 versus KY019601 = 386/391 bp) and 98.67% (MZ329337 versus KY019601 = 373/378 bp); and *TEF1*, 98.91% (MZ329336 versus KY019396 = 452/457 bp) and 98.89% (MZ329334 versus KY019396 = 444/449 bp), respectively. The phylogenetic tree of the combined four sequences showed that both isolates clustered with *N. oryzae*. Based on morphological characteristics and the multigene phylogenetic analysis, GUCC19-5105 and GUCC19-5192 isolates were identified as *N. oryzae*. Pathogenicity tests were performed twice by spraying conidial suspension (1×10^5 conidia/ml) of the two isolates (GUCC19-5105 and GUCC19-5192) on leaves of 10 (five per isolate) healthy 5-week-old kidney bean Dabailong plants. Two plants sprayed with sterile water served as controls. After inoculation, all the plants were kept moist in plastic bags for 24 h and incubated in a greenhouse at 25°C for 20 days. Leaf spots similar to those observed in the field were observed 20 days postinoculation but no lesions were observed on control plants. *N. oryzae* was reisolated from the infected tissues of inoculated kidney bean plants and the identity of the reisolated pathogen was confirmed as *N. oryzae* through morphology and sequencing ITS, LSU, *TUB*, and *TEF1* loci. In recent years, *N. oryzae* has been reported to infect a variety of plants such as *Aloe vera*, *Citrullus lanatus*, and *Costus speciosus* (Begum et al. 2018; Chen et al. 2019; Sun et al. 2021). To our knowledge, this is the first report of leaf spot disease on kidney bean caused by *N. oryzae* in the world and provides a basis for diagnosticians and researchers to identify the disease and develop disease management strategies.

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