Diseases Caused by Nematodes

Occurrence of *Pratylenchus coffeae* Causing Root Rot of Soybean in Shandong Province of China

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Soybean (*Glycine max* L.) is a very important commercial crop in China (Li et al. 2019). *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev & Schuurmans Stekhoven, 1941 is one of the most important root-lesion nematodes that invade the roots of many crops. In August 2018, five root and soil samples were collected in a soybean field near Xipan village in Linshu county of Linyi City, Shandong Province, China, to investigate the occurrence of root-lesion nematodes. The collected plants (cv. Lindou No.10) were growing poorly, and the roots showed distinct brown lesions. *Pratylenchus* spp. were extracted using the modified Baermann funnel method for 2 days (Hooper et al. 2005). On average, 395 root-lesion nematodes per kilogram of soil and 36 root-lesion nematodes per gram of fresh roots were extracted. The extracted root-lesion nematodes were disinfected with 0.3% streptomycin sulfate and cultured on carrot disks for propagation at 25°C. The species identification was based on morphological and molecular criteria. Key morphological features were determined for females and males. Measurements of females ($n = 16$) included body length = 561.0 ± 37.6 μm (standard deviation) (520.5 to 654.0 μm), tail length = 30.0 ± 1.9 μm (27.0 to 33.5 μm), stylet = 16.0 ± 0.6 μm (15.0 to 17.5 μm), $a = 28.2 \pm 2.3$ (23.7 to 31.5), $b = 6.4 \pm 0.5$ (5.7 to 7.3), $c = 18.7 \pm 1.8$ (15.7 to 23.8), and $V = 80.8\% \pm 2.1$ (76.5 to 83.8%). Measurements of males ($n = 16$): body length = 511.0 ± 28.1 μm (range = 475.5 to 566.0 μm), tail length = 26.0 ± 1.3 μm (23.5 to 28.5 μm), stylet = 15.0 ± 0.5 μm (14.5 to 16.0 μm), spicule length = 17.0 ± 0.9 μm (16.0 to 18.5 μm), $a = 30.8 \pm 1.5$ (28.0 to

33.2), $b = 6.1 \pm 0.4$ (5.6 to 6.9), and $c = 19.8 \pm 1.3$ (18.1 to 22.2). All the morphological features of this population matched the description of *Pratylenchus coffeae* (Castillo and Vovlas 2007). DNA was extracted from an individual female as described previously (Wang et al. 2011). The rDNA-internal transcribed spacer (ITS) region and the D2/D3 region of the 28S rRNA gene were amplified by primers 18S/26S (Vrain et al. 1992) and D2A/D3B (De Ley et al. 1999), respectively. The PCR products were purified and sequenced. The obtained sequences of the ITS region (1,253 bp) and the D2/D3 region of 28S rRNA (781 bp) were deposited in GenBank. The ITS sequences of the root-lesion nematode obtained in this study (GenBank accession no. MT879294) exhibited 99% identity with several *P. coffeae* sequences available in GenBank (e.g., KR106219, MT586756, KY424205, and MN749379), and the obtained D2/D3 region sequence (MT879295) exhibited 100% identity with several *P. coffeae* sequences (e.g., MT586754, MN750755, MK829009, and MH730447). Both morphological and molecular data confirmed the presence of *P. coffeae*. To confirm reproduction on soybean, the obtained root-lesion nematode population was used in a greenhouse (25°C) assay to fulfill modified Koch's postulates. About 20 days after sowing, eight pots, each with one soybean plant (Lindou No.10) were inoculated with 1,000 *P. coffeae*. The inoculated plants were kept in 1.5-liter pots containing 1.2 liters of sterilized soil. Eight pots of uninoculated soybeans were used as the control. Ten weeks later, the inoculated roots were washed, and brown lesions were observed. The number of nematodes per pot was approximately 7,360 in soil and 796 in roots, and the reproduction factor was 8.16. Root-lesion nematodes and symptoms were not observed in control groups. *P. coffeae* has only been reported on soybean in Zhejiang (Wei et al. 2013) and Henan Province (Li et al. 2019) of China. To our knowledge, this is the first report of *P. coffeae* infecting soybean in Shandong Province, China. Because the root-lesion nematode can cause considerable damage to soybean, care should be taken to prevent the spread of *P. coffeae* to other regions in China.

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