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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

**Table 1**
Sample collection sites for sweet potato in Kenya.

| Province | County | Sub-County | Latitude | Longitude | Village | *Meloidogyne* species | Sweet potato variety |
|---|---|---|---|---|---|---|---|
| Nyanza | Kisii | Mosocho | 0°38′58.6″S | 34°44′56.8″E | Nyabungututu | *M. incognita* | KS1 |
| | | | | | | | Mosambi 1 |
| | | | 0°38′13.9″S | 34°44′16.4″E | Kiaboega | *M. incognita* | Oburi |
| | | | | | | | Nyakamoro |
| | | | | | | | KS2 |
| | | | 0°39′35.4″S | 34°45′16.4″E | Bonyagatenyi | *M. incognita* | KS3 |
| | | | 0°39′33.8″S | 34°45′11.5″E | Nyakobaria | *M. incognita* | KS4 |
| | | | 0°35′50.8″S | 34°42′58.4″E | Mwamasarore | *M. incognita* and *M. enterolobii* | KS5 |
| | | | 0°36′04.2″S | 34°42′46.2″E | Mwabagaka | *M. incognita* | KS6 |
| | | | | | | | Mosambi 2 |
| | | | | | | | Mbwayo |
| | | | | | | | KS7 |
| | | | 0°36′15.5″S | 34°43′05.9″E | Mwamoja | *M. incognita* and *M. hapla* | KS8 |
| | | | | | | | Mosambi 3 |
| Western | Busia | Matayos | 0°21′31.2″N | 34°10′15.7″E | Namikoe | *M. incognita* | Kampala 1 |
| | | | | | | | Miezi tatu |
| | | | | | | | BS1 |
| | | | 0°21′32.7″N | 34°10′17.6″E | Buroboi A | *M. incognita* | BS2 |
| | | | | | | | BS3 |
| | | | 0°21′38.2″N | 34°10′08.8″E | Buroboi B | *M. incognita* | BS4 |
| | | | 0°22′21.4″N | 34°09′24.1″E | Nabisiongo | *M. incognita* | BS5 |
| | | | | | | | BS6 |
| | | | | | | | BS7 |
| | | | | | | | Kampala 2 |
| | | | 0°25′21.6″N | 34°08′46.0″E | Emaseno | *M. incognita* | BS8 |
| | | | | | | | BS9 |
| | | | | | | | BS10 |
| | | | | | | | BS11 |
| | | | | | | | BS12 |
| | | | | | | | BS13 |
| | | | | | | | BS14 |
| | | | 0°26′36.8″N | 34°06′44.3″E | Buruba | *M. incognita* and *M. enterolobii* | BS15 |
| | Teso | Teso South | 0°29′53.9″N | 34°07′41.7″E | Angorom (KALRO, ALupe) | *M. incognita* and *M. hapla* | Vitaa |
| | | | | | | | Kenspot 6 |
| | | | | | | | Kenspot 4 |
| | | | | | | | Kenspot 1 |
| | | | | | | | Kenspot 3 |
| | | | | | | | Kenspot 2 |
| | | | | | | | Kabode |
| | | | 0°30′46.3″N | 34°08′57.9″E | Andungos | *M. incognita* | TS1 |
| | | | | | | | TS2 |
| | | | | | | | TS3 |
| | | | 0°30′51.3″N | 34°08′54.6″E | Andukumut | *M. incognita* | TS4 |
| Eastern | Embu | Manyatta | 0°31′09.4″S | 37°26′55.7″E | Njukiri | *M. incognita* | EM1 |
| | | | | | | | EM2 |
| | | | | | | | EM3 |
| | | | 0°30′03.4″S | 37°26′50.8″E | Mariamairi | *M. incognita* | EM4 |
| | | | | | | | EM5 |
| | | | | | | | EM6 |
| | | | 0°29′27.7″S | 37°27′14.1″E | Githungururu | *M. incognita* | EM7 |
| | | | | | | | EM8 |
| | | | 0°30′21.0″S | 37°27′26.6″E | Kangaru (KALRO, Embu) | *M. incognita* and *M. hapla* | SPK 004 |
| | | | | | | | Cape 10 |
| | | | | | | | Bungoma |
| | | | 0°30′54.1″S | 37°27′59.0″E | Iveche | *M. incognita* and *M. hapla* | EM9 |
| | | | | | | | EM10 |
| | Makueni | Nzaui | 1°56′54.9″S | 37°31′52.0″E | Kiinze | *M. incognita* | MK1 |
| | | | | | | | MK2 |
| | | | 1°56′35.3″S | 37°32′06.1″E | Kinui | *M. incognita* | MK3 |
| | | | | | | | MK4 |
| | | | 1°57′22.6″S | 37°33′58.9″E | Kikuu | *M. incognita* | MK5 |
| | | | | | | | MK6 |
| | | | 1°58′41.8″S | 37°35′43.2″E | Kalima | *M. incognita* | MK7 |
| | | | | | | | MK8 |
| | | | | | | | MK9 |
| | | | | | | | MK10 |
| | | | | | | | MK11 |
| | | | | | | | MK12 |
| | | | 1°59′48.0″S | 37°35′54.0″E | Inyeke | *M. incognita* and *M. hapla* | MK13 |
| | | | | | | | MK14 |
| | | | | | | | MK15 |
| | | | 1°58′12.1″S | 37°34′41.4″E | Kyanguu | *M. incognita* | MK16 |
| Central | Murang'a | Kiharu | 0°44′21.4″S | 37°09′14.1″E | Kongoini | | SPK 004 |

**Table 1** (*continued*)

| Province | County | Sub-County | Latitude | Longitude | Village | *Meloidogyne* species | Sweet potato variety |
|---|---|---|---|---|---|---|---|
| | | | | | | *M. incognita*, *M. enterolobii* and *Meloidogyne* sp. | |
| | | | 0°46′56.8″S | 37°11′11.5″E | Maragua | *M. incognita* | SPK 004 |
| | | | 0°44′18.0″S | 37°07′51.8″E | Maragi | *M. incognita* | SPK 004 |

varieties were included as controls in the trial (provided by Benjamin Kavuva-KALRO, Muguga). Five plants per sweet potato variety were planted in sterilized soil (50:50, sand:soil mixture) in perforated 500 $cm^3$ plastic pots arranged in a completely randomized design. Plants were watered as required and the greenhouse temperature maintained at 25 ± 3 °C. Two weeks after planting, approximately 10,000 *M. incognita* race 1 eggs per pot were inoculated into the soil and covered with a moist layer of sand (Cervantes-Flores and Yencho, 2002). Plants were harvested at eight weeks after inoculation and rated visually for the number of galls using a 0–5 galling index (GI) as follows: 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 > 100 galls (Taylor and Sasser, 1978). Nematode eggs were extracted from 20 g root subsamples as previously described and counted using a stereomicroscope. Resistance and susceptibility of sweet potato varieties was based on a reproduction index (RI), which was calculated as number of eggs $gram^{-1}$ of sweet potato root divided by the number of eggs $gram^{-1}$ of susceptible control roots multiplied by 100. The RI rating was as follows: RI = 0 (immune), RI < 1 (highly resistant), 1 ≤ RI < 10 (very resistant), 10 ≤ RI < 25 (moderately resistant), 25 ≤ RI < 50 (slightly resistant) and RI ≥ 50 (susceptible) (Taylor, 1967). The experiment was carried out twice in different years, using the same set of cultivars to determine the consistency of differences in nematode resistance.

### 2.5. Statistical analysis

Data on GI, RI and number of eggs from trial and 1 and 2 were combined and treated as a single trial during subsequent analyses and transformed using log (x + 1) before analyses. The data from each of trials I and 2 were subjected to analysis of variance (ANOVA) using Genstat (Payne et al., 2011) before combining them into a single analysis. The relationship between GI and number of eggs was determined using Pearson's correlation coefficient in SPSS (Norusis, 1995).

## 3. Results

### 3.1. RKN survey

The nematode species *M. incognita*, *M. hapla*, *M. enterolobii* and an unknown *Meloidogyne* sp. were identified by analyses of mtDNA. Primers C2F3/MRH106 were able to robustly differentiate *M. hapla* (662 base pair (bp) amplicon), *M. enterolobii* (841 bp amplicon) and an unknown *Meloidogyne* sp. (399 bp amplicon). PCR products >1700 bp restricted with Hinf1, yielded three fragments robustly identified as *M. incognita*. Representative sequences have been deposited in GenBank: *M. incognita* (accession KX214347, 1661 bp), *M. hapla* (accession KX214348, 592 bp; accession KX214349, 585 bp), *M. enterolobii* (accession KX214350, 759 bp) and the unknown *Meloidogyne* sp. (accession KX214351, 353 bp). The latter *Meloidogyne* sp. sequence had 99% homology (*e*-value: 2*e*-145) to *M. javanica* (GenBank accession KP202352) (Humphreys-Pereira and Elling, 2015), however a deletion > 1376 bp spanned an intron and the tRNA-His gene region.

*Meloidogyne incognita* was observed in all fields in the sampled regions whereas *M. hapla* was recorded in all sub-counties except Kiharu and Matayos, and *M. enterolobii* was observed in Kiharu, Matayos and Mosocho. The unidentified *Meloidogyne* sp. was recorded in Kiharu sub-county (Fig. 1; Fig. 2). Angorom (Western Province), Mwamoja (Nyanza Province), Inyeke, Iveche and Kangaru (Eastern Province) were the only five villages where *M. hapla* was recorded, while *M. enterolobii* was observed in Buruba (Western Province), Kongoini (Central Province) and Mwamasorore (Nyanza Province). The unidentified *Meloidogyne* sp. was recorded only in Kongoini village in Kiharu sub-county.

### 3.2. Resistance screening of sweet potato to M. incognita

In the resistance screening experiment, the susceptible control, Beauregard had a GI of 4.7 ± 0.15 and 552.6 ± 12.13 eggs. The resistant control Tanzania had a GI of 1.5 ± 0.17 and 15.3 ± 2.70 eggs and an RI of 2.73. There was a significant difference in the GI ($F_{71,359}$ = 25.68; P < 0.001), number of eggs ($F_{71,359}$ = 51.5; P < 0.001) and RI ($F_{71,359}$ = 25.14; P < 0.001) among the varieties. A GI greater than 3 was recorded in 23.6% of the varieties while the RI ranged from 1 to 90%. The sweet potato variety EM7 had the highest number of eggs. The varieties MK10, EM9, and SPK 004 had a GI ≤ 1 and RI ≤ 1. On the other hand, EM6, EM7, Kenspot 4, Kenspot 6, MK15, MK16, MK9 and MK5 had a GI ≥ 4 and an RI ≥ 50 (Table 2). Based on the RI, 68.1%, 15.3%, 5.6% and 11.1% of the varieties were very resistant, moderately resistant, slightly resistant and susceptible respectively. GI was positively correlated to the number of eggs (r = 0.880, P < 0.01).

## 4. Discussion

### 4.1. RKN survey

Nematode abundance and composition is significantly influenced by soil properties, rainfall and temperature (Nielsen et al., 2014). The most prevalent species in all the sampled regions was *M. incognita*, which is a common RKN in Kenyan agroecosystems (Desaeger and Rao, 1999; Kimenju et al., 1999; Nzesya et al., 2014; Van den Berg et al., 2001). The geographic distribution of *M. incognita* is largely dependent on environmental factors such as temperature and moisture (Sasser, 1977). Due to their poikilothermic nature, elevated temperatures will likely lead to an increase in cumulative relative development rates of *M. incognita* in some cropping systems (Waals et al., 2013). Such a scenario may exacerbate existing problems in the five sub-counties where *M. incognita* population density was found to be relatively high and may yet initiate problems for locations where *M. incognita* is, in comparative terms, currently relatively low. Thus, the projected increase in temperature and rainfall (Christensen et al., 2007) may expose areas of food production in East Africa to significantly greater impact of RKN (and other nematode species), leading to national food security issues (Hassan et al., 2013).

Association of *M. hapla* and *M. enterolobii* with Kenyan sweet potato observed in this study and identification of a *M. javanica*-like *Meloidogyne* sp. sequence with a large deletion has not been previously reported. *Meloidogyne incognita*, *M. javanica* and *M. arenaria*

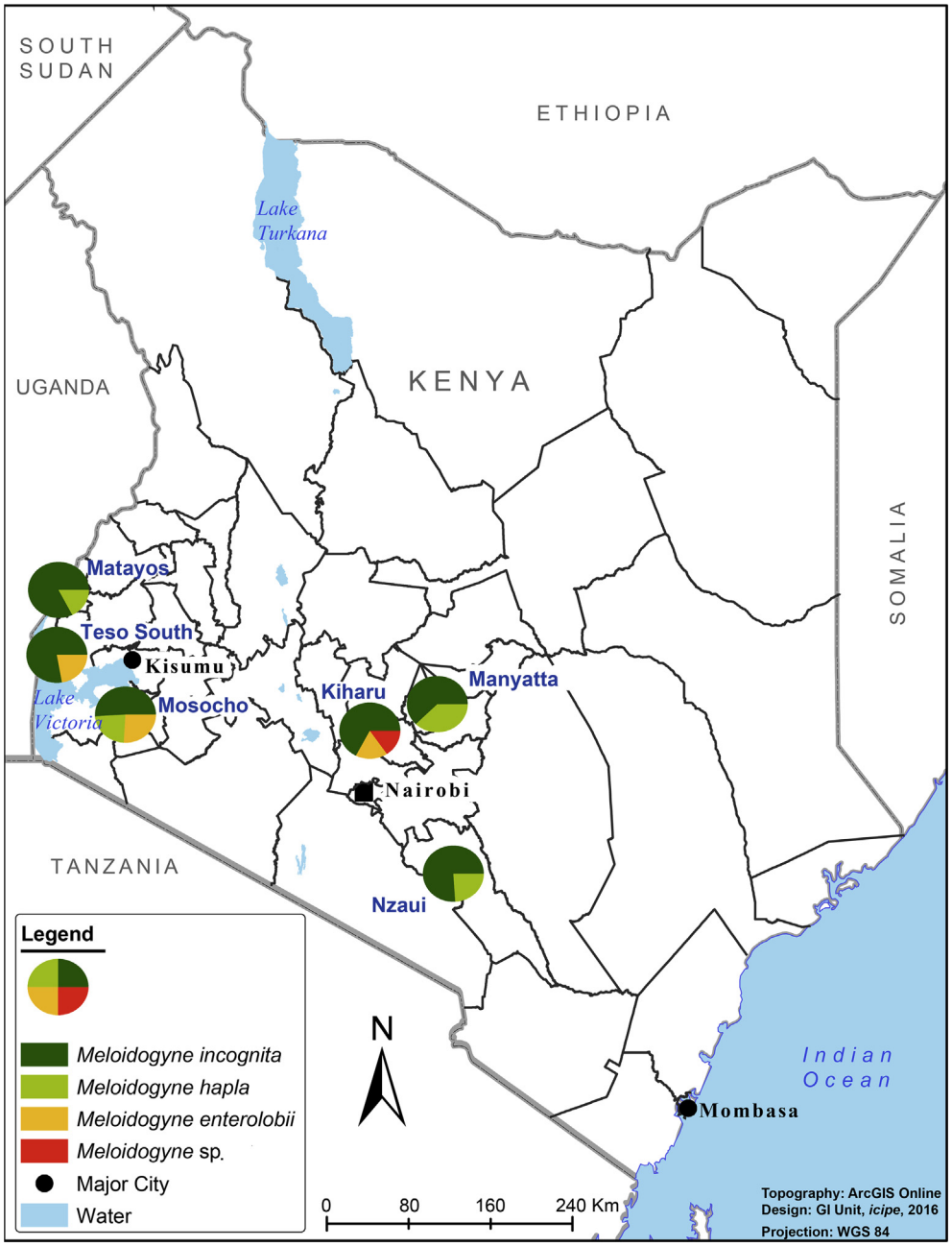


**Fig. 1.** Map of Kenya showing the distribution of *Meloidogyne* species in Kiharu, Manyatta, Matayos, Mosocho, Nzaui and Teso South sub-counties, Kenya.

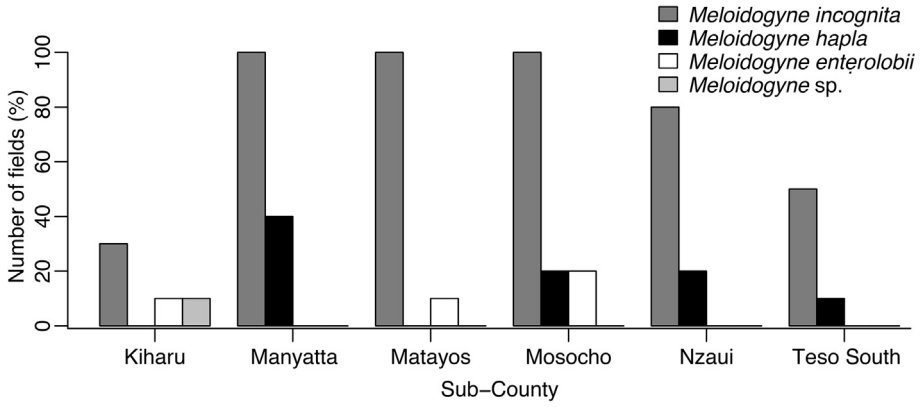


**Fig. 2.** Occurrence of *M. incognita*, *M. hapla*, *M. enterolobii* and *Meloidogyne* sp. in sweet potato growing regions of Kiharu, Manyatta, Matayos, Mosocho, Nzaui and Teso South sub-counties, Kenya.

**Table 2**
Galling index, number of eggs, reproduction index (RI) and resistance level of sweet potato varieties infected with *Meloidogyne incognita*. Data (±SE) are back-transformed means from two trials.

| Sweet potato variety | [a]Galling index | Number of eggs | [b]RI | [c]Resistance level |
|---|---|---|---|---|
| BS11 | 2.95 ± 0.08 | 60.66 ± 0.3 | 11 ± 0.37 | MR |
| Kenspot 1 | 2.78 ± 0.06 | 60.38 ± 0.17 | 12 ± 0.75 | MR |
| EM2 | 2.78 ± 0.06 | 67.39 ± 0.2 | 13 ± 0.68 | MR |
| Cape 10 | 3.18 ± 0.05 | 85.7 ± 0.1 | 14 ± 1.02 | MR |
| Kabode | 3 ± 0 | 81.04 ± 0.03 | 15 ± 0.58 | MR |
| MK14 | 3 ± 0 | 73.82 ± 0.03 | 16 ± 1.12 | MR |
| MK4 | 3 ± 0 | 92.97 ± 0.04 | 18 ± 1.02 | MR |
| TS1 | 3.78 ± 0.05 | 99.93 ± 0.1 | 17 ± 1 | MR |
| MK2 | 3.37 ± 0.06 | 98.08 ± 0.08 | 19 ± 0.97 | MR |
| MK12 | 3.57 ± 0.06 | 113.55 ± 0.11 | 21 ± 1.2 | MR |
| Miezi tatu | 3.37 ± 0.06 | 109.66 ± 0.28 | 21 ± 1.08 | MR |
| Beauregard (Susceptible control) | 4.7 ± 0.15 | 552.6 ± 12.13 | 100 ± 2.28 | S |
| MK15 | 4 ± 0 | 310.17 ± 0.1 | 54 ± 0.63 | S |
| MK16 | 4.58 ± 0.05 | 326.34 ± 0.18 | 56 ± 1.33 | S |
| EM6 | 4.78 ± 0.04 | 420.7 ± 0.19 | 71 ± 1.5 | S |
| Kenspot 6 | 4.78 ± 0.04 | 463.52 ± 0.02 | 83 ± 1 | S |
| Kenspot 4 | 4.78 ± 0.04 | 497.88 ± 0.01 | 86 ± 0.51 | S |
| MK9 | 5 ± 0 | 495.59 ± 0.02 | 87 ± 1.2 | S |
| MK5 | 5 ± 0 | 516.61 ± 0.01 | 89 ± 2.23 | S |
| EM7 | 5 ± 0 | 517.8 ± 0.02 | 92 ± 0.75 | S |
| BS7 | 3.57 ± 0.06 | 152.46 ± 0.14 | 26 ± 0.68 | SR |
| BS1 | 4.19 ± 0.04 | 185.64 ± 0.02 | 34 ± 1.16 | SR |
| Kampala 1 | 3.96 ± 0.07 | 184.78 ± 0.21 | 35 ± 1.02 | SR |
| EM3 | 4.19 ± 0.04 | 230.74 ± 0.15 | 38 ± 1.62 | SR |
| BS9 | 1.17 ± 0.08 | 8.51 ± 0.2 | 2 ± 0.24 | VR |
| Mbwayo | 1.17 ± 0.08 | 7.69 ± 0.28 | 2 ± 0.58 | VR |
| Mosambi 3 | 1.17 ± 0.08 | 6.41 ± 0.28 | 2 ± 0.58 | VR |
| EM9 | 1 ± 0 | 6.69 ± 0.22 | 1 ± 0 | VR |
| MK10 | 1 ± 0 | 6.53 ± 0.31 | 1 ± 0 | VR |
| SPK 004 | 1 ± 0 | 6.59 ± 0.26 | 1 ± 0 | VR |
| BS15 | 1.35 ± 0.1 | 12.43 ± 0.15 | 2 ± 0.2 | VR |
| Kenspot 3 | 1.17 ± 0.08 | 11.45 ± 0.18 | 2 ± 0.49 | VR |
| KS2 | 1.17 ± 0.08 | 7.11 ± 0.15 | 2 ± 0.49 | VR |
| BS12 | 1.17 ± 0.08 | 11.11 ± 0.23 | 2 ± 0.51 | VR |
| KS3 | 1.17 ± 0.08 | 5.82 ± 0.28 | 2 ± 0.51 | VR |
| KS1 | 1.17 ± 0.08 | 5.85 ± 0.11 | 3 ± 0.6 | VR |
| Tanzania (Resistant control) | 1.5 ± 0.17 | 15.3 ± 2.70 | 3 ± 0.49 | VR |
| Mosambi 1 | 2.37 ± 0.07 | 16.5 ± 0.05 | 3 ± 0.37 | VR |
| BS6 | 1.55 ± 0.1 | 13.76 ± 0.23 | 3 ± 0.58 | VR |
| BS10 | 1 ± 0 | 11.42 ± 0.09 | 2 ± 0.32 | VR |
| BS5 | 1.17 ± 0.08 | 7.89 ± 0.31 | 2 ± 0.55 | VR |
| TS2 | 1.17 ± 0.08 | 7.95 ± 0.31 | 2 ± 0.55 | VR |
| Bungoma | 1.55 ± 0.1 | 19.09 ± 0.3 | 3 ± 0.2 | VR |
| Mosambi 2 | 1.17 ± 0.08 | 9.3 ± 0.25 | 4 ± 0.75 | VR |
| KS6 | 1.55 ± 0.1 | 18.86 ± 0.36 | 4 ± 0.73 | VR |
| MK3 | 1.77 ± 0.08 | 19.23 ± 0.22 | 3 ± 0.32 | VR |
| KS8 | 1.17 ± 0.08 | 10.35 ± 0.32 | 3 ± 0.45 | VR |
| BS3 | 1.17 ± 0.08 | 9.57 ± 0.15 | 3 ± 0.63 | VR |
| Nyakamoro | 1.77 ± 0.08 | 19.23 ± 0.1 | 3 ± 0.84 | VR |
| TS3 | 1.17 ± 0.08 | 10.51 ± 0.18 | 3 ± 0.89 | VR |
| BS8 | 1.35 ± 0.1 | 17.79 ± 0.22 | 4 ± 0.73 | VR |
| EM1 | 2.18 ± 0.06 | 25.3 ± 0.08 | 4 ± 0.8 | VR |
| EM4 | 1.77 ± 0.08 | 25.06 ± 0.22 | 4 ± 0.51 | VR |
| MK7 | 1.55 ± 0.1 | 17.45 ± 0.18 | 4 ± 0.87 | VR |
| EM8 | 1.77 ± 0.08 | 28.11 ± 0.28 | 5 ± 0.24 | VR |
| BS14 | 1.77 ± 0.08 | 17.84 ± 0.32 | 5 ± 0.6 | VR |
| Oburi | 1.77 ± 0.08 | 17.45 ± 0.07 | 5 ± 0.81 | VR |
| KS7 | 1.77 ± 0.08 | 22.07 ± 0.27 | 5 ± 1.02 | VR |
| BS2 | 1.55 ± 0.1 | 16.74 ± 0.3 | 4 ± 0.77 | VR |
| MK8 | 1.77 ± 0.08 | 27.71 ± 0.17 | 5 ± 0.73 | VR |
| BS4 | 1.77 ± 0.08 | 25.92 ± 0.23 | 5 ± 0.8 | VR |
| Kenspot 2 | 1.77 ± 0.08 | 28.92 ± 0.27 | 6 ± 0.24 | VR |
| MK1 | 1.77 ± 0.08 | 20.23 ± 0.1 | 6 ± 0.75 | VR |
| Vitaa | 1.77 ± 0.08 | 25.98 ± 0.21 | 6 ± 0.75 | VR |
| MK11 | 2 ± 0 | 25.61 ± 0.11 | 6 ± 0.98 | VR |
| Kampala 2 | 1.77 ± 0.08 | 23.43 ± 0.2 | 5 ± 0.89 | VR |
| EM5 | 2 ± 0 | 35.64 ± 0.08 | 6 ± 0.37 | VR |
| MK6 | 2.18 ± 0.06 | 35.98 ± 0.28 | 6 ± 0.75 | VR |
| TS4 | 2 ± 0 | 33.59 ± 0.06 | 7 ± 0.8 | VR |
| EM10 | 2.57 ± 0.07 | 38.63 ± 0.07 | 8 ± 0.68 | VR |
| KS5 | 2.78 ± 0.06 | 41.17 ± 0.09 | 8 ± 0.58 | VR |
| KS4 | 2.57 ± 0.07 | 33.83 ± 0.11 | 7 ± 0.89 | VR |
| BS13 | 2.78 ± 0.06 | 51 ± 0.15 | 9 ± 0.24 | VR |
| MK13 | 2.57 ± 0.07 | 41.46 ± 0.21 | 9 ± 1.05 | VR |

[a] Galling index: 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; 5 > 100 galls (Taylor and Sasser, 1978).
[b] RI: Reproduction index = (number of eggs gram$^{-1}$ root of each sweet potato variety)/(number of eggs gram$^{-1}$ of root of susceptible Beauregard) × 100 (Taylor, 1967).
[c] Resistance level based on the RI where VR-Very Resistant, MR-Moderately Resistant, SR-Slightly Resistant and S-Susceptible.

are considered as the most damaging nematode species in sweet potato (Cervantes-Flores and Yencho, 2002; Giamalva et al., 1963). However, the impact of *M. enterolobii* and *M. hapla* on Kenyan sweet potato yields should also be considered. *Meloidogyne enterolobii*, a highly aggressive species that is well adapted to warm climates, causes more damage than other *Meloidogyne* species and has the ability to reproduce in diverse crop types, including those with resistance genes (Castagnone-Sereno, 2012). Although *M. hapla* and *M. enterolobii* were not recorded in all sub-counties, predicted climate change (Hoang et al., 2014) may affect the density and population dynamics of these species in Kenya, leading to increased sweet potato yield losses.

*Meloidogyne incognita* was recorded in all regions, including Kisii County, which is among the largest sweet potato production areas in Kenya (Kwach et al., 2010). Recently, increased rainfall and temperature has been recorded in Kisii County (Mugalavai and Kipkorir, 2003). Sustained changes in temperature and moisture may thus result in higher populations of *M. incognita*, with a potential concomitant reduction in sweet potato yield, especially if susceptible varieties are cultivated.

### 4.2. Resistance screening of sweet potato to M. incognita

The tested sweet potato varieties showed different responses to infection with *M. incognita*. Similar results were observed in other RKN resistance screening studies of sweet potato (Cervantes-Flores and Yencho, 2002). In this study, 11.1% of the tested sweet potato varieties were classified as susceptible. Among them was the variety EM7, which had the highest absolute number of eggs. Susceptibility of sweet potato varieties to RKN is probably due to the presence of previously reported unfavorable alleles that reduce the level of resistance (Cervantes-Flores et al., 2008). Sweet potato varieties Kenspot 4 and 6 were also classified as susceptible. These varieties were collected from KALRO, Alupe and were part of a breeding program aimed at producing orange fleshed sweet potato with high β-carotene. Due to the high susceptibility of these varieties, it would be important to cross them with *M. incognita* resistant varieties in order to get higher yields.

A large number (68.0%) of the tested sweet potato varieties were very resistant (RI < 10%) to *M. incognita*. This is an indication of a high level of resistance to *M. incognita* in the sweet potato varieties presently cultivated in Kenya. Such resistance may break down at high soil temperatures associated with climate change (Dropkin, 1969; Williamson, 1998). In addition, the durability of this resistance may be compromised by continuous cropping of resistant or moderately resistant varieties due to an increase in highly pathogenic *Meloidogyne* races.

Very resistant sweet potato varieties with low GI and RI (GI $\leq$ 1, RI $\leq$ 1) were collected from Nzaui (MK10) and Manyatta (EM9 and SPK 004) sub-counties. GI is an important measure of nematode infection (Sasser et al., 1984) and has been previously used in selection of *M. incognita* resistant sweet potato (Cervantes-Flores et al., 2008), yam (Mudiope et al., 2012) and cassava (Talwana et al., 1997). In the current study, a strong correlation was observed between GI and number of eggs. Similar positive correlations have been previously reported in sweet potato varieties Beauregard, Excel, Hernandez and Porto Rico (Cervantes-Flores and Yencho, 2002). RI is also considered a good indicator of resistance as it measures nematode establishment and reproduction in the host (Hadisoeganda and Sasser, 1982). It has been successfully used in selection of RKN resistant sweet potato (Gomes et al., 2015; Marchese et al., 2010). An RI of 1 in sweet potato varieties is indicative of low *M. incognita* reproduction. Resistance to *M. incognita* is qualitatively inherited and controlled by multiple genes (Ukoskit et al., 1997). The resistance observed in these varieties may have been due to additive effects of multiple *M. incognita* resistance genes (Cervantes-Flores et al., 2008). In addition, the inability of infective juveniles to form feeding sites in the plant and/or production of pre-infection compounds that prevent penetration of nematodes into the plant roots (Jatala and Russell, 1972) may have also contributed to the observed resistance.

With exception of SPK 004, which was collected from Kiharu sub-county and KALRO, Embu, the very resistant sweet potato varieties were collected from farmers' fields and their agronomic characteristics have not been determined and they require further analysis. In contrast, SPK 004 is a well characterized variety released in Kenya in 2000 (Mcharo and Ndolo, 2013), with its morphological and genetic diversity previously characterized and known to be resistant to sweet potato virus disease (Karuri et al., 2009). It has a high dry matter content (Karuri et al., 2009) and β-carotene content of 6200 μg100 $g^{-1}$, 7800 μg100 $g^{-1}$, 4630 μg100 $g^{-1}$ when raw, boiled and roasted, respectively (Kidmose et al., 2007). In addition, it is highly palatable with yields that are stable across different environments (Mcharo and Ndolo, 2013). Thus, SPK 004 is a candidate sweet potato variety that could be cultivated in regions where *M. incognita* population density is high. The other resistant sweet potato varieties identified in this study can also be used as an economically feasible means of RKN management. In addition to producing high yields, roots from the resistant sweet potato varieties will have a high commercial value due to reduced root cracking.

## 5. Conclusion

The identification of sweet potato varieties in this study with putative resistance to *M. incognita* infection will be of utility in future breeding programs to maintain yields of a key nutritional crop under increasing constraints from changing land use and changing climate. The information on *Meloidogyne* species diversity in Kenyan sweet potato fields will also be useful in designing and implementing nematode management programs.

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