Diseases Caused by Nematodes

First Report of Cyst Nematode (*Heterodera elachista*) on *Zea mays* in Gansu Province, China

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Cyst nematodes are important plant-parasitic nematodes and make detrimental economic impacts worldwide. *Heterodera elachista* attacks rice in Japan (Ohshima 1974), Iran (Tanha Maafi et al. 2004), and China (Ding et al. 2012) and also attacks corn in Italy (De Luca et al. 2013) and northeast China (Xiao et al. 2019). Considering this nematode can infect these two most important crops with high reproduction rates, special attention should be paid to avoid its spread to new areas by soil, water, and agricultural practices. A survey for cyst nematodes was performed in fields of corn seed for propagation in September 2018 in Zhangye City, Gansu Province, which is the biggest corn seed production base in China. One hundred seventy-four samples were collected by the zig-zag method in about 25 ha of corn fields. Each soil sample containing approximately 1 kg of soil in total was from the mixture of 20 cores (20 cm in depth and 0.2 cm in diameter). Cysts were detected in six out of 174 samples using the sieving-decanting method (Byrd et al. 1983). The six samples from ~1.2 ha of corn fields in which plants displayed symptoms of dwarf, yellow leaves, and earlier wilting had an average of 14 ± 2.8 cysts per 100 g of soil. The cysts ($n = 30$) were dark brown, spherical to lemon shaped, protruding, tapered vulva with ambifenestrae separated by a vulva slit, obvious and medium-sized underbridge, few dark-brown vesicles, and clear swollen protrusion around underbridge and cyst cuticle with a zig-zag pattern. Morphological measurements of the cysts included body length (excluding neck) (range = 359.4 to 498.6 μm , mean = 429.5 $\mu\text{m} \pm \text{SD } 39.3 \mu\text{m}$), vulva slit length (34.3 to 42.8 μm , $37.9 \pm 2.5 \mu\text{m}$), fenestral length (31.2 to

43.8 μm , $38.0 \pm 4.4 \mu\text{m}$) and width (26.1 to 31.4 μm , $29.3 \pm 1.6 \mu\text{m}$), and underbridge length (74.7 to 82.9 μm , $78.3 \pm 2.8 \mu\text{m}$). Second-stage juveniles (J2s) ($n = 20$) had the following measurements: body length (386.6 to 454.9 μm , $425.3 \pm 21.8 \mu\text{m}$), stylet length (17.1 to 22.2 μm , $19.2 \pm 1.6 \mu\text{m}$), stylet knob width (3.4 to 4.3 μm , $3.9 \pm 0.3 \mu\text{m}$), tail length (50.5 to 60.5 μm , $55.5 \pm 3.3 \mu\text{m}$), and hyaline tail length (25.0 to 34.6 μm , $29.1 \pm 3.1 \mu\text{m}$). The morphological characteristics are consistent with the Italian population of *H. elachista* through the comparison of the measurements. DNA was extracted from a single cyst randomly chosen ($n = 5$) using the protocol described by Subbotin et al. (2001). The ITS-rDNA and D2/D3 fragments of the 28S rRNA were respectively amplified with universal primers pairs TW81/AB28 (Subbotin et al. 2001) and D2A/D3B (Castillo et al. 2003) and sequenced (Tsingke Biological Technology, Beijing, China). The consensus ITS sequence (GenBank accession no. MN699476, 1,037 bp) had 98.37 to 99.60% similarity with the *H. elachista* from corn in Italy (KC618469) and in China (MH712504) and from rice in China (HM560778) and in Iran (AF498391). The D2/D3 sequence (GenBank accession no. MN699477, 746 bp) exhibited 99.60 to 99.73% similarity with that of *H. elachista* from rice in China (JN202922, HM560842) and from corn in Italy (KC618462). This nematode population was identified as *H. elachista* according to the morphological and molecular features. Twenty-day-old corn seedlings (cv. Tiannuo) were inoculated with 3,000 J2s and eggs in a pot filled with sterile soil. Eight plants were inoculated, and two uninoculated plants were used as controls. J2s were found in the stained roots at 10 days after inoculation, and J4s were detected at 30 days. These inoculated plants exhibited yellowing and dwarfing similar to the symptoms observed initially in the field, and the control grew normally. After 60 days, an average of 28 cysts were detected from each inoculated plant. This is the first report of *H. elachista* in Gansu Province, northwest China.

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