Diseases Caused by Nematodes

First Report of *Meloidogyne arenaria* Infecting Maidong (*Ophiopogon japonicus*) in China

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Maidong (*Ophiopogon japonicus*) is a perennial evergreen plant of the Asparagaceae, occurring mainly in China, Japan, Vietnam, and India. It grows in the damp places on the hillside below 2,000 m above sea level, under the forest or beside the stream. It has been widely cultivated in Sichuan Province of China for medicinal uses, and it is included in the Chinese pharmacopoeia. During April 2019, maidong plants exhibiting symptoms of stunting, leaf wilting, and multiple galls in the roots associated with root-knot nematode (*Meloidogyne* sp.) were detected in a commercial field near the city of Mianyang (N30.93°, E105.42°), Sichuan, China. The second-stage juveniles (J2s) were collected from the soil in the root zone, and adult females were dissected from roots. Population densities of J2s ranged from 190 to 255 per 100 cm³. Subsequently, individual females ($n = 20$) were extracted from root samples and submitted to *Meloidogyne* species identification by perineal pattern morphological analysis ($n = 20$) and by morphometric measurements of J2s ($n = 20$). The J2s showed the following morphometric characters: body length = 475.5 ± 24.2 μ m, tail length = 55.2 ± 6.43 μ m, stylet

length = 12.4 ± 1.56 μ m, and distance from dorsal esophageal gland opening to the stylet knot (DGO) = 2.97 ± 0.44 μ m; perineal patterns of females showed a low dorsal arch, with lateral field marked by forked and broken striae, and no punctate markings between anus and tail terminus were observed. These morphological characteristics are consistent with *Meloidogyne arenaria* (Neves et al. 2017). In addition, to confirm species identification, DNA was extracted from females (Blok et al. 1997), and D2/D3 fragments of the 28S rRNA were amplified using the universal primers D2A/D3B. The DNA fragment obtained showed a 754-bp length (GenBank accession no. MW965614) that was sequenced and analyzed, and sequences were 99.8% identical to the MH359158, KX151138, and EU364889 *M. arenaria* sequences. Furthermore, species-specific sequence characterized amplified region (SCAR) primers Far/Rar were used as described by Zijlstra et al. (2000). The PCR produced approximately 420-bp sequences, which were identical to that previously reported for *M. arenaria* (Zijlstra et al. 2000). Morphological and molecular characterization supports the identification of the isolate found on *O. japonicus* as *M. arenaria*. To verify the nematode pathogenicity on maidong plants, maidong seeds were planted in 20-cm-diameter, 10-cm-deep plastic pots containing 1,000 cm³ of sterilized soil and infested with 2,000 *M. arenaria* J2s per seedling, using a sterilized micropipette. Plants were maintained at 20 to 25°C in a greenhouse. Control plants received sterile water, and the pathogenicity test was repeated three times. After 60 days, all inoculated plants showed reduced growth compared with the control. The symptoms were similar to those observed in the field, and a large number of galls (38.5 ± 2.4) and egg masses (18.5 ± 0.2) were found on each root system. Maidong was considered a good host for *M. arenaria* in Mianyang. *M. arenaria* is one of the most important plant-parasitic nematodes with a wide geographic distribution and causes great losses in many crops around the world (Perry et al. 2009). Through investigation, this is the first report worldwide of *M. arenaria* infecting *O. japonicus*.

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