Diseases Caused by Nematodes

Discovery of Root-Lesion Nematode (*Pratylenchus scribneri*) on Corn in Hainan Province of China

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Corn (*Zea mays* L.) is a very important cereal crop and serves as food, feed, and industrial material (Liu et al. 2016). Root-lesion nematodes (RLNs) are considered one of the most important plant-parasitic nematodes and can cause economic losses in agriculture worldwide (Jones et al. 2013). In January 2020, five samples were collected from a corn field in Lingshui Lizu Autonomous County, Hainan Province, China. The collected corn plants (cv. Denghai 685) were growing poorly, and roots showed distinct lesions and rot. Corn fields with symptoms of stunted plants and brown lesions on roots were widespread. This corn disease was severe in Lingshui Lizu Autonomous County. RLNs were extracted from soil samples by the modified Baermann funnel (Hooper et al. 2005). All samples contained RLNs, ranging from 9 to 82 (average 39) RLNs per 100 cm³ of soil and 113 to 257 (average 194) RLNs per 5 g of roots. The extracted RLNs were sterilized and cultured on carrot disks at 25°C for 90 days. Afterward, seeds of corn (cv. Denghai 685) were sown in pots containing 1.8 liters of sterilized soil. Eight plants, one per pot, were infested with 1,000 RLNs, eight pots of noninfested corn plants were used as controls, and plants were kept in a greenhouse at 25°C. At 75 days after inoculation, symptoms were like those initially observed in corn fields, whereas no symptoms were observed in the control plants. Nematodes in the soil and roots were extracted using the same method as previously described (Hooper et al. 2005). The average number of RLNs per pot was

approximately 4,250 in soil and 820 in roots. The reproduction factor (final number of nematodes/initial number of nematodes) was 5.07. No RLNs were found in the control. The experiment was conducted twice. Morphological and molecular studies of RLNs were performed to confirm species identification. The main morphological measurements ($\pm$ standard error) of adult females ($n = 15$) included body length = 526.0 ± 17.1 μ m (range = 498.0 to 560.5 μ m); stylet = 16.0 ± 0.3 μ m (15.5 to 16.5 μ m); tail length = 29.0 ± 1.5 μ m (26.5 to 31.0 μ m); a = 23.6 ± 0.6 (22.6 to 24.4); b = 5.6 ± 0.3 (5.2 to 6.0); c = 18.3 ± 0.9 (16.4 to 19.7); V = $78.2 \pm 0.6\%$ (77.4 to 79.2%); and a lip region with two annules. No males were found in the samples. This population was identified as *Pratylenchus scribneri* based on the morphological characters (Castillo and Vovlas 2007). DNA was isolated from individual nematodes followed by proteinase K-based lysis (Wang et al. 2011). The D2/D3 expansion region of the 28S rRNA gene, rDNA-internal transcribed spacer (ITS) region, and mitochondrial cytochrome oxidase I (mtDNA-COI) gene were amplified with primers D2A/-D3B (De Ley et al. 1999), TW81/AB28 (Vovlas et al. 2011), and JB3/JB5 (Liu et al. 2018), respectively. The PCR products were purified and ligated into pJET 1.2/blunt cloning vectors, transformed to *Escherichia coli* strain DH5 α , and sequenced. The obtained 28S rRNA gene D2/D3 region sequences (785 bp), ITS sequences (886 bp), and mtDNA-COI (447 bp) were submitted to GenBank. The D2/D3 region of the 28S rRNA sequences (MZ701843) showed 99.75% identity with *P. scribneri* sequences in GenBank (KX842628 and KX842625). The ITS sequences (MZ701842) showed the highest identity of 97.06% with *P. scribneri* sequences (KX842626). The mtDNA-COI sequences (OK040228) showed 100% identity with *P. scribneri* (MN366409). Both morphological and molecular data confirmed the identity of *P. scribneri*. *P. scribneri* has been reported on corn in Inner Mongolia, Hebei, Shanxi, Shandong, Henan, Jiangsu, and Liaoning provinces of China (Li et al. 2019). As far as we know, this is the first report of *P. scribneri* on corn in Hainan Province, China. Since RLNs can cause considerable damage to corn, strategic measures should be taken to prevent the spread of *P. scribneri* to other regions in China.

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