RESEARCH/INVESTIGACIÓN

MOLECULAR IDENTIFICATION, RACE DETECTION, AND LIFE CYCLE OF *ROTYLENCHULUS RENIFORMIS* IN EGYPT

M. Adam[1]*, S. F. Diab[1], A. Farahat[1], A. A. Alsayed[1], and H. Heuer[2]

[1]Department of Zoology and Nematology, Faculty of Agriculture, Cairo University, Egypt; [2]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany; *Corresponding author: mohamed-adam2007@agr.cu.edu.eg

ABSTRACT

Adam, A., S. F. Diab, A. Farahat, A. A. Alsayed, and H. Heuer. 2018. Molecular identification, race detection, and life cycle of *Rotylenchulus reniformis* in Egypt. Nematropica 48:59-67.

Molecular and bioassay methods were used to characterize an isolate of *Rotylenchulus reniformis* collected from Fowa-Kafr-El-Sheihk, Egypt, and maintained for 40 years under greenhouse conditions. The identification of this isolate was rapidly determined by PCR with species-specific primers developed for amplifying the D2–D3 of 28S rRNA gene. The nested PCR allowed direct sequencing without cloning the fragments. All sequences of the D2–D3 of 28S rRNA fragments obtained were identical and exhibited 99% similarity with a partial 28S ribosomal RNA gene of *R. reniformis* in GenBank. Phylogenetic analysis revealed relationships of our isolate with *R. reniformis* from other countries and a sister relationship with *R. macrosoma*. The isolate's reproductive potential was tested using different hosts. The isolate infected and reproduced on cowpea, castor, and cotton with differences among the plants. Cowpea was the most preferred host. Low reproduction was achieved on cotton. After penetration, the minimum time for the appearance of mature females was 3 days on cowpea, 7 days on castor, and 14 days on cotton. Complete formation of egg masses was observed after 7, 14, and 21 days on cowpea, castor, and cotton, respectively. Juvenile females of the second generation were recorded after 14 days on cowpea and 21 days on castor and cotton.

Key words: D2–D3 of 28S rRNA gene, Egypt, life cycle, molecular diagnosis, pathogenicity, *Rotylenchulus reniformis*

RESUMEN

Adam, A., S. F. Diab, A. Farahat, A. A. Alsayed, and H. Heuer. 2018. Identificación molecular, detección de razas y ciclo de vida de *Rotylenchulus reniformis* en Egipto. Nematropica 48:59-67.

Se usaron métodos moleculares y de bioensayo para caracterizar un aislado de *Rotylenchulus reniformis* recogido de Fowa-Kafr-El-Sheihk, Egipto, y se mantuvo durante 40 años en condiciones de invernadero. La identificación de este aislado se determinó rápidamente por PCR con cebadores específicos de especie desarrollados para amplificar el gen D2-D3 del rRNA 28S. La PCR anidada permitió la secuenciación directa sin clonar los fragmentos. Todas las secuencias de D2-D3 de los fragmentos de rRNA 28S fueron idénticas y exhibieron un 99% de similitud con un gen de ARN ribosómico 28S parcial de *R. reniformis* en GenBank. El análisis filogenético reveló las relaciones de nuestro aislado con *R. reniformis* de otros países, y tuvo una relación hermana con *R. macrosoma*. El potencial reproductivo de los aislamientos se probó usando diferentes hospederos. El aislamiento se infectó y reprodujo en caupí, ricino y algodón con diferencias entre las plantas. El caupí fue el anfitrión más preferible. Se logró una baja reproducción en algodón. Después de la penetración, 3 días fueron el tiempo mínimo para la aparición de hembras maduras en el caupí, 7 días en el ricino y 14 días en el algodón. La formación completa de masas de huevos se observó después de 7, 14 y 21 días en caupí, ricino y algodón, respectivamente. Se registraron hembras juveniles de la segunda generación después de 14 días con caupí y 21 días con ricino y algodón.

Palabras claves: ciclo de vida, D2-D3 del gen 28S rRNA, diagnóstico molecular, Egipto, patogenicidad, *Rotylenchulus reniformis*

INTRODUCTION

The reniform nematode, *Rotylenchulus reniformis*, is one of the most common plant-parasitic nematode species in tropical and subtropical regions (Robinson *et al*., 1997). This nematode is an obligate sedentary semi-endoparasite on the roots of plants and is considered a major pathogen of cotton and other crops in Egypt and several other countries (Ibrahim *et al*., 2010). Successful nematode management requires accurate information on the species and pathogenicity of a given nematode population causing the crop damage. Most *Rotylenchulus* species can be identified using distinctive morphological characters of immature females (Robinson *et al*., 1997). However, high intraspecific variability of some diagnostic features makes identification to species level of this group based on morphology very difficult.

Currently, DNA-based approaches have become the preferred means for identification of *Rotylenchulus* species as these methods are faster and more accurate than morphology (Agudelo *et al*., 2005; Leach *et al*., 2012; Nyaku *et al*., 2013; Van Den Berg *et al*., 2016). During the past few decades, several studies have been conducted to characterize nuclear ribosomal RNA (rRNA) genes of *R. reniformis* (Subbotin *et al*., 2006; Zhan *et al*., 2011; Palomares-Rius *et al*., 2017). Conventional and real-time PCR with species-specific primers were developed for diagnostics of *R. reniformis* using sequence differences between species in rRNA and β-tubulin genes (Showmaker *et al*., 2011; Sayler *et al*., 2012). The 18S internal transcribed spacer (ITS) and the D2 and D3 expansion segments of 28S rRNA genes have been shown to be markers for the molecular diagnostics and diversity of species of *Rotylenchulus* (Subbotin *et al*., 2007; Palomares-Rius *et al*., 2017). Based on differences in the D2-D3 of 28S rRNA gene sequences, PCR with species-specific primers was developed for rapid diagnostics of *R. reniformis* (Van Den Berg *et al*., 2016).

The life cycle of *R. reniformis* is host type, host susceptibility, temperature, and nematode population dependent. The life cycle of *R. reniformis* ranged from 14 to 29 d on different hosts (Lim and Castillo, 1978; Rodriguez, 1978; Vadhera *et al*., 2001; Rashid and Khan, 2013). *Rotylenchulus reniformis* has been documented to be quite variable in pathogenicity (McGawley and Overstreet, 1995; Nakasono, 2004; Arias et *al.,* 2009). Marked variation in reproduction among different cultivars of a single host species has been documented for a single population of *R. reniformis* (Koenning *et al*., 2000; Robbins *et al*., 2001; Robbins *et al*., 2002; Usery *et al*., 2005).

Additionally, multiple populations of *R. reniformis* document variation in reproduction and pathogenicity across a variety of crops (McGawley *et al*., 2010). Observations by Birchfield and Brister (1962) led them to postulate the existence of races or pathotypes of *R. reniformis*. The first definition of *R. reniformis* races was reported by Dasgupta and Seshadri (1971a), who designated race A and race B based on differential reproduction patterns on cotton, castor, or cowpea. Vadhera and Shukla (1999) identified another population of *R. reniformis* that failed to reproduce on castor and considered it as another race. Rao and Ganguly (1996) reported the existence of four physiological races of the reniform nematode that differed in their ability to reproduce on cotton, castor, cowpea, bajra, and mustard. McGawley *et al*. (2010) found, among 6 isolates of *R. reniformis,* that there were differences in their reproduction and pathogenicity on cotton and soybean.

In the Nile Delta region of Egypt, 13 populations of *Rotylenchulus* spp. were collected in nematological surveys undertaken during the 1970s on several crops, including cotton, sugar beet, wheat, rice, soybean, and ornamental plants such as jasmine. These populations were divided into two different pathotypes based on their physiological reaction on 13 soybean cultivars (Farahat, 1979). Of them, only one population that showed highest reproduction rate was able to survive so far under greenhouse conditions. This population was recovered from severely damaged roots of jasmine plants grown in fields located at Kafr-El-Sheikh Governorate, one of the most important agricultural areas for cotton cultivation. Therefore, the objective of this research was to characterize a population of *Rotylenchulus* spp. maintained on pigeon pea (*Cajanus cajan*) under greenhouse conditions since 1977 i) molecularly and ii) biologically.

MATERIALS AND METHODS

Nematode source

The reniform nematode used in this study was obtained from a pure culture reared on pigeon pea (*C. cajan*) under greenhouse conditions at the Zoology and Agricultural Nematology Department, Faculty of Agriculture, Cairo University. The initial inoculum was taken from

naturally infected jasmine plants (*Jasminum grandiflorum*) collected in 1977 from Fowa region in Kafr-El-Sheikh Governorate, Egypt. The pure culture was established from a single egg mass, and morphological characters of the corresponding female suggested similarity to *R. reniformis* (Robinson *et al*., 1997). For culture propagation, single egg-masses picked from the females were individually inoculated onto pigeon pea plants grown in 25-cm-diam. clay pots filled with sterilized loamy sand soil. To maintain the stock culture, the plants were watered, fertilized, and renewed as needed.

Molecular characterization
DNA extraction

Nematode DNA was extracted from individual juvenile (IJ), female (IF) and several juveniles (SJ) and females (SF) using the procedure developed by Holterman *et al*. (2006). Nematodes were transferred to a 1.5-ml Eppendorf tube containing 25-µl of sterile water. An equal volume of lysis buffer containing 0.2-M NaCl, 0.2 M Tris–HCl (pH 8.0), 1% (v/v) β-mercaptoethanol, and 800 mg/ml proteinase K was added. Lysis took place in a Thermomixer at 65°C at 750 rpm for 2 hr, followed by 5 min incubation at 100°C. Lysates were used immediately as template DNA for PCR or stored at -20°C.

PCR and sequencing were completed in Colors Medical Laboratory, Egypt. For rapid identification of *R. reniformis*, PCR with species-specific primers developed for the D2–D3 of 28S rRNA gene was used. The forward primer D2A (5′ACA AGT ACC GTG AGG GAA AGTT 3ˈ) and reverse primer R_renif_R2A (5ˈCCC GAT ACC ATT TCC ATA CAA G3ˈ) as designed by Van Den Berg *et al*. (2016) were used for amplification of the fragment of the 28S rRNA gene. The PCR mixture was prepared as described by Maafi *et al*. (2003). Shortly thereafter, PCR was conducted in a 0.2-ml Eppendorf PCR tube containing 25-µl total volume of 3 µl of template DNA, 12.5 µl Taq PCR Master Mix, 1.5 µl of each primer (10 µM), and double-distilled water to the final volume. The PCR amplification profile consisted of 4 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C, followed by a final step of 10 min at 72°C. A 5-µL aliquot of PCR product was electrophoresed on a 1.5% agarose gel and visualized using ultraviolet light (E-Gel® Imager Camera Hood, life technology, USA) after staining with 0.5 µg/mL ethidium bromide.

To sequence directly from the PCR products, 5 µl of the primary PCR products were used as template in a second PCR with identical amounts of the reaction mixture mentioned above. The nested PCR products were purified by using the QIAquick PCR purification kit (Qiagen, Clinilab, Egypt). PCR products were analyzed on an ABI 373 automated DNA sequencer and sequenced in both directions using the same primers. The obtained sequences were compared with nucleotide sequences in the NCBI GenBank using the Basic Local Alignment Search Tool (BLAST) Software (http://blast.ncbi.nlm.nih.gov) to determine similarities. The obtained sequences were submitted to the GenBank database under accession number MG189905.1 (Adam *et al*., 2017). These sequences were aligned using Mega 5.1 program (Tamura *et al*., 2011) with the D2-D3 region of 28S rRNA gene sequences of several *R. reniformis* obtained from GenBank for phylogenetic analysis.

Life cycle and reproduction

Cowpea, castor, and cotton were used as differential hosts to determine *R. reniformis* reproductive potential. Post-infection development of *R. reniformis* was studied on cowpea cv. ‘Kareem7’, cotton cv. ‘Giza 94’, and castor (wild variety) under greenhouse conditions at temperatures of 30±5ºC from pre-infective female to the first deposited egg.

Seeds of each plant were planted in 10-cm-diam. plastic pots filled with steam-sterilized loamy sand soil at a ratio of 1:1. After germination, pots were thinned to one healthy seedling, and 15 days after germination, each plant was inoculated with 1,000 juvenile females of *R. reniformis* by pipetting the inoculum suspension in four holes of 3-cm depth at 3-cm distance from the stem of each plant. Pots were placed on a greenhouse bench, cleaned with fresh water, at 30±5°C at ambient light. All plants were horticulturally treated the same. Developmental stages, mature females, and egg-laying females per root system were estimated at 24, 48, and 72 hr after inoculation then every week for a period of 28 days. Eight root samples of each plant species were taken for examination. Roots were submerged for 1 hr in water contained in plastic pans to remove the adhering soil, washed gently, stained with acid fuchsin (Goody, 1957), and developmental stages were counted under a binocular at 10× to 40× magnification. Twenty eight days after inoculation, a separate set of eight plants of each species was taken, and nematodes on roots and in the soil were extracted by sieving (60, 200, 325, 500 mesh) (Hooper *et al*., 2005) and counted to estimate the reproductive potential (Pf/Pi) of *R. reniformis*.

Statistical analysis

Data are presented as the means value ± standard deviation of eight replicates. Statistical analyses were performed by IBM SPSS Statistics Version 16. Data were subjected to an analysis of variance (One-way AVOVA). Differences between means were reported as significant if $P < 0.05$ using Tukey's test.

RESULTS

Molecular characterization

The rapid diagnostics of *R. reniformis* were performed by PCR with species-specific primers developed for amplifying the D2–D3 region of the 28S rRNA gene. Amplification of the D2–D3 of 28S rRNA region using primer pair was obtained from the genomic DNA extracts of individual and several nematodes (IJ, IF, SJ, and SF). All DNA samples of nematode isolate AAD 1977 gave consistent amplification with a single PCR product of 320 bp (Fig. 1). Four sequences of the D2-D3 region of 28S rRNA genes were obtained in this study. These sequences were submitted to NCBI GenBank database (Accession No. MG189905.1, Adam *et al*., 2017) and compared with those in GenBank. The four sequences exhibited 99% similarity with a partial 28S ribosomal RNA gene of *R. reniformis* in GenBank. Sequences alignment showed that the four sequences were identical to those that were obtained from GenBank (except one sequence differed only in one nucleotide) (Fig. 2). A phylogenetic tree using the D2–D3 region sequences of *Rotylenchulus* species is presented in Fig. 3. In the Neighbor joining tree, two clades were formed. The first clade was formed by sequences of *R. reniformis* isolate AAD 1977 and *R. reniformis* of USA, Spain, and China with similarities < 99%. The four sequences of our

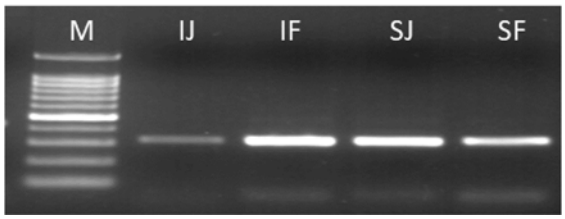

Fig. 1. PCR product (320 bp) obtained by amplification of DNA from individual juvenile (IJ), female (IF), and several juveniles (SJ) and females (SF) using species-specific primers developed for the D2–D3 region of the 28S rRNA gene (M=100 bp DNA marker (Promega)).

Table 1. Percentages of parasitic-stage durations of *Rotylenchulus reniformis* on cowpea, castor, and cotton at temperature degree of 30 ± 5ºC.

Host	Days after inoculation	Developmental stages/root	Mature female/root	Egg laying female/root
Cowpea cv. Kareem7	1	0	0	0
	2	100	0	0
	3	50	50	0
	7	7	81	12
	14	0	12	88
	21	0	2	98
	28	0	1	99
Castor wild variety	1	0	0	0
	2	0	0	0
	3	100	0	0
	7	57	43	0
	14	0	67	33
	21	0	7	93
	28	0	13	88
Cotton cv. Giza 94	1	0	0	0
	2	0	0	0
	3	100	0	0
	7	100	0	0
	14	0	100	0
	21	0	67	33
	28	0	29	71

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1
R.reniformis|IJ          CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|IF          CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|SJ          CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|SF          CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|KY992809.1 CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|KT003745.1 CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|HM131882.1 CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC
R.reniformis|KP054126.1 CGGACAGAGT CGGCGTATCT AGCCTGTATT CAACTGGAGA CCCAAGCACA TTGGGTAGGC

61
R.reniformis|IJ          CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|IF          CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|SJ          CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|SF          CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|KY992809.1 CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|KT003745.1 CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|HM131882.1 CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG
R.reniformis|KP054126.1 CTTTTCCAGA TTGGACAGGC AATCCAACTG TTTGGGAGTC TACAGTGCAT TTGCAAGTGG

121
R.reniformis|IJ          AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|IF          AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|SJ          AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|SF          AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|KY992809.1 AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|KT003745.1 AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|HM131882.1 AGTGCGCTGA GGTGGTCGGG TTAGCTGCGT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG
R.reniformis|KP054126.1 AGTGCGCTGA GGTGGTCGGG TTAGCTGCTT GATCTCGGGT TTGAGGCCAG CCCCTCGGGG

181
R.reniformis|IJ          TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|IF          TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|SJ          TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|SF          TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|KY992809.1 TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|KT003745.1 TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|HM131882.1 TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
R.reniformis|KP054126.1 TATGGTACCC GGCTCGGGGG AGTGCTGTTT AGCCTGTTCA A
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Fig. 2. Sequences alignment of fragments of the D2–D3 region of the 28S rRNA gene obtained from *Rotylenchulus reniformis* isolate AAD 1977 with others from NCBI GenBank.

isolate formed a sub-clade with 100% similarity. The second clade was formed by sequences of *R. macrosoma* of Greece with similarities < 78%.

Post-infection development

The first unswollen females in the roots were observed 48 hr after inoculation on cowpea, and 72 hr after inoculation on castor and cotton (Table 1). Further examination of the whole infected root system showed that the majority of the embedded nematodes were detected 2 to 3 days after inoculation on cowpea and after 3 to 7 days on castor and cotton. After penetration, the nematode developed rapidly on cowpea with a minimum of 3 days elapsing for the appearance of swollen females. That time was prolonged to 7 days on castor and 14 days on cotton. The maximum percentages of the completely kidney-shaped female occurred after 7 days on cowpea and after 14 days on castor and cotton. Seven days after inoculation were sufficient for the formation of egg masses on cowpea, but 14 and 21 days were needed on castor and cotton, respectively. Emergence of second-stage juveniles was observed after 14 days on cowpea and 21 days on castor and cotton. An infection of cowpea was already observed after 2 days, suggesting that development on cowpea is proceeding at a faster pace than on castor or cotton. Compared to cowpea, delayed development of *R. reniformis* on cotton has been attributed to contributing to resistance. Penetration was delayed by 1 day, development of mature females by 1 wk, and of egg-laying females by 1 additional week.

Reproductive potential

Data (Fig. 4) showed that the population of *R. reniformis* was able to penetrate, develop, and

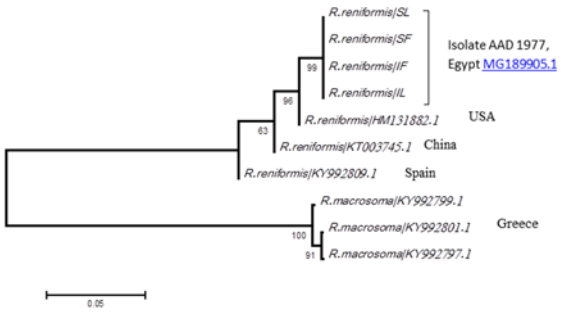

Fig. 3. Neighbor Joining tree showing the degree of relationship of the D2–D3 region of the 28S rRNA gene sequence of *Rotylenchulus reniformis* isolate AAD 1977 and other sequences of *R. reniformis* and *R. macrosoma* from different countries.

reproduce on cowpea, castor, and cotton with significant differences in reproduction among the plants. Cowpea supported the greatest numbers of nematodes in the roots, soil, and total final population resulting in the greatest population increase of the three host plants evaluated (14.4). *Rotylenchulus reniformis* developed and reproduced at a lower capacity and increased only 2.6 times on castor. In cotton, very low numbers of *R. reniformis* invaded the roots, developed, and were unable to increase to the inoculation population. Cowpea was the most favorable host followed by castor. Cotton was a less suitable host than castor. Such being the case, the nematode was able to reproduce on the three hosts.

DISCUSSION

The reniform nematode is one of the most widespread and economically damaging nematodes to most crops grown in old and reclaimed lands in Egypt (Ibrahim *et al*., 2010). PCR with specific primers developed for the D2-D3 region of the 28S rRNA gene was a useful means to rapidly identify *R. reniformis*. The identical sequences of all fragments of the D2-D3 of 28S rRNA gene proved that this population is homogenous, but genetic variations often occur in the field under environmental constraints (Grant, 1994). The nested PCR allowed us to directly sequence without cloning the fragments indicating that our procedure is less time consuming and costly. The phylogenetic analysis showed relationships of our isolate with *R. reniformis* from other countries, which formed a main cluster and had a sister relationship with *R. macrosoma*. These findings are in agreement with the published data (Van Den Berg *et al*., 2016; Palomares-Rius *et al*., 2017).

The juvenile females of *R. reniformis* were able to penetrate cowpea roots after 2-3 days and 3-7 days on castor and cotton. Seven, 14 and 21 days were sufficient for complete egg-mass formation on cowpea, castor and cotton, respectively. Many reports indicated that temperature and host susceptibility or host preference determined the nematode life cycle duration. These results are in accordance with those of Lim and Castello (1978), Farahat (1979) on soybean, Al-sayed (1983) on jasmine, Vadhera *et al*. (2001) on vegetables and Rashid and Khan (2013) on coleus. These results support that

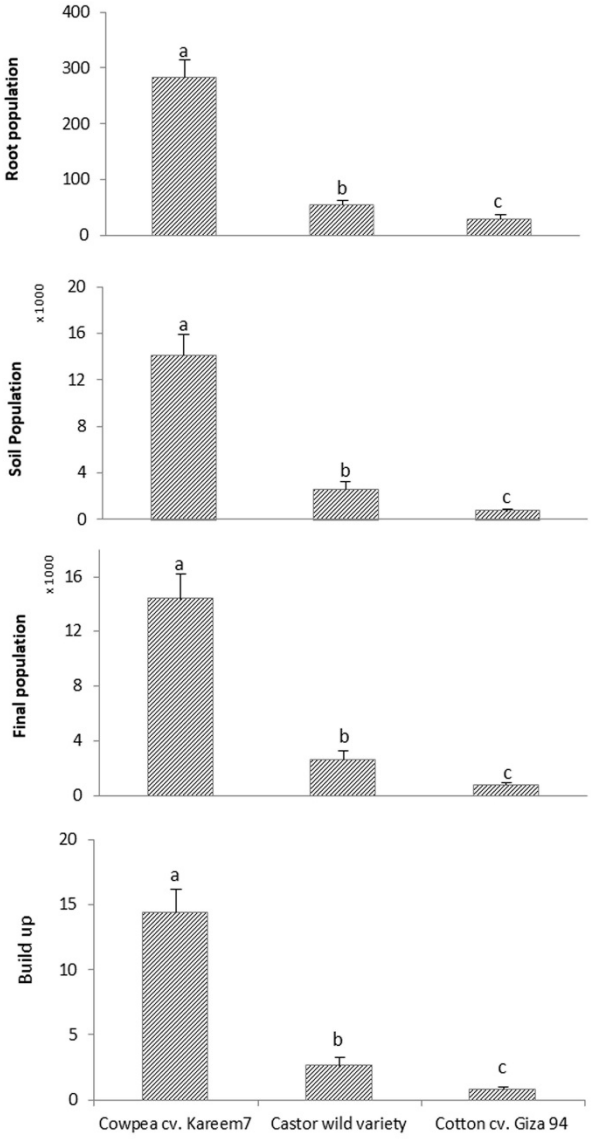

Fig. 4. Reproduction of *Rotylenchulus reniformis* infecting cowpea, castor, and cotton. Error bars represent standard deviations. Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test (n = 8).

extended warm growing seasons, in part due to climate change, will shorten nematode generation time on susceptible hosts or break resistance of plant species and cultivars (Araujo *et al*., 1982a, 1982b; Araujo *et al*., 1983; Ammati *et al*., 1986). Consequently, the number of generations per year will likely increase and virulent or aggressive nematode populations will prevail.

Cowpea, castor, and cotton have been used as differential hosts to determine the race of *R. reniformis* (Dasgupta and Seshadri, 1971a, 1971b; Rao and Ganguly, 1996; Vadhera *et al*., 1999; McGawley *et al*., 2010; Singh and Azam, 2012). Host races of *R. reniformis* may account for some of the differences in host status (Carter, 1981; Heald and Meredith 1987; Nakasono, 2004). Dasgubta and Seshadri (1971b) have proposed the existence of discrete races of *R. reniformis* A and B, but this has not been widely adopted. Race A multiplied on cowpea, castor, and cotton while race B on cowpea only. Those findings support our results that indicated that the Fowa population of *R. reniformis,* which reproduced on cowpea, castor, and cotton with significant differences, is rated as race/pathotype A. However, Vadhera *et al*. (1999) noticed a different race of *R. reniformis*, which failed to reproduce on castor. In conclusion, our study on use of PCR species-specific primers, nested PCR to directly sequence, and different plant hosts to determine the life cycle are useful, rapid, and accurate techniques for characterization of *R. reniformis*.

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