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A survey of root knot nematodes and resistance to *Meloidogyne incognita* in sweet potato varieties from Kenyan fields

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ABSTRACT

The root knot nematode, *Meloidogyne* is one of the most economically damaging plant parasitic nematode groups, and are widely distributed in Kenyan agro-ecosystems. The aim of this study was to determine the diversity of *Meloidogyne* species in Kenyan sweet potato fields and identify sweet potato varieties that exhibit resistance to *M. incognita*. *Meloidogyne* species were collected from Nyanza, Western, Eastern and Central Provinces of Kenya. Mitochondrial DNA was used to differentiate *Meloidogyne* species. The most common species in all sampled regions was *M. incognita*. *Meloidogyne hapla* was recorded for the first time in Kenyan sweet potato growing areas (Mosocho, Matayos, Teso South, Manyatta, and Nzaui sub-counties), while *M. enterolobii* was observed in Kiharu, Matayos and Mosocho sub-counties and a novel *Meloidogyne* sp. was identified in Kiharu sub-county. Seventy-two sweet potato varieties collected from both agricultural fields and research stations in Kenya were evaluated for resistance to *M. incognita* under greenhouse conditions in two separate trials. Known susceptible (Beauregard) and resistant (Tanzania) sweet potato varieties were included as controls. Responses of sweet potato varieties to *M. incognita* infection was assessed by the number of eggs present and level of galling on a scale of 1–5, where 0 = 0 galls and 5 ≥ 100 galls. The reproduction index (RI) was used to classify the varieties as resistant or susceptible. There was a significant difference ($P < 0.001$) in the number of eggs, GI and RI among the varieties tested. Forty nine sweet potato varieties were considered very resistant and may be used in breeding programs to incorporate resistance against *M. incognita* into commercial cultivars of sweet potato or to use them in crop rotation programmes for management of RKN. The results on *Meloidogyne* species diversity in Kenyan sweet potato fields will also be useful in nematode management programs.

1. Introduction

Root knot nematodes (RKN; *Meloidogyne* spp.) are ranked as the most economically damaging group of plant parasitic nematodes (Jones et al., 2013) with a global distribution (Sasser, 1977). The genus *Meloidogyne* is composed of about 100 species, with *M. arenaria*, *M. incognita*, *M. hapla* and *M. javanica* being considered as "major" species (Elling, 2013). Out of the 100 identified *Meloidogyne* species, 22 occur in Africa, posing a significant threat to crop production by small-holder farmers. Among these, *M. incognita*, *M. javanica*, *M. hapla*, *M. africana*, *M. acronea*, and *M. kikuyensis* have been previously reported in Kenya (Onkendi et al., 2014) in various cropping systems (Desaeger and Rao, 1999; Kimenju et al., 1999; Nzesya et al., 2014; Van den Berg et al., 2001).

Africa is the second largest producer of sweet potato (10.6%) after Asia (86.5%). The area under sweet potato cultivation in Kenya has increased from 30,000 ha in 1994 to the current 90,000 ha (Tedesco and Stathers, 2015). Sweet potato production in Kenya in 2013 was 1.2 million tonnes compared to 285,000 tonnes in 1994 (FAOSTAT, 2015). In Kenya, sweet potato is an important staple food

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and feed crop and it is also a source of income for many families who sell it as a cash crop (Claessens et al., 2009; Mukras et al., 2013). Sweet potato constitutes an important part of dietary components in urban and rural Kenya due to its cheap price compared with other crops (Tedesco and Stathers, 2015). The major sweet potato growing regions are in Nyanza and Western Province, Kenya (Kaguongo et al., 2012). In addition to cultivation of white and cream flesh sweet potato, Kenyan small holder farmers also grow orange fleshed varieties which are a rich source of β-carotene with a high potential of improving vitamin A status (Hagenimana et al., 2001; Tumwegamire et al., 2004). Sweet potato yields are reduced by various biotic and abiotic factors with the major production constraint being *M. incognita* (Jatala and Russell, 1972). This species causes root galling which reduces the uptake of water and nutrients. It also causes necrosis and cracking of roots which reduces their marketable quality (Lawrence et al., 1986).

Projections by the Intergovernmental Panel for Climate Change (IPCC) indicate that there will be an increase in mean annual temperature and rainfall in East Africa (Christensen et al., 2007). The elevated temperature and moisture may result in an increase in the rate of RKN reproduction, development and infection, and cause shifts in their abundance and geographic distribution. Such effects may have a detrimental impact on sweet potato production in Kenya. Current RKN management in sweet potato is mainly through crop rotation, and the use of nematode free propagation material coupled with the use of nematicides. However, nematicides increase the cost of production and pose a risk to human health and the environment. The use of resistant sweet potato varieties has been cited as the most economical, effective, and environmentally safe method of managing *Meloidogyne* spp. as it significantly decreases production costs (Clark and Moyer, 1988). Resistant varieties produce better yields with tubers of good quality hence increasing their market value.

Information on the diversity of *Meloidogyne* species associated with a crop is important since the interaction of these species during concomitant infections affects plant defense responses (Ogallo and Mcclure, 1995). There are taxonomic limitations in differentiating *Meloidogyne* species using perineal patterns due to similarities in morphological characteristics among species. Molecular techniques are more reliable and have been successfully applied in identification of *Meloidogyne* species (Blok et al., 2002; Hu et al., 2011; Niu et al., 2012) and in assessing their evolutionary relationships (Chen et al., 2003; Janssen et al., 2016; Tenente et al., 2004; Tigano et al., 2005) and genetic diversity (Devran and Sogut, 2009; Janssen et al., 2016; Navas et al., 2001; Pokharel et al., 2007). The aim of this study was to determine the distribution and prevalence of *Meloidogyne* species in sweet potato fields in Kenya and to identify sweet potato varieties that exhibit resistance to *M. incognita*.

2. Materials and methods

2.1. Collection of samples

Soil and sweet potato vines that were used in resistance screening experiments were collected from 60 fields in 30 villages within Mosocho, Matayos, Teso South, Manyatta, Nzaui and Kiharu sub-counties of Kenya (Table 1). Sampling sites were farmers' fields and research stations (Kenya Agricultural and Livestock Research Organization -KALRO) and were located in the main sweet potato growing regions in Kenya. Soil samples from each field were collected from 30 sampling points close to sweet potato roots along three W shaped "sample walks" (Wiesel et al., 2015).

2.2. Extraction and molecular identification of Meloidogyne

Meloidogyne species present in soil samples collected from the field (Table 1) were extracted by planting individual nematode free tomato plants (*Lycopersicon esculentum* Mill. cv. Rutgers) in 500 cm^3 pots containing soil from each field. The tomato plants were maintained in the greenhouse at 25 ± 3 °C. Populations of each *Meloidogyne* species were obtained by randomly picking ten egg masses from the roots of each tomato plant after 60 days. In order to obtain a pure population, ten tomato plants were each inoculated with a single egg mass and uprooted after 60 days. Roots were washed and a female nematode teased out of a gall using forceps. DNA was extracted from a single female nematode by crushing it in 300 μL cell lysis buffer (10 mM Tris pH 8, 0.5% SDS and 5 mM EDTA). The mixture was incubated for 30 min at 65 °C before adding 100 μL protein precipitation solution (8 M ammonium acetate and 1 mM EDTA) at room temperature. Samples were vortexed for 30 s, placed on ice for five minutes and centrifuged for 3 min at 25,000 relative centrifugal force (rcf). The supernatant was removed and placed in a tube containing 300 μL isopropanol, mixed several times and centrifuged at 25,000 rcf for five minutes. The resultant supernatant was discarded and 300 μL 70% ethanol was added followed by centrifugation for 2 min at 25,000 rcf. The ethanol was then pipetted off and the DNA pellet air dried, hydrated with 200 μL water and stored at −20 °C until processing.

Primers Mel_C2F3_F (GGTCAATGTTCAGAAATTTGTGG) (Powers and Harris, 1993) and Mel_MRH106_R (AATTTCTAAAGACTTTTCTTAGT) (Stanton et al., 1997) targeting mtDNA were used in the PCR reactions. Amplification of DNA was performed in a volume of 20 μL containing 13 μL nuclease free PCR water, 3 μL 5X HOT FIREpol Blend Master Mix (Solis BioDyne, Estonia), 0.5 μl of each primer and 3 μL of DNA. PCR conditions consisted of initial denaturation at 95 °C for 15 min; 50 cycles of 95 °C for 1 min, 56 °C for 1 min, and 66 °C for 2 min and 30s; and a final extension of 66 °C for 12 min. PCR products were separated by electrophoresis in TAE buffer and visualized under UV light. PCR products>1700 base pairs were restricted using Hinf1 restriction enzyme (New England Biolabs) as first described by Powers and Harris (1993). The digestion mixture included 1 μL Hinf1, 1 μL 10X NE buffer and 10 μL PCR product. The restriction digestion was then performed at 37 °C for 15 min with inactivation at 80 °C for 15 min. Restriction fragments were visualized beside 100 bp DNA ladders on 2% agarose gel in TAE buffer. Before outsourcing capillary sequencing (Macrogen, Seoul, Korea) of PCR products, they were purified using EXoSAP-IT (Thermo Scientific). Sequences were edited and aligned with *Meloidogyne* sequences available on GenBank using Geneious software v8.1.4 (Biomatters, San Francisco, CA, USA). Species identification was confirmed based on sequences with ≥99% homology to reference sequences from GenBank.

2.3. Nematode inoculum for resistance screening experiments

Pure populations from a single egg mass of *M. incognita* race 1 were multiplied on tomato (*L. esculentum* Mill. cv. Rutgers). Nematode eggs were extracted by cutting the tomato roots into 10–20 mm sections and agitating them in 0.6% NaOCl for four minutes (Hussey and Barker, 1973). The homogenate was then washed with distilled water through an 80 and 500-mesh sieve.

2.4. Nematode resistance screening of sweet potato varieties

Vine cuttings of 72 sweet potato varieties collected from sites described in Table 1 were transferred to a greenhouse at the University of Nairobi (Kenya) for subsequent experiments. Known susceptible (Beauregard) and resistant (Tanzania) sweet potato