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Research Article

Characterization of *Meloidogyne* species and the reaction of tomato *Solanum lycopersicum* L. cultivars to *Meloidogyne incognita* and *Meloidogyne javanica*

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Abstract: The study was conducted to determine the distribution of the common *Meloidogyne* species in research stations and vegetable farms in Ibadan, south-western Nigeria. Galled roots were collected from inoculum plots of four research stations and two vegetable farms. Identification of species was based on juvenile and female morphological characters and specific SCAR primers for *Meloidogyne* species. The pathogenicity of *M. incognita* and *M. javanica* was evaluated at different inoculum levels on tomato in a screenhouse study. *M. incognita* was the dominant species encountered in research plots, although it often occurred in mixed population with *M. javanica* and other unidentified species. Growth parameters such as plant height, number of leaves, and yield responded negatively to increasing inoculum levels for all the cultivars except Small Fry and Celebrity. Both cultivars were categorized as resistant to *M. incognita* and tolerant to *M. javanca*. The most popularly grown tomato cultivars, Ibadan Local, Roma (Roma type) and Beske were susceptible to both species of root-knot nematodes.

Keywords: celebrity, host rating, pathogenicity, root-knot nematodes, SCAR-PCR, vegetables

Introduction

Tomato *Solanum lycopersicon* L. is a herbaceous, usually sprawling plant in the nightshade or Solanaceae family, widely cultivated for its edible fruit. The most popular fruit vegetables grown in West Africa are tomatoes, peppers and eggplant (Braima *et al*., 2010). Vegetables are important components of daily diets in Africa and important source of income, especially in urban and peri-urban areas (Braima *et al*., 2010). Tomato is the most popular vegetable crop in Nigeria dominating the largest area under production among vegetable crops (Ramalan, 1994). Nigeria is the second largest producer of tomato in Africa and its cultivation is mainly for domestic consumption in soups, stews and salads (Poysa, 2000).

Nematodes are the most wide spread microorganisms that limit world agricultural productivity (Sasser and Carter, 1982; Taylor *et al.,* 1982). They attack almost all cultivated plants but vegetable crops are their most preferred hosts (Sasser, 1980). *Meloidogyne* species, *Rotylenchulus reniformis*, *Globodera rostochiensis* and several ecto-parasitic species of nematodes are known to attack tomato in different parts of the world. Tomato is regarded as the most favourable host for root-knot nematodes (Dropkin, 1980). Root-knot nematode (*Meloidogyne* spp.) infestations on tomato are common in Nigeria. The yield of okra, tomato, and eggplant suffered 90.9, 46.2 and 23% losses,

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respectively, due to root-knot nematode infestation at the rate of 3-4 juveniles/g soil under field conditions (Bhati and Jain, 1977). Characterization of specific *Meloidogyne* species is important in order to deploy management using resistance. Morphological methods are the most often used in developing countries. Several molecular methods which detect DNA polymorphisms between species have been used with DNA extracted from nematode samples. DNA analysis has the advantage of potentially identifying any life stage of *Meloidogyne* to the species level (Blok *et al*., 2002). This method also eliminates the ambiguity that often occurs with morphological identification.

The city, Ibadan hosts about eight Federal institutions and one international institution with an agricultural mandate which conduct research on plant-parasitic nematodes and rely on field populations of *Meloidogyne* for inoculum. Research on *M. incognita* (Adegbite and Agbaje, 2007) and *M. javanica* (Olowe, 2004) have been reported within the locations. The study aimed to characterise and determine the distribution of the most common *Meloidogyne* species in and around Ibadan area in Oyo State, Nigeria and to determine the pathogenicity of identified *Meloidogyne* species on five selected tomato cultivars in the screenhouse.

Materials and Methods

Source of root-knot nematode populations

Field sampling was conducted in and around Ibadan at four research stations and two vegetable farms. Galled roots of tomato, jews mallow (*Corchorus olitorus)* and cockscomb (*Celosia argentea)* plants were obtained from Akobo 7º25'49.13"N; 3º56' 55.44"E (a peri-urban vegetable farm), Ijaye 7º50'48.60"N; 3º34'11.23"E (field vegetable farm), National Institute for Horticultural Research 7º24'19.13"N; 3º50'57.01"E (NIHORT), International Institute of Tropical Agriculture 7º29'52.05"N; 3º54' 19.16"E (IITA), Institute of Agricultural Research and Training (IAR & T), and crop garden of the Department of Crop Protection and Environmental Biology, 7º27'4.14"N; 3º53' 49.19"E University of Ibadan (UI).

Root samples were collected using a systematic sampling method following a W-shaped path marked on each plot. Samples from various fields were collected into labelled polythene bags and taken to the Nematology laboratory of IITA. In the laboratory, roots from each plant were thoroughly examined for the presence of galls and egg masses and samples without galls were discarded.

Maintenance of inoculum and pure culturing

Two cultivars of tomato (Ibadan Local and Roma) obtained from National Institute for Horticultural Research (NIHORT) and Department of Agronomy, University of Ibadan, respectively, were used to raise the inoculum. Seeds of the two tomato cultivars were planted in small pots (5 cm diameter) containing autoclaved soil in a screenhouse at IITA. Single egg masses were picked from the roots collected from various locations and inoculated on a single tomato seedling. This was in order to make pure cultures from the field populations. The seedlings were transferred to 3000 cm^3 pots with sterilized soil three weeks after inoculation in order to provide enough space for the roots to grow.

Morphological identification

Eight weeks after inoculation, plants were removed from the pots and 20 galls were selected per pot for morphological characterization. For each population, the galls were teased in a small amount of water inside a glass petri-dish while observing under a dissecting microscope at ×10 magnification. Adult females released from the roots were picked into 2 cm^2 glass blocks while the egg masses attached were picked separately for molecular studies. The adult females were placed on a Perspex block and cut with a scalpel. The cut posterior end was then cleaned and trimmed and then placed on a glass slide in a drop of cotton blue in glycerol and observed at × 40 and × 100 mag. Egg masses from each population were picked from the galled roots of tomato and collected in sterile distilled water in separate

glass blocks. These were incubated at room temperature for 7-10 days in order to collect juveniles. Juveniles were measured for length of nematode body, stylet, hyaline portion of tail and body width and compared to reference measurements in Eisenback *et al*. (1981).

Extraction of DNA and PCR amplification

Egg masses from each population were picked from the galled roots of tomato and collected in sterile distilled water in separate 1 ml tubes. The eggs were allowed to hatch at room temperature for 7-10 days before DNA was extracted from the bulk juveniles following the method of Castagnone-Sereno *et al*. (1995). The DNA was re-suspended in sterile distilled water and stored at- 20 ^{o}C as stock. PCR amplifications using the universal SCAR primer 194/195 (5S-18S ribosome region) (Blok *et al*., 1997) were carried out following the method of Adam *et al*. (2007). The primers selected for specific *Meloidogyne* species were Fjav/Rjav specific for *M. javanica*; Far/Rar specific for *M. arenaria* and MI-F/MI-R specific for *M. incognita* (Meng *et al.*, 2004; Adam *et al.;* 2007). The universal primer was used first to separate the common tropical species from other *Meloidogyne* spp., while the specific primers were used to identify the specific tropical species. The nucleic acid was size-fractioned in 1% agarose gel and stained with 4µl ethidium bromide which was incorporated during the gel preparation. Electrophoresis was carried out on the gel which was examined under ultraviolet light and photographed. All amplification tests included a no-template (DNA) control. The PCR amplification conditions used for each primer set were as described by Adam *et al.* (2007).

Evaluation of cultivars

Three local (Ibadan Local, Beske, Roma [locally adapted type]), and two improved (Small Fry and Celebrity) tomato cultivars were evaluated for their reaction to *Meloidogyne incognita* and *M. javanica*. Experiments were conducted in plastic pots containing 5000 cm^3 of steam-sterilized soil. Three week old tomato seedlings of each cultivar was transplanted per pot. One week after transplanting, seedlings were inoculated with juveniles hatched from egg masses picked from established pure cultures maintained in the screenhouse. Inoculation was by exposing the area around the roots into which the nematodes were introduced. The inoculation points were then covered over with soil. The pots were arranged in a completely randomized design in the screenhouse with four replicates. Plants were inoculated with two population densities (0.5 and 1.0 J2/cm 3 soil) of both species of *Meloidogyne*, while the control was uninoculated. Plants were maintained in the screenhouse at 27 ± 2 ^{o}C, 80% relative humidity and 12h day light. The experiment was terminated ten weeks after inoculation.

Observations on plant height, and number of leaves were recorded weekly, and the number of days to anthesis was recorded per treatment. After harvest, fresh and dry shoot weight, fresh root weight and fruit weight per plant were taken using a weighing balance. Plant shoots were dried in an oven at 70 °C for 48h to a constant weight. The nematode populations of second stage juveniles per 200 cm^3 of soil were counted after extraction using the pie-pan extraction method (Coyne *et al*., 2007) per pot. The total root system was chopped into 1-2 cm pieces and put into a conical flask. A preparation of 0.5% sodium hypochlorite solution was made and added to the roots in the conical flasks and shaken vigorously for four minutes. Extracted eggs were collected in 25 µm sieves after several rinses in water (Hussey and Barker, 1985). The reproductive factor of the nematode (RF) was determined with the formula, RF = Pf / Pi (where Pf = final population [summation of juveniles from soil and eggs from roots] and Pi = Initial inoculum introduced into each unit) Root galling index was rated on a 1-5 scale, where 1 = no galling; 2 = 1-10% galling; 3 = 11-30% galling; 4 = 31-60% galling and 5 = 61-100% (Coyne *et al*., 2007).

Data on nematode counts were transformed using square root of x+0.5 and all data were analyzed using analysis of variance (SAS 9.2) and means were separated using LSD at 5% level of probability. Root gall index and nematode population were correlated with fruit yield.

Results

Identification and distribution of *Meloidogyne* species

The root-knot nematode species collected from infected plants in various locations in and around Ibadan were identified to include *M. incognita, M. javanica* and two unidentified *Meloidogyne* spp. *Meloidogyne arenaria* was not found in any of the research station or farm locations**.** Specimens identified as *M. incognita* had J2 with an average body length of 353.7 µm (336.4-366.4), and the hyaline tail terminus of 11.1 µm (9.3-12.2) which fall in the range used to describe the species (Table 1). Female perineal patterns had a high dorsal arch and wavy striae. Lateral lines were not distinct, typical of this species. Body length of J2 specimens identified as *M. javanica* was 449 µm (336.9-506), hyaline tail length was 12.6 µm (6.2-16.6). The hyaline tail terminus was conspicuous and the tail had a conical rounded tip tapering to a fine point. Perineal patterns of females were typical for *M. javanica* with a rounded to flattened dorsal arch and conspicuous lateral lines that clearly separated the dorsal and ventral regions of the patterns. Perineal patterns of females in other samples were highly variable, showing indistinct patterns that could not be corroborated with corresponding J2 measurements.

The non-transcribed spacer region between the 5S and 18S DNA genes was routinely amplified from bulk second-stage juveniles of all species of *Meloidogyne* with the 194/195 primers which produced amplification products of 720-bp from the tropical species populations of *Meloidogyne* spp. (Figure 1). Further identification to species level using specific primers produced products at 999 bp for *M. incognita* (Figure 2) and 670 bp for *M. javanica* (Figure 3). No amplification was observed in any sample where *M. arenaria* specific primers were used.

Table 1 Identification of *Meloidogyne* species from each location using morphological characters.

Species	Number of isolates (n = 10)	Average measurements of J2		Perineal pattern fit
		Body length (µm)	Hyaline tail portion	
M. incognita	57	353.7 (336.4-366.4)	10.8 (9.3-12.2)	High dorsal arch, no lateral lines
M. javanica	16	449 (336.9-506.0)	12.6 (6.2-16.6)	Prominent Lateral lines
Meloidogyne spp.	14	389.3 (358.7-425.3)	11.9 (9.7-13.6)	Generally rounded, indistinct, lateral lines
Meloidogyne spp.	9	393.1 (351.9-429.8)	12.0 (10-13.9)	Slightly high dorsal arch with faint lateral lines
Meloidogyne spp.	4	409.8 (370.5-443.2)	9.5 (7.7-10.8)	Fine, rounded annulations with indistinct lines

n = number of samples per isolate

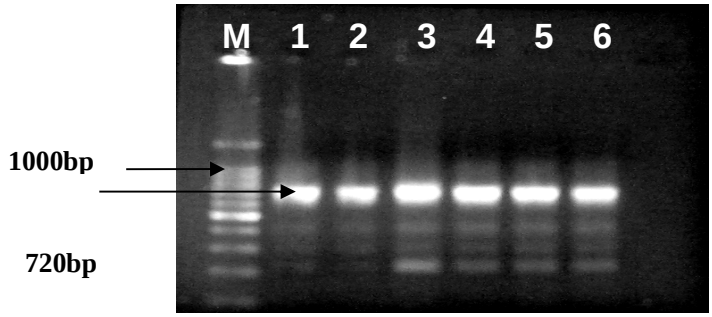

Figure 1 Amplified fragments of *Meloidogyne* isolates by universal 194/195 primer.
Lane labels: M = Marker (1000 bp-promega), 1 = I-034 UI, 2 = R-029 UI, 3 = I-032 UI, 4 = I-015 IITA, 5 = R-023 IITA, 6 = I-013 IITA.

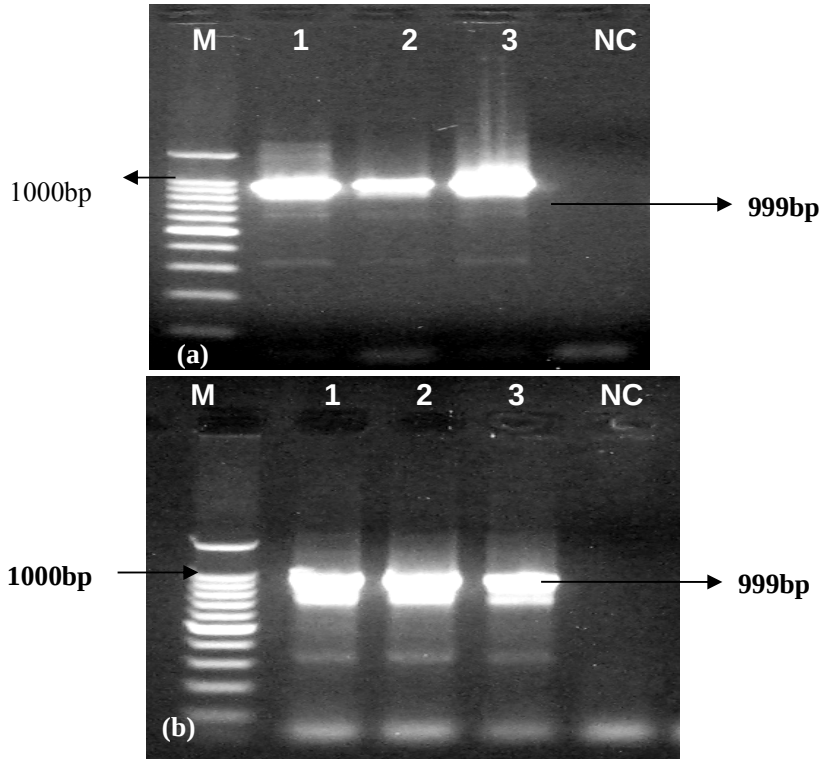

Figure 2 Amplified fragments from *Meloidogyne incognita* populations by using (MI-F/MI-R) specific primer.
Lane labels (a) M = Marker (1000 bp-promega), 1 = I- 015 IITA, 2 = I-021 IITA, 3 = I-013 UI, NC = Negative control; (b) M = Marker (1000 bp-promega), 1 = I-006 NIHORT, 2 = I-008 Ijaiye, 3 = R-032 IAR & T, NC = Negative control.

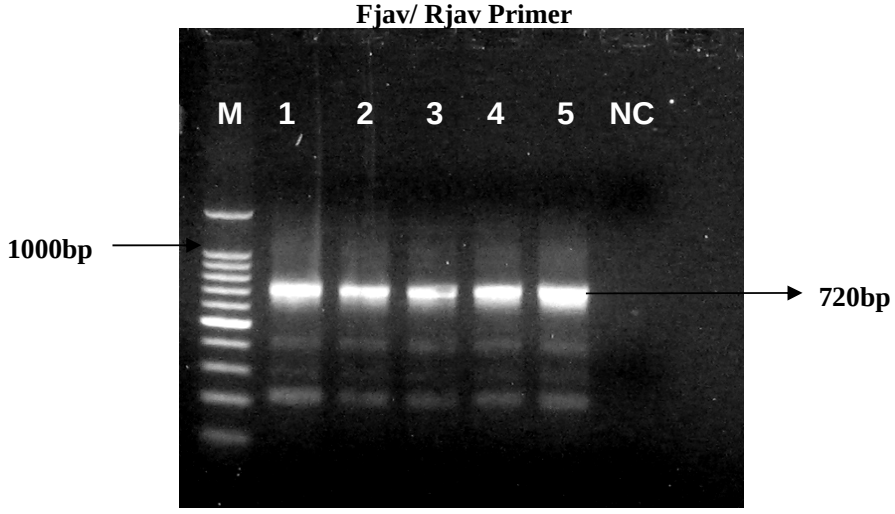

Figure 3 Gel photograph showing amplified fragments obtained from *Meloidogyne javanica* using Fjav/Rjav primer from four locations.
Lanes M = Marker (1000 bp-promega), 1 = R- 029 UI, 2 = R-032 UI, 3 = R-034 IAR & TI, 4 = I-018 IITA, 5 = R- 025 Akobo, NC = Negative control.

The most frequently occurring species was *M. incognita* across all locations followed by unidentified *Meloidogyne* species and *M. javanica*. Mixed populations were found to occur in samples collected from IITA, UI, IAR & T, and the vegetable farms at Akobo and Ijaiye (Table 2). *Meloidogyne incognita* was the most frequently occurring species in all the sampled locations and it was found most frequently in samples collected from NIHORT. While only *M. incognita* was identified from samples in NIHORT, two and seven populations were identified as *Meloidogyne javanica* and *M. incognita* respectively in samples from the UI plots (Table 2). In IITA vegetable plots, 12 populations were *M. incognita* and three were *M. javanica*. Ten, sixteen, five and seven populations were identified as *M incognita* in vegetable fields of IAR & T, NIHORT and farms at Ijaiye and Akobo respectively. *M. javanica* was not detected at NIHORT however, it was identified in three populations among those collected from vegetable field of IAR & T, and four populations from Ijaye and Akobo vegetable farms. In all the locations, some samples could not be identified because they did not amplify with the specific SCAR primers.

Evaluation of cultivars

Uninoculated plants of all the tomato cultivars were significantly taller than plants inoculated with *M. javanica* and the plants with higher population (5000 eggs) were the most stunted. When plants were inoculated with *M. incognita*, inoculated plants at both rates had significantly reduced plant height in Ibadan local, Roma and Beske whereas, Small Fry and Celebrity were not significantly different from uninoculated plants (Fig. 4a). Number of leaves in the five tomato cultivars was significantly ($P \leq 0.05$) reduced when tomato plants were challenged with *M. javanica*. The number of leaves in Ibadan local, Roma and Beske were also significantly reduced with *M. incognita* inoculation. No significant differences were observed in *M. incognita*-inoculated Small Fry and Celebrity compared to the control (Fig. 4b).

Table 2 Populations of *Meloidogyne* species from single egg masses collected at field locations.

Location	Species	Number of positive populations	
		Morphological identification	Molecular identification
Crop Garden, UI	*M. incognita*	7	3
Crop Garden, UI	*M. javanica*	8	4
Crop Garden, UI	*Meloidogyne* spp	2	-
Inoculum plots, IITA	*M. incognita*	12	8
Inoculum plots, IITA	*M. javanica*	3	3
Inoculum plots, IITA	*Meloidogyne* spp	-	-
Vegetables field IAR & T	*M. incognita*	10	9
Vegetables field IAR & T	*M. javanica*	3	3
Vegetables field IAR & T	*Meloidogyne* spp	4	-
Vegetable plots NIHORT	*M. incognita*	16	12
Vegetable plots NIHORT	*M. javanica*	-	-
Vegetable plots NIHORT	*Meloidogyne* spp	2	-
Ijaye tomato farm	*M. incognita*	5	7
Ijaye tomato farm	*M. javanica*	4	3
Ijaye tomato farm	*Meloidogyne* spp	7	-
Akobo Celosia farm	*M. incognita*	7	6
Akobo Celosia/Corchorus farm	*M. javanica*	4	4
Akobo Celosia/Corchorus farm	*Meloidogyne* spp	6	-

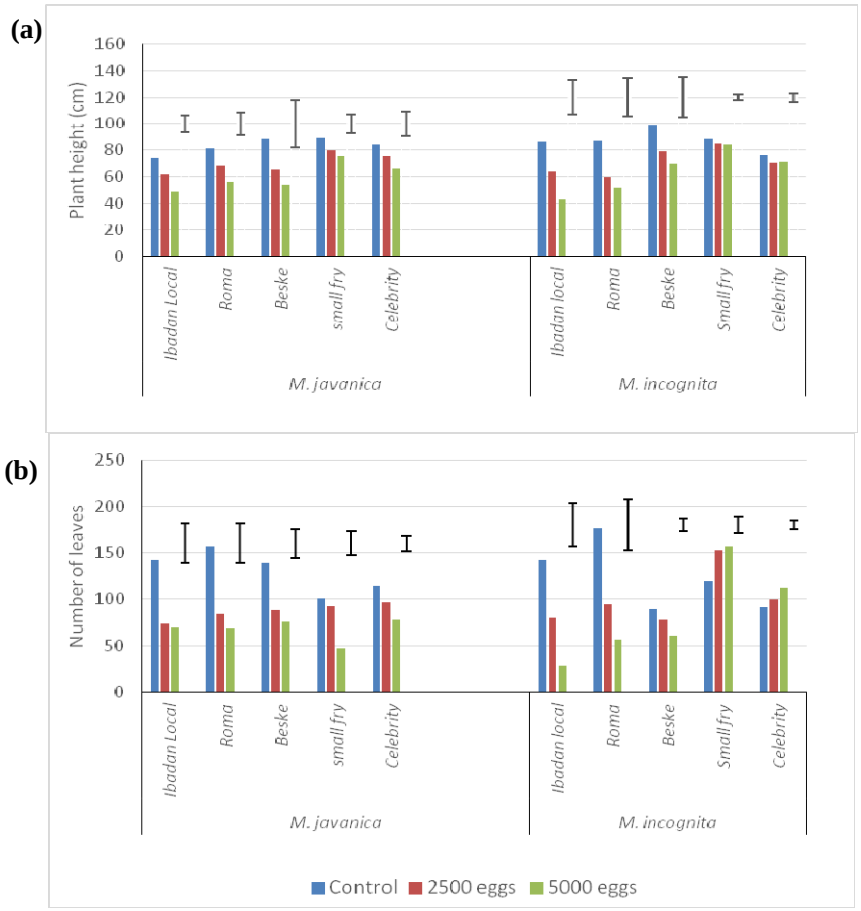

Figure 4 Plant height (a) and number of leaves (b) of five tomato cultivars inoculated with populations of *Meloidogyne javanica* and *M. incognita* 12 weeks after planting.
Bars = standard error of means.

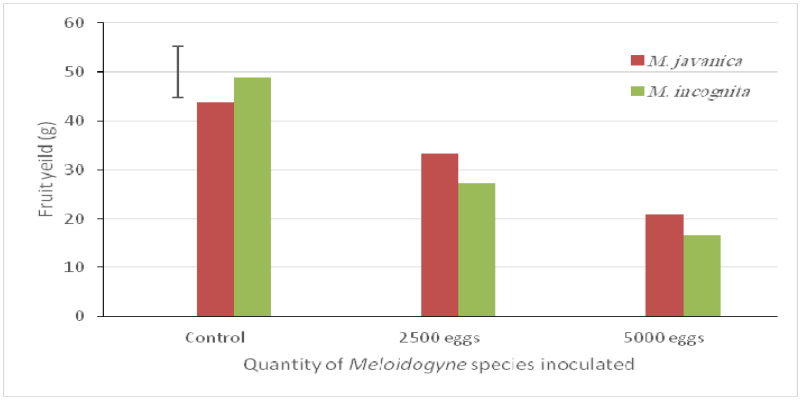

Figure 5 Fruit yield of tomato in response to inoculation with 2500 and 5000 eggs of *Meloidogyne javanica* and *M. incognita*.
Bar = LSD.

Table 3 Yield, galling index (GI) and nematode populations of tomato cultivars inoculated with 2500 and 5000 *Meloidogyne incognita* and *M. javanica*.

Treatment	Tomato cultivar	Quantity of Nematode Inoculum												
		Control	2500	5000	LSD	2500	5000	LSD	2500	5000	LSD	2500	5000	LSD
		Fruit yield				GI			Total No. of nematodes			RF		
M. javanica	Ibadan Local	65.1	55.6	25	21.0	3.0	4.8	1.3	328.1	543.4	152.2	35.9	49.2	9.4
	Roma	57.9	39.0	26	16.0	2.0	4.3	1.6	260.1	408.7	105.1	27.8	22.5	3.7
	Beske	74	51.9	35.7	19.2	2.0	4.0	1.4	205.1	457.5	178.5	14.0	34.9	14.8
	Small Fry	15.4	13.4	11.6	2.0	1.8	2.8	0.7	91.3	195.4	73.6	2.8	6.4	2.5
	Celebrity	8.9	6.3	5.9	2.6	2.3	3.3	0.7	118.5	410.0	206.2	4.7	28	16.5
	SE	13.7	10	5.4		0.2	0.4	0.2	202.4	304.7	72.3	6.5	7.1	2.8
M. incognita	Ibadan Local	80.7	32.1	10.8	35.8	3.0	5.0	1.4	374.1	567.3	136.6	56	64.4	5.9
	Roma	67.7	37.3	23.5	22.7	2.5	4.2	1.2	232.6	473.1	170.1	21.6	44.8	16.4
	Beske	73.9	51.8	35.7	19.2	1.7	4.0	1.6	177.6	490.6	221.4	12.6	48.1	25.1
	Small Fry	15.2	11.9	11.6	1.9	1.0	1.0	0.0	76.9	112.8	25.4	0.4	1.5	0.1
	Celebrity	6.8	2.5	1.45	2.9	1.0	1.0	0.0	44.8	129.7	60.0	0.8	2.4	1.8
	SE	15.7	8.9	5.9	6.4	0.4	0.9	0.4	237.1	374.8	97.3	10.1	12.6	4.8

Values are means of 4 replicates. Transformed nematode populations presented.
LSD = least significant difference at $P \leq 0.05$); RF = reproductive factor (RF = Pi/Pf); GI was 1-5 scale (1 = no galling; 2 = 1-10% galling; 3 = 11-30% galling; 4 = 31-60% galling and 5 = 61-100%).

Tomato fruit weight was reduced significantly at both nematode inoculation populations, with the higher inoculum causing greater loss in yield (Fig. 5). *M. incognita* had more detrimental effect on the yield of tomato compared to *M. javanica*. Fruit yield of Beske was significantly reduced with inoculation of *M. javanica* at both population densities of inoculation. However, only inoculation with 5000 *M. javanica* caused significant yield losses in the other cultivars. Small Fry and Ibadan local inoculated with 2500 *M. javanica* showed the smallest yield loss of 12.9% and 14.5% respectively (Table 3). The yield losses of cultivars inoculated with 5000 *M. incognita* were significantly ($P \leq 0.05$) greater than those of plants inoculated with 2500 juveniles and ranged from 23% to 86% with Small Fry having the lowest loss and Ibadan local having the greatest loss.

At the higher level of inoculation, numerous and massive galls were observed in Ibadan local, Roma and Beske for both root-knot nematode species, while Celebrity and Small Fry had few galls of *M. javanica* and no galls of *M. incognita*. Higher populations of both nematodes were recovered from plants inoculated with 5000 nematodes compared to those inoculated with 2500 nematodes, except for Celebrity and Small Fry inoculated with *M. incognita*. The reproductive factor for both species of root-knot nematodes was lowest in Small Fry followed by Celebrity while the other cultivars greatly enhanced the reproduction of the nematodes. There was a positive correlation between gall index and nematode population and a negative correlation between nematode population and fruit yield in susceptible tomato cultivars.

Discussion

The correct identification of a specific nematode pest is central to decision making in nematode disease management. Accurate identification of root-knot nematodes is very useful for efficient and successful utilization of resistance. The cultivation of nematodes for research purposes is a standard procedure for institutes conducting such research and any output has to be placed in the context of the

species of root-knot nematode used in the research. The most frequently identified nematode in all sampled areas was *M. incognita*. This is not different from the finding of Olowe (2004) where *M. incognita* was identified in 82% of cowpea farms samples in southern Nigeria. *Meloidogyne incognita* was also the most abundant nematode identified from yam field sampled in the south-western region of Nigeria (Adegbite *et al*., 2005). As was observed in this study, Olowe (2004) found that there was often a mixed population in field plots. This is not surprising for the two farms samples but it is a point of concern for inoculum and field plots meant to serve nematology research. There are implications for the occurrence of mixed populations in plots meant for research. Where mixed populations occurred it was mainly with *M. javanica* usually at a lower population density and occasionally with unidentified species. Among the species of *Meloidogyne* recorded in association with crops of agricultural importance in subtropical and tropical regions, *M. incognita* and *M. javanica* are considered as common and wide-spread (Sasser, 1979; Moens *et al*., 2009).

In this study, 73% of the 100 samples were identified to species level using morphological characters whereas only 61% were positively identified using molecular tools. While the gap in number of positively identified samples using these two methods may not be entirely due to the higher accuracy of the latter method i.e. some of the difference can be due to non amplification of correctly identified samples. It was observed that some morphologically identified *M. incognita* did not amplify with the designated primer suggesting that those isolates may not be *M. incognita*. Also some uncategorised species were identified as *M. incognita* using the SCAR-PCR primers for the species (observed in IART & T, IITA and Ijaye farm). Morphological methods are heavily relied on for the identification of root-knot nematode in most sub-Saharan African countries and Nigeria is not an exception. Morphological identification is heavily dependent on the skill of the researcher and the facilities available in the Institution. Where funds are a challenge and experienced nematologists are unavailable, the use of morphology to identify root-knot nematode species becomes risky. This is especially so where resistance is the choice for management. The sensitivity of nucleic acid-based detection systems has improved over time and the sequence characterised amplified region-polymerase chain reaction (SCAR-PCR) method has been accurately used to detect and identify single specimens (Fourie *at al*., 2001; Adam *et al*., 2007). The use of species-specific primers was useful in giving confidence in the identification of the root-knot nematode species. It served to support and confirm identification using morphological characters of juveniles and perineal patterns of females. However, since the primers are species-specific for *M. incognita*, *M. javanica* and *M. arenaria*, they could not amplify other nematode species. Perineal patterns are considered important for differentiating *Meloidogyne* species although, in this study, some samples were variable and indistinguishable and could not be used for identification on their own. This was observed where some specimens had the characteristic patterns of *M. incognita* in addition to visible lateral lines and could easily be confused as *M. javanica*. The same confusion was reported by Rammah and Hirschmann (1990).

Plant growth and yield were severely affected in susceptible cultivars inoculated with both species of root-knot nematode. For Small Fry and Celebrity there were no significant differences in yield between inoculated and uninoculated plants. The loss in yield due to root–knot nematode infection has been reported by several workers on different plants (Chitwood, 2003; Hisamuddin *et al.* 2004; Niyaz 2008). Significant differences in root damage on each of the five cultivars indicate different levels of susceptibility. The level of susceptibility is controlled by the presence of resistance genes such as the *Mi* gene and the genetic background of the tomato cultivar (Castagnone-Sereno 2006; Jacquet *et al*. 2005). According to the rating of Soriano *et al*., 2000, Ibadan Local was the most

susceptible among all the cultivars while Small Fry and Celebrity were moderately resistant to *M. incognita*. However, following the resistance rating based on Afolami (2000) where resistance means RF $\leq$ 1, GI $\leq$ 2 accompanied by no significant yield loss, both Small Fry and Celebrity cultivars sustained their resistance to *M. incognita.* With *M. javanica,* Small Fry and Celebrity had the lowest gall indices, indicating that they were tolerant based on Afolami (2000) resistance rating. There is sophisticated interaction between the host plant and root- knot nematodes and a number of studies have found resistance breaking pathotypes of root-knot nematodes that are able to parasitize root-knot nematodes resistant plants (Baicheva *et al.* 2002; Abad *et al*. 2003) which is a major limiting factor in using plant resistance as a means for controlling root-knot nematodes. The high gall indices of the three local cultivars Ibadan Local, Roma and Beske tomatoes indicated that they were the most susceptible cultivars. The highly susceptible host plants allow the juveniles to enter the roots, reach maturity and produce many eggs while the resistant plants suppress their development and thus do not allow reproduction (Karssen and Moens 2006). This agreed with the present findings whereby Ibadan Local, Roma and Beske recorded the highest mean values for total nematode population, gall index and nematode reproductive factor. Although resistant, Small Fry and Celebrity had comparatively lower yields in uninoculated plants. The explanation could be that, as exotic cultivars, they may not be adaptated to fit into the local environment or should be tested in environments that are more suitable for their productivity within the country. Tomato cultivars have varying degrees of resistance to root-knot nematodes and differences in quality and quantity of fruit production. Several cultivars of tomatoes have been developed in an attempt to produce root-knot nematode resistant cultivars (Milligan *et al.* 1998; Jacquet *et al*., 2005; Tisserat 2006).

The higher initial inoculum produced the highest final nematode population per plant, this shows that when the inoculum levels are high, greater number of juveniles are able to infect the plant roots, resulting in reduced nutrient and water uptake by the roots and consequently poor plant growth (Karssen and Moens, 2006). The increase in the nematode populations and the subsequent reduction in the yield of crops are directly influenced by the initial density of the nematodes in the soil (Azam 2008). This view holds true with the present findings where plant growth and yield were proportionately affected with increase in the gall index and final nematode population.

In conclusion, this study further established that *M. incognita* is the most prevalent root-knot nematode in the south-western region of Nigeria. Resistance was identified in two cultivars which seem poorly adaptated to the tropical environment or they could be used in breeding programmes to develop local cultivars. The occurrence of mixed populations of root-knot nematodes in research station plots has implications for the research outputs from these stations. The recommendation for this is that single populations arising from a single egg mass should be raised for specific nematology research. Further research needs to be conducted to identify the uncharacterised populations using a wider range of primers to identify more species. Other methods of identifying root-knot nematodes such as isozyme analysis, especially esterase phenotyping (Carneiro *et al*., 2000) could be explored.

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تعیین هویت گونه‌های نماتد ریشه گرهی و واکنش کولتیوارهای گوجه‌فرنگی به *Meloidogyne incognifa* و *Meloidogyne javanea*

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چکیده: این مطالعه به‌منظور تعیین پراکنش گونه‌های رایج نماتد ریشه گرهی در ایستگاه‌های تحقیقاتی و مزارع سبزی‌کاری Ibadan در جنوب غربی نیجریه انجام شد. ریشه‌های گال‌دار از کرت‌های آلوده در چهار ایستگاه تحقیقاتی و دو مزرعه جمع‌آوری شد. شناسایی گونه‌ها براساس صفات ریخت‌شناسی نماتدهای ماده و لاروها و با استفاده از پرایمرهای SCAR اختصاصی گونه‌های ملویدوجاین انجام شد. بیماری‌زایی هر دوگونه نماتد در یک بررسی گلخانه‌ای بیماریزایی هر دو گونه نماتد در گلخانه با مایه‌زنی سطوح مختلف اینوکولوم نماتد روی گوجه‌فرنگی مورد ارزیابی قرار گرفت. نتایج نشان داد که در کرت‌های تحقیقاتی *Meloidogyne incognita* گونه غالب بود، اگرچه اغلب با *Meloidogyne javanea* و گونه‌های ناشناخته دیگری مخلوط و همراه بود. پارامترهای رشد گیاه شامل ارتفاع، تعداد برگ و محصول گوجه‌فرنگی در مورد همه کولتیوارها به استثنای Small Fry و Celebrity با افزایش سطوح اینوکولوم نماتد کاهش یافت. هر دو کولتیوار مذکور مقاوم به گونه *M. incognita* و متحمل به *M. javanea* بودند. مشتری پسندترین کولتیوار شامل Ibadan Local، Roma و Beske به هر دو گونه نماتد حساس بودند.

واژگان کلیدی: رقم سلبریتی، رتبه‌بندی میزبان، بیماری‌زایی، نماتدهای گره ریشه، SCAR-PCR، سبزیجات