Meloidogyne arenaria *attacking eggplant in Souss region, Morocco*

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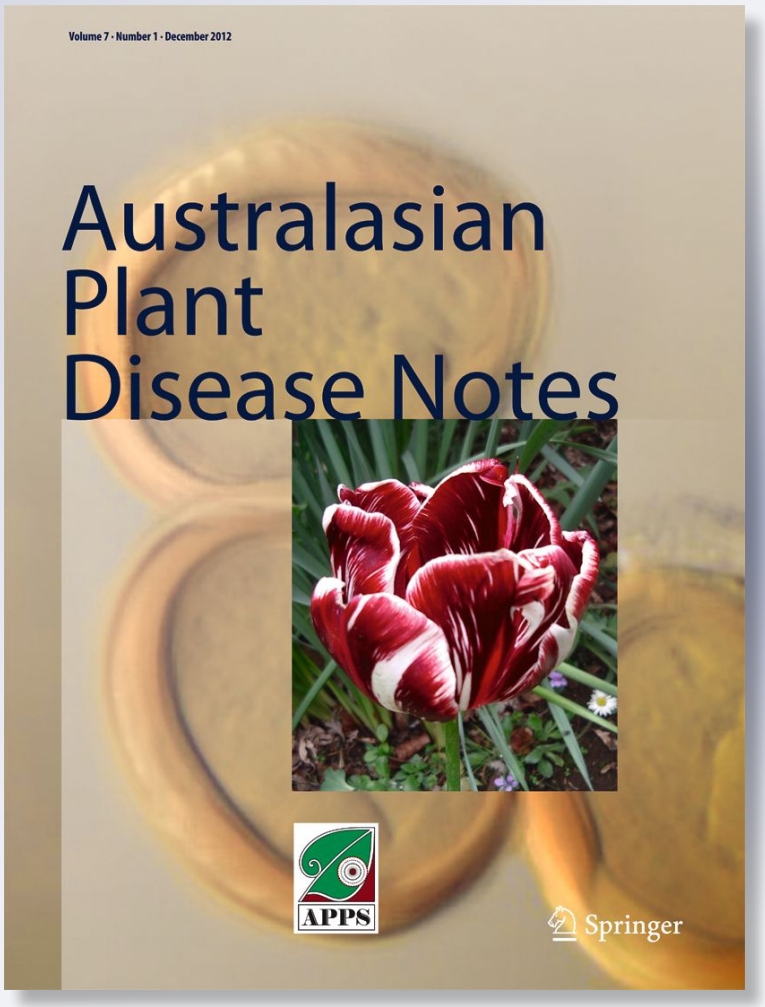

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Meloidogyne arenaria attacking eggplant in Souss region, Morocco

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Abstract

Root-knot nematodes extracted from eggplant (*Solanum melongena cv.* Black beauty) root samples collected from plantations in the Souss region of Morocco, was identified as *Meloidogyne arenaria* based on female perineal pattern and sequence-characterized amplified region polymerase chain reaction (SCAR-PCR) technique.

Keywords Eggplants · Galls · Identification

Meloidogyne spp. are the most damaging plant-parasitic nematodes in vegetable production (Jones et al. 2013). In Morocco, nematode species of the genus *Meloidogyne* are among the most relevant group of plant parasitic nematodes and are widely distributed throughout the country (Mokrini 2016; Janati et al. 2018). Both *Meloidogyne javanica* and *M. incognita* are the most common species in different agricultural areas of Morocco (Janati et al. 2018). In June 2016, eggplants (*Solanum melongena cv.* Black beauty) with above-ground symptoms of stunting and leaf wilting were recorded in a field in the Souss region of Morocco (Latitude: 30.320739; Longitude: −9.482632). Examination of the root samples taken from plants showing above-ground symptoms revealed the presence of root-knot nematodes (galls) (Fig. 1). Egg masses ($n = 4$) were picked up individually from infested roots using a small needle. These egg masses were surface-sterilized in 0.5% NaOCl as described by Dababat and Sikora (2007) then rinsed in tap water 3 times and prepared for inoculation. To obtain pure cultures, seedlings of susceptible tomato plant (*Solanum lycopersicum*, cv. Prystila F1) were transplanted singly into 500 mL plastic pots containing sterilized sandy loam soil and sand (2: 1, *v*/v) and were allowed to grow for 5–7 days before each was inoculated with a single egg mass. Plants were maintained in a glasshouse at temperature rate of 25 ± 5 °C, artificial light of 16 h per day, and 60 to 90% of relative humidity. Sixty days after inoculation, plants were removed from pots, root systems were gently washed to free of adhering soil particles, and adult females were recovered from roots with a fine needle. The collected adult females were identified by the female perineal pattern, and by sequence-characterized amplified region polymerase chain reaction (SCAR-PCR) technique as *Meloidogyne arenaria*. Female ($n = 20$) perineal patterns had the characteristic rounded to flattened, low dorsal arch, sometimes flat with dorsal striae abruptly to the lateral field and forming a small shoulder (Fig. 2). Measurements and morphological observations of 20 s-stage juveniles (J2 s) were: body length = 435 ± 18.4 μm; stylet length = 10.1 ± 0.9 μm; DGO = 2.6 ± 0.5 μm; tail length 51 ± 1.2 μm; hyaline tail terminus = 9.1 ± 1.1 μm. The morphology and morphometry of this species conformed with descriptions and measurements provided by Hunt and Handoo (2009). Microscope slides of *M. arenaria* perineal patterns were deposited at the Nematology laboratory at National Institute of Agricultural Research (INRA), Agadir, Morocco, with the following accession numbers: MF322019, MF332019, and MF342019. From each morphologically investigated female, DNA was extracted from single J2 ($n = 4$) by incubating them in a lysis buffer (200 mM NaCl, 200 mM

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Fig. 1 Eggplant fields in Kliaa with above-ground symptoms of stunting and leaf wilting

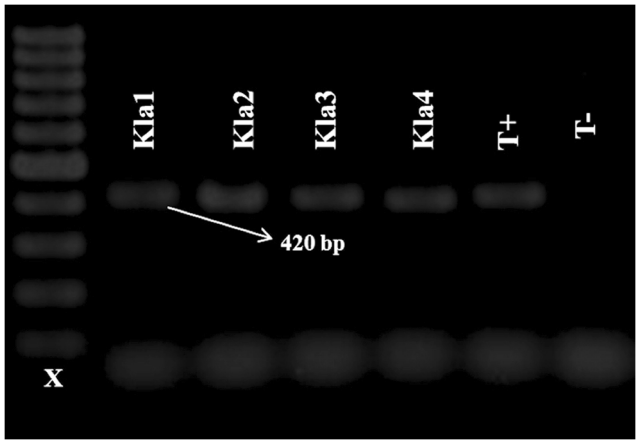

Fig. 3 Amplification product (420 bp) with Far/Rar primers on *Meloidogyne* population from the Souss region of Morocco. X = 100 bp DNA ladder; T- negative control; T+: positive control (obtained from Turkey)

Tris-HCl (pH 8), 1% ß-mercaptoethanol and 800 µg/ml Proteinase K) for 1.5 h at 65 °C and 5 min at 99 °C in a thermocycler. One µL of crude DNA extract was used for PCR. The juveniles' DNA was amplified via PCR using primers Far (5'-TCGGCGATAGAGGTAAATGAC-3′) and Rar (5'-TCGGCGATAGACACTACAACT-3′), which are specific for amplification of *Meloidogyne arenaria* (Zijlstra et al. 2000). The amplified PCR product was run on a 1% agarose gel, and a 420-bp fragment was observed under an ultraviolet light (Fig. 3), confirming the population to be *M. arenaria* (Zijlstra et al. 2000). The pathogenicity test was conducted under greenhouse condition at an average temperature of 25 °C. Seedlings ($n = 10$) of eggplant were maintained in pots (12 cm high and 8 cm diam) filled with sterilized sand and subsequently each plant was inoculated with a suspension of 1000 eggs and J2 s obtained from the pure single egg mass culture of the original *M. arenaria* population. Pots of non-inoculated seedlings served as controls ($n = 8$). Seventy days after inoculation, plants were uprooted and roots were galled similarly to plants encountered in the field. Nematode eggs were extracted from tomato roots by cutting the entire root system into approximately 2.5-cm pieces, placing the pieces in a 1.5-l flask, and agitating for 4 min in a 1% NaOCl (Hussey and Barker 1973). Eggs were collected and rinsed with tap water on nested 75- and 25-µm-pore sieves and counted. Nematode population densities were in the ranges of 2256 to 4234 eggs per 10 g of root. The reproduction factor (final nematode population/initial population) was 6.4, demonstrating this species multiply on this host. The nematodes were re-extracted from plant tissue and identified as aforementioned. No symptoms were observed on control plants. Further studies on root-knot nematodes in the Souss region will be necessary to determine whether there are other species of nematodes causing damage to eggplant, to assess crop losses and to develop effective control and management strategies in the region.

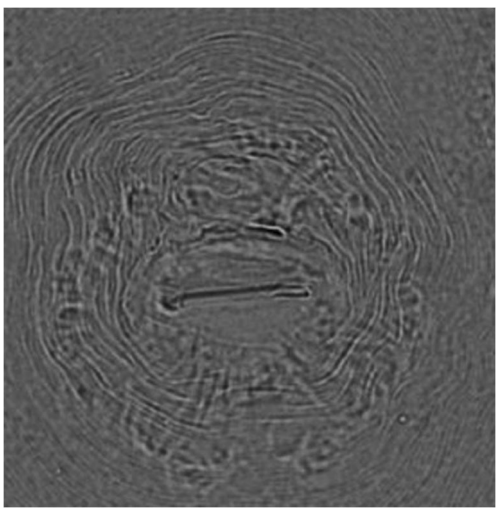

Fig. 2 Perineal patterns for *Meloidogyne arenaria* collected from eggplant roots in Souss region of Morocco

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