Diseases Caused by Nematodes

First Report of *Meloidogyne graminicola* on Rice in Henan Province, China

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Rice (*Oryza sativa*) is an important food crop in China, and root-knot nematode *Meloidogyne graminicola* has been one of the most important diseases on rice in past 5 years (Ju et al. 2021). In August 2020, rice plants were found to be maldeveloped, having yellow leaves and hooked root tips, in an irrigated paddy field of Yuanyang County, Xinxiang City, Henan Province. Fifty rice plants were randomly collected, and 84.0% of plants were infected with root-knot nematodes, with a root-gall index of 56.0. Then nematodes from rice roots were isolated with 100- and 25- μ m sieves. A large number of females, some third-stage juveniles (J3s), and a small number of males of *Meloidogyne* spp. were found in root galls of all samples after dissection, and then they were identified and measured under the microscope. In females ($n = 20$), the perineal pattern was dorsoventrally oval with low and round dorsal arch, and the lateral field was not obvious or absent, striae are usually smooth, with occasional short and irregular striatal fragmentation. The morphological data of females are as follows: body length (BL) = 516.9 ± 72.5 μ m (424.2 to 611.6 μ m), body width (BW) = 328.4 ± 80.7 μ m (232.1 to 437.4 μ m), stylet length = 11.2 ± 1.3 μ m (7.7 to 13.9 μ m), dorsal pharyngeal gland orifice to stylet base (DGO) = 3.9 ± 0.5 μ m (3.2 to 4.5 μ m), vulval slit length = 24.3 ± 4.6 μ m (15.2 to 31.4 μ m), and vulval slit to anus distance = 16.2 ± 2.5 μ m (10.1 to 20.2 μ m). Males are long cylindrical, wormlike, with a short round tail. Morphological measurements of males ($n = 20$) were BL = $1,218.0 \pm 150.7$ μ m (1,085.7 to 1,692.2 μ m), BW = 34.2 ± 4.6 μ m (28.5 to 39.7 μ m), stylet = 17.4 ± 0.7 μ m (15.9 to 19.3 μ m), DGO = 3.6 ± 0.7 μ m (2.5 to 4.5

μ m), tail = 10.8 ± 2.1 μ m (8.0 to 14.8 μ m), and spicule = 30.3 ± 2.6 μ m (24.7 to 36.3 μ m). The egg masses from the females were incubated at 28°C for 48 h. Measurements of J2s ($n = 20$) were BL = 444.2 ± 37.8 μ m (315.7 to 547.5 μ m), BW = 21.2 ± 2.7 μ m (16.7 to 26.4 μ m), stylet = 14.2 ± 0.3 μ m (13.6 to 14.8 μ m), DGO = 3.5 ± 0.5 μ m (2.7 to 4.5 μ m), tail = 70.8 ± 5.1 μ m (61.3 to 80.8 μ m), and hyaline tail length = 21.0 ± 2.5 μ m (16.3 to 26.1 μ m). These morphological features are consistent with the original description by Golden and Birchfield (1965). DNA of a single female from each sample was extracted for molecular identification. Primer pairs D2A/D3B (5'-ACAAGTACCGTGAGGGAAAGTTG-3'/5'-TCGGAAGGAA CCAGCTACTA-3') (De Ley et al. 1999) and the species-specific primers Mg-F3/Mg-R2 (5'-TTATCGCATCATTTTATTG-3'/5'-CGCTTTGTT AGAAAATGACCT-3') (Htay et al. 2016) were used to amplify the D2/D3 region of 28S rRNA and the internal transcribed spacer (ITS) region, respectively. The amplified sequences of the D2/D3 region (GenBank MW490724, 766 bp) shared 99.9 and 99.7% homology with the sequences of *M. graminicola* (MN647592, MT576694) isolated from Guangxi and Anhui Province (Ju et al. 2021), respectively, whereas ITS region sequences (MW487239, 369 bp) shared 100 and 99.7% homology to *M. graminicola* isolates GXL3 (MN636702) and FQJ01 (MT159690), respectively. To verify the pathogenicity of nematodes, about 300 J2s were inoculated on ten 14-week-old rice (*Oryza sativa* cv. Nipponbare) planted in pots with sterilized sandy soil, respectively, and maintained in a greenhouse at 28°C/26°C with a 16-h/8-h light/dark photoperiod and 75% relative humidity. At 14 days postinoculation, obvious symptoms of hook galls were observed on roots in all inoculated rice plants, and females and males in the same shape as the collected samples were found in the root galls under the stereoscopic microscope. No symptoms were observed on noninoculated rice plants. After 28 days, the growth of the inoculated rice plants was significantly worse than that of uninoculated ones, with yellow leaves and short plants. These results confirmed the pathogenicity of *M. graminicola* on rice and indicated that *M. graminicola* had already spread from the main rice-producing areas to the wheat and rice rotation areas. To our knowledge, this is the first report of *M. graminicola* in Henan Province of China.

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