Community Analysis of Nematodes Associated with Banana, Identification of root knot nematode and Evaluation the Susceptibility of Some Cultivars to Infection

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Abstract

Background Plant-parasitic nematodes are extremely dangerous pests in a variety of economically important crops. The purpose of this study was a survey of all nematode species existing in banana from three sites in Assiut Governorate, Egypt and to characterize the most common species by morphological, morphometric and molecular techniques (PCR with species-specific primers). Then, study of resistance or sensitivity of some banana cultivars to root-knot nematodes.

Methods and Results Four nematodes, *Meloidogyne*, *Rotylenchulus reniformis*, *Helicotylenchus* and *Pratylenchus* were isolated and identified from soil and root samples collected from banana plants. Most frequently occurring of plant parasitic nematode species in banana was *Meloidogyne*. Former research found differences in species and in resistance to root-knot nematodes among the examined plant cultivars. Identification of Root-knot nematodes by Characterize of morphometric, molecularly, morphological isolate of *Meloidogyne* related to banana plants. The results revealed that the identified nematode species, *Meloidogyne javanica*, is the most common plant-parasitic nematodes in all locations. Data on the susceptibility of the tested banana cultivars to *M. javanica* revealed that Grand Naine was highly susceptible (HS) however, Magraby was susceptible (S) but Williams and Hindi cultivars were moderately resistant (MR).

Conclusions we concluded that a survey revealed the significant prevalence of *Meloidogyne javanica*, the most important nematodes on banana in Assiut. The morphometric, morphological, and molecular identification were harmonic with one another. In addition to the host response of certain banana cultivars, to *M. javanica* that resistance is of significance and can be helpful to incorporate through planning control measures for root- knot nematodes.

Introduction

Banana (*Musa* sp.) is one of the world's most economic tropical fruit crops. Bananas thrive in a wide range of soil conditions, also an important source of carbohydrates, fiber, proteins, vitamins, and minerals. Its cultivation area in Egypt reached approximately 447299 ha, with an average yield of 1359297 tons/ha. [1]. Nematodes are one of the world's most significant limitations on banana production, with 146 species recorded in 43 genera. A most dangerous nematode species are that, devastate the primary roots, causing the anchorage system to fail and the plant to fall over [2]. The major nematode pests of banana include burrowing nematode, *Radopholus similis*; root knot nematodes, *Meloidogyne arenaria*, *M. incognita* and *M. javanica*; spiral nematodes, *Helicotylenchus multicinctus*; and *H. dihystra*; root lesion nematodes, *Pratylenchus coffeae*, *P. reniformi* and *P. brachyurus* and *P. goodeyi*; and reniform nematode, *Rotylenchulus reniformis* [3, 4]. The perineal pattern is predominantly uncertain when used alone to make diagnostic deduction, but when used as an integral device in conjunction with morphometric characterization or molecular techniques, it is substantial for screening the morphological matchmaking of the identification [5]. So that, molecular diagnostics of *Meloidogyne* sp. have been research as a surrogate. PCR-based disclosure methods, such as species specific or sequence

characterized amplification region (SCAR) primers, have been advanced and vastly used for nematode identification [6]. SCAR markers are preferred over RAPD markers because they detect only a single locus and are more specific. Furthermore, their PCR amplification is low sensitive to reaction constraints and thus more reproducible. Nematode relationships with bananas, including damage, depend on environmental conditions, susceptibility of the host and pathogenicity of the nematode considered [7]. Due to recent concerns about pesticide contamination of the environment, the search for both plant resistance and/or tolerance to plant-parasitic nematodes of bananas is now a major challenge to supplement the existing IPM approach, gaining new interest and involving many research teams [8].

The objective of the present study was a survey of all nematode species existing in banana from three sites (Assiut Center, Al-Fath, Sahel Saleem) in Assiut Governorate, Egypt and to characterize the most common species by morphological, morphometric and molecular techniques (PCR with species-specific primers). Then, study of resistance or sensitivity of some banana cultivars to root-knot nematodes.

Materials And Methods

Plant-parasitic nematodes associated with banana plants in Assiut Governorate:

Three sites (Assiut, Sahel-Selim and El-Fath) were selected for the nematode screening based on contrasting soil types and the availability of diverse *Musa* species under commercial cultivation. All sites had been commercial banana have favorable climatic conditions for *Musa* agricultural production.

Sampling and Collection:

Two to four banana plants were sampled for each accession at each site preferentially from recently flowering plants. Root and soil samples were collected from an approximately 30 cm x 30 cm area on two sides of each plant and placed into a plastic bag properly identified with the date, location, and cultivar name. All samples were transported in insulated boxes to protect samples from direct sunlight and temperature fluctuations. Samples were stored at 4°C until processing for nematode extraction.

Nematode extraction:

Cobb's decanting and sieving method [9] was used to extract the nematodes from a 100g combined soil sample, accompanied by amended Baermann procedure [10].

Taking 20g of pencil-thick banana feeder roots, gently wash them under tap water, cut them into little bits of 2-3 cm in length, split them longitudinally, and place them on double-layered facial tissue paper, then follow the amended Baermann procedure [10]. Keep this society to sit unmoved for 48 hours before collecting nematode suspension to examine under a microscope. After differential staining with NaOCl -

acid fuchsin technique, the root samples were analyzed using a stereoscopic microscope (Carl Zeiss-Stemi 2000C) [11].

Estimate of nematode populations:

Flask containing stable nematode suspension was taken in a measuring cylinder to measure the total quantity of nematode suspension acquired from 100g of soil. Nematodes stayed in the constant suspension were calculated under binocular microscope using multichambered counting plate. The nematode suspension from each location was spotted and mean of three aliquots was taken for calculating the population density per 100g of soil.

Processing of nematodes

The fixed nematode samples from each population were analyzed when using glycerol-ethanol method for morphological and morphometric studies [12]. The processed specimens were permanently placed in pure anhydrous glycerol. A small drop of glycerol was placed in the center of a clean glass slide (Borosil brand) that measured 76 mm 26 mm 1.25 mm. Approximately 8-10 processed nematode specimens were picked up and located in the middle of a glycerol drop with their heads leading in the same direction, ensuring that they were resting on the surface of the glass slide and not hovering on the surface of the drop. A microscopic cover glass measuring 18 mm was placed over the specimen and sealed with a paraffin wax ring [13].

Identification of Plant parasitic nematodes from different banana growing areas:

Important morphological and morphometric features of taxonomic significance have been studied in detail for each population of Plant parasitic nematodes obtained from different banana growing areas. The parameters used to characterize nematode species were developed initially by [14] and added to, modified and amended by [15,16] and others.

Identification of root-knot nematodes:

Morphometrical characterization:

Morphometric dimensions of *Meloidogyne* were specified on ten individual J2 from three centers. J2 was tentatively mounted in water on glass slides before being spotted and measured at 100 magnifications with a compound light microscope (OMAX 40X-2000X digital binocular biological compound microscope) linked to a computer working Scope-Image-9.0 Professional Imaging software. The optical

microscope was used to measure five morphometric variables (stylet length, tail length, body length, hyaline terminus length, and the distance between the stylet base and the dorsal esophageal gland orifice (DEGO)).

Morphological characterization:

Sections of infected roots should be immersed in 0.9% NaCl. Using a dissecting microscope, separate females from roots by needle and a scalpel and transfer the females to a petri dish with a small drop of 45% lactic acid. Push a female body out of a drop in a small isthmus of lactic acid solution, so that surface tension holds it in place. Insert the razor blade fragment into the slide and use a paper cutter to cut off the nematode's posterior. Using a dissecting needle, gently remove body tissue from the posterior section. In a small drop of glycerin, place the perineal pattern on a microscope slide. The internal surface of the cuticle should place against the glass then, cover slip placed on the glycerin drop. [17].

Molecular characterization:

DNA extraction:

The CTAB (cetyltrimethylammonium bromide) method was used to extract DNA from nematode isolates [18] with some modifications. Many adult females gained from each isolate were frozen in liquid nitrogen then, crushed using a suitable pestle and mortar. 600µl of CTAB extraction buffer was added to each sample and the mixture was then transferred to 1.5 ml Eppendorf tube. A volume of 50µl β-mercaptoethanol was added and all tubes were well vortexed for 15 sec and then incubated for about 40 min at 65° C in a water bath. After incubation, the tubes were kept at room temperature for 5-10 min, and 600µl chloroform: isoamyl alcohol solution (24:1 v/v) was then added to each tube, and the solution was gently mixed. The tubes were then subjected to a centrifugation (8,000 rpm at 4° C for 15 min). After the centrifugation, approximately 500µl of the upper aqueous phase (without any solid material) was transferred to a new 1.5 tube and an equal volume (500 µl) of cold isopropanol was added to each tube. The tubes were then slowly inverted several times and stored in the refrigerator overnight. A centrifugation (13,000 rpm at 4° C for 10min) was performed for the tubes. After the centrifugation, the supernatant was discarded and the DNA pellet was then washed by adding 1 ml of 70% cold ethanol, and a centrifugation (13,000 rpm at 4° C for 5 min) was performed. The tubes were kept at room temperature to allow the DNA pellet to air-dry (approximately 15 min). The dried DNA pellet was then resuspended in 100 µl TE buffer. DNA concentration (µg/ml) was determined for each sample by using spectrophotometer, and required dilutions were then performed to be used later for PCR.

Species-specific PCR assay:

A species-specific SCAR primer collection Table 1, selected from previous studies [19] as a specific marker for *Meloidogyne javanica*, namely Fjav/Rjav, was used to confirm morphological identification of nematode isolates. Amplifications were carried out in 25µl reaction mixtures containing 5-10 ng of genomic DNA, 1X PCR buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 0.8 µM of each primer, and 1 U Taq DNA-polymerase and using the following PCR software in a Senso Quest Lab Cyclor (SensoQuest GmbH, Göttingen, Germany): 5 minutes at 95° C, then 35 intervals of 1 minute at 94° C, 1 minute at 58° C, and 1 minute at 72° C, followed by one final extension period at 72° C for 10 minutes. PCR products were separated on a 1.5 percent agarose gel stained with ethidium bromide in 0.5 X TBE buffer using a horizontal gel electrophoresis unit. The size of each amplified DNA fragment was determined using a DNA ladder. The gel was run for about an hour at a constant voltage of almost 80 V, and then photographed using a gel documentation device under UV light. For each SCAR marker, the same band with the predicted size was then detected separately.

Table 1

SCAR primers were used to identify *M. javanica* at the molecular level.

Name of Primer	Fragment size (bp)	Sequence (5'-3')	Reference
Fjav/Rjav	670	GGTGCGCGATTGAACTGAGC CAGGCCCTTCAGTGGA ACTATAC	[19]

Susceptibility of certain banana cultivars to *M. javanica*:

This experiment was conducted at the greenhouse of Plant Pathology Department, faculty of Agriculture, Assiut University. Nematode free seedlings, of four rootstocks; (Hindi, Magraby, Williams and Grand Naine) were used for evaluating their susceptibility to the Root-Knot nematode, *M. javanica*. They were obtained from the Horticultural research Centre, Giza. Three - month old seedlings of each cultivar were grown in 40cm pots filled with sterilized sandy-clay soil (4 Kg soil to each pot).

Inoculation of root-knot nematode was taken from the stock culture and nematode eggs were extracted from the roots using sodium hypochlorite 0.5 % solution [20]. After 25 days of seedling transplanting inocula was added as 5000 J2 per plant according to [21] with modify. The inocula were added in three holes around plant roots with micropipette and pots were watered daily and fertilized every week with 1 g /plant of NPK salt (1:1:1).

Data were recorded after 90 days from nematode inoculation. Nematode population was estimated using Baermann pan technique [22] as previously mentioned.

Plants with 0-2 egg-masses/plant were considered resistant (R), 3-10 egg-masses/plant moderately resistant (MR), 11-30 egg-masses/plant moderately susceptible (MS), 31-100 egg-masses/ plant susceptible (S) and < 100 egg-masses/plant highly susceptible (HS), [23].

Results

In the sampled areas, phytonematodes occurred in polyspecific communities comprising a mixture of *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, and *Rotylenchulus reniformis*. The root-knot nematode (*Meloidogyne*) was dominant in all localities (Assiut Center, Sahel-Selim and El-Fath). Higher frequencies were observed in Sahel-Selim and El-Fath, with frequencies of 1150 and 1125 J2/250g soil respectively (Table 2). *Rotylenchulus reniformis* presented a low frequency 125 J2/250g soil in Sahel-Selim Table 2.

Table 2

Survey of plant-parasitic nematodes associated with banana in Assiut Governorate.

Locality	No. of samples	Nematode genera	Average no. of J2/250g. soil
Assiut Center	150	<i>Meloidogyne</i>	800
		<i>Pratylenchus</i>	125
		<i>Helicotylenchus</i>	163
		<i>Rotylenchulus reniformis</i>	0
Sahel-Selim	100	<i>Meloidogyne</i>	1150
		<i>Pratylenchus</i>	400
		<i>Helicotylenchus</i>	150
		<i>Rotylenchulus reniformis</i>	125
El-Fath	75	<i>Meloidogyne</i>	1125
		<i>Pratylenchus</i>	325
		<i>Helicotylenchus</i>	0
		<i>Rotylenchulus reniformis</i>	0

Morphometric characters of second-stage juveniles of *Meloidogyne* spp.

Second-stage juveniles were vermiform and slender ranged from 400-550 µm in length and a head that was not offset from the body. Stylet knobs transversely elongate and are offset from the stylet shaft, stylet length ranged from 9.6-12.4 µm. The distance between the dorsal esophageal gland and the base of the stylet was 3-4 m. Tail length was 50-62.2 µm with rounded tip. The hyaline tail length ranged from 10.2 to 18.4 µm, with a long slender tapering tail and a delicately curved tail tip, which corresponded to the characterization set for *M. javanica* by [24,25]. Morphometric performed on J2 are reported in Table 3.

Table 3

Morphometric comparison of second-stage juveniles of the isolates and *Meloidogyne javanica* reported values.

Characteristic	Isolates	<i>M. javanica</i> reported values
Body length	400 - 550 (475) µm	400 – 560 µm
Stylet length	15 – 17 (16) µm	14 -18 (16) µm
Tail length	50.0 - 62.2 (56.1) µm	51.0 – 63.0 (57) µm
Hyaline terminus	10.2 – 18.4 (14.3) µm	10.0 – 19.0 (14.5) µm
Dorsal esophageal Gland Orifice (DEGO)	3 – 4 (3.5) µm	3 - 4 (3.5) µm
All measurements in micrometers with range (mean).All measurements in micrometers with range (mean).		

Perineal Pattern Morphology:

When comparing to previous reports, assessment of the perineal pattern’s morphology of adult females from three localities, selected by hand from infected banana roots, revealed brow model of *M. javanica* [24]. *M. javanica* was dominant in the three localities. The perineal patterns of *M. javanica* are unrivaled because they consist of side ridges that part the dorsal and ventral lines. In generally, the ridges run the entire width of the pattern, but progressively die out near the tail end. The dorsal arch is faint and rounded to high and squarish and often contains a whorl in the tail terminal area. The striae are sleek to little adverse, and several striae may curvature across the vulval edges Fig. 1.

Molecular identification of *Meloidogyne javanica*:

One species-specific SCAR primer pairs, namely Fjav/Rjav were used for molecular diagnosis of nematode isolates to further confirm species identification. The PCR assay was performed on one sample from each location. The three nematode isolates clearly amplified the expected specific DNA fragment of 670 bp Fig. 2 which confirms the identification of *M. javanica* as recorded by [19].

Susceptibility of certain banana cultivars to *M. javanica*

Data on the susceptibility of the tested banana cultivars to *M. javanica* revealed that Grand Naine was highly susceptible (HS), Magraby was susceptible (S), but Williams and Hindi were moderately resistant (MR) Table 3.

The number of galls and egg masses of root-knot nematode were counted. Plants were graded based on the number of egg masses found on their roots.

Table 3

Susceptibility of certain banana cultivars to *M. javanica*.

Banana cultivars	J2/100 g soil	No. of galls/1 g. roots	No. of egg masses /1 g. roots	Status of cultivars
Hindi	850	56.3	28.7	MS
Magraby	1130	161	98.7	S
Williams	980	61.7	23.3	MS
Grand Naine	3150	183.7	122.3	HS

Discussion

This study identified the most frequent and abundant nematode species isolated from soil and banana roots from two long-term banana plantings in Assiut Governorate. *Meloidogyne*, and *Pratylenchus* are known to cause the serious economic damage to banana plantations, it is agree with [7]. *Meloidogyne* were, in general, the most abundant phytonematodes recovered from soil and root samples.

These results relative to root-knot nematode population are in accordance with those reported by several investigators as an important nematode pest attacking banana in different countries, [26] in West and Central Africa, [27] in Crete, [3] in Hawaiian Islands, [28] in West Bengal, [29] in Rusitu Valley, [30] in Egypt, [31] in Republic of Congo, [32] in Ethiopia, [33] in South Africa, [34] in Turkey, [35] in Lasbela Balochistan and [36] in Southern Florida.

Morphometric and morphological studies necessitate a significant amount of endeavor and are not always simple, attributed to the prevalence of intra-specific variations. As the previously reported, the morphometric values overlap, and the morphology of the perineal pattern, while extra helpful but, remains indecisive due to individual variability, the assorted practice of those describing the patterns, and the increased numbering of species. The combination of morphology and morphometric may provide a small

hint toward species identification. This result is agreement with [37, 38, 39, 40]. *M. javanica* is the most frequent *Meloidogyne* sp. found in tropical and subtropical areas [41], such as Egypt, where annual temperatures range between 17–32°C. It is important to note that, while PCR is quick, simple, and capable of determining species identity regardless of developmental phase and from tiny portions of tissue. According to this study, Because of intraspecific variability and species closeness, its dependability is uncertain. Thus, morphology, morphometric, and molecular analysis work in tandem to provide more accurate and reliable identification. The PCR examination for the nematode isolates with the specific SCAR primer Fjav/Rjav clearly produces a specific DNA piece of 670 bp (Fig. 2) which confirms the identification of *M. javanica* such results were in harmony with those [19, 42, 43, 44]. SCAR markers have been to a large degree used in molecular identity of root-knot nematodes, both to prove morphological identifications and to set apart unknown isolates in genomic analysis [19, 45, 42, 46, 43].

Susceptibility of four banana cultivars (Hindi, Magraby, Williams and Grand Naine) to *M. javanica*. The glasshouse experiment indicated some differences in the response between the cultivars. 'Grand Nain' was most sensitive to *M. javanica*, whereas 'Williams' allowed less reproduction of this nematode. Such results agree with those reported by [47, 48, 49]. However, disagree with reported by [33].

Conclusions

From the results we concluded that a survey revealed the significant prevalence of *Meloidogyne javanica*, the most important nematodes on banana in Assiut. As, these species are known to cause severe damage and yield losses throughout most banana growing areas in the tropical and subtropical regions of the world. The morphometric, morphological, and molecular identification were harmonic with one another, implying that molecular analysis of root-knot nematodes using SCAR markers could be used as a supplement to morphometric and morphological identification. In addition to the host response of certain banana cultivars, to *M. javanica* that resistance is of significance and can be helpful to incorporate through planning control measures for root- knot nematodes.

Declarations

Author contributions Radwa G. Mostafa:

Methodology, Validation, Formal analysis, Investigation, Writing-original draft. Aida M. El-Zawahry: Supervision, Conceptualization, make substantial contributions to conception and design, Resources, Validation, Writing-review& editing. Ashraf E. M. Khalil: Supervision, Conceptualization, Resources, make substantial contributions to conception and design. Ameer E. Elfarash: participate in drafting the article or revising it critically for important intellectual content. Ali D. A. Allam: Supervision, Conceptualization, Resources. All authors give confirmation of their consent to participate in this manuscript sent for publication. All authors also give final approval to the submitted version and any revised versions

Data availability

The data supporting the study's findings are available upon request from the corresponding author. Due to privacy and ethical concerns, the data is not publicly available.

Conflict of interest:

The authors state that they do not have any conflicts of interest.

Ethical statement:

Not all author conducted any studies with human subjects or animals for this article.

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Figures

Figure 1

Perineal pattern of *M. javanica*.

Figure 2

Amplification items (670 bp) for *M. javanica* species – Fjav/Rjav SCAR primers.

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