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Diversity of *Meloidogyne incognita* populations from cotton and aggressiveness to *Gossypium* spp. accessions

C. M. L. Lopes[ab]*, J. E. Cares[a], F. J. Perina[c], G. F. Nascimento[b], J. S. F. Mendonça[b], A. W. Moita[d], P. Castagnone-Sereno[e] and R. M. D. G. Carneiro[b]

[a]*Programa de Pós-Graduação em Fitopatologia, Universidade de Brasília, Brasília, DF 70910-900;* [b]*Embrapa Centro de Recursos Genéticos e Biotecnologia, Brasília, DF 70849-970;* [c]*Embrapa Algodão, Campina Grande, PB 58428-095;* [d]*Embrapa Hortaliças, Brasília, DF 70359-970, Brazil; and* [e]*INRA, INRA-UMR 1355, UNS, CNRS-UMR 7254, Institut Sophia Agrobiotech, 400 route des Chappes BP167, 06903 Sophia Antipolis, France*

The root-knot nematode (RKN) *Meloidogyne incognita* is the main nematode causing losses to the cotton crop in Brazil. In order to implement control strategies within integrated management, an accurate identification of the nematode populations prevailing in the cotton production areas is necessary. This study aimed to assess the genetic variability and aggressiveness of RKN populations from cotton production areas in Bahia state, Brazil. All populations were characterized biochemically and molecularly and identified as *M. incognita*. RAPD and AFLP markers detected 44% of polymorphic fragments among the 13 populations of this species. The 10 *M. incognita* populations collected in Bahia presented 33.7% of diversity when compared to each other, and 25% when the population from Barreiras (the most polymorphic) was excluded. This polymorphism increased when populations from other Brazilian states were included. The aggressiveness and virulence among populations from Bahia towards different cotton accessions (susceptible/resistant) was also studied. None of the populations showed virulence against the moderately resistant (Clevewilt 6, Wild Mexican Jack Jones and LA 887) and highly resistant (CIR1348 and M-315 RNR) cultivars. Two *M. incognita* populations from Barreiras were the most aggressive, reaching reproduction factors of 539 and 218, respectively, in the susceptible cultivar FiberMax 966. The most aggressive population (8) was also the most genetically divergent in phylogenetic analyses. These results demonstrate that diversity of *M. incognita* populations from cotton farms in Bahia is not related to virulence against resistant accessions, which suggests that cultivars containing one or two resistance genes with good agronomic characteristics could be used in infested commercial areas in Bahia state, Brazil.

Keywords: AFLP, *Gossypium* spp., pathogenicity, RAPD, resistance, RKN management

Introduction

Several diseases and pests affect cotton (*Gossypium* spp.) yield worldwide. In Brazil, the main nematode causing yield losses in the cotton crop is the root-knot nematode (RKN) *Meloidogyne incognita*, which stands out due to its wide distribution, survival capacity, and a wide range of host plants. The yield losses caused by this RKN species are higher in sandy soils with low fertility, and when it is found in association with the cotton wilt-causing agent, the fungus *Fusarium oxysporum* f. sp. *vasinfectum*, inducing the *Fusarium*–nematode disease complex (Wang & Roberts, 2006). The occurrence of high levels of *M. incognita* populations can make cotton cultivation unfeasible, with reports of abandonment of infested areas in São Paulo, Paraná and Goiás states (Galbieri *et al.*, 2015). Bahia is the second largest cotton-growing state in Brazil, with 94% of the production in the western region, and has suffered severe losses due to the attack of this nematode (CONAB, 2018). In Brazil, most commercial cotton cultivars are susceptible to this RKN species, and only a few are moderately resistant or resistant (Silva *et al.*, 2014).

The cotton American line Auburn 623 RNR, resistant to *M. incognita*, has been available for over 40 years (Shepherd, 1974), from which the M-315 RNR line is derived, widely used in studies as a source of high resistance to this pathogen. The high resistance observed in Auburn 623 RNR has an oligogenic inheritance that is determined by at least two genes located on chromosomes 11 and 14. This line originated from the cross between two moderately resistant accessions: Clevewilt 6 and Wild Mexican Jack Jones (PI 593649). The quantitative trait locus (QTL) qMi-C11, originating from Clevewilt 6, has an additive gene effect and is located in the CIR069–CIR316 interval on chromosome 11, whereas QTL qMi-C14, which originated from Wild Mexican Jack Jones, has an additive-dominant gene effect and is located in the BNL3545–BNL3661 interval on chromosome 14 (Shepherd, 1974; McPherson *et al.*, 2004;

*E-mail: carinallopes@hotmail.com

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Jenkins *et al.*, 2012; He *et al.*, 2014). New sources of resistance are desirable for the development of cultivars with higher levels of resistance to this RKN and less likely to be supplanted by variants of the pathogen. Recently, the accession of *Gossypium barbadense* (CIR1348) was identified as a new source of high resistance to *M. incognita* (Mota *et al.*, 2013; Silva *et al.*, 2014). In accession CIR1348 two QTLs were identified, one on chromosome 11 and another on chromosome 15, which are responsible for the high level of resistance to the nematode (Silva *et al.*, 2014; M. Giband, CIRAD, France, personal communication).

The use of resistant cultivars as a strategy in integrated management requires the correct characterization and identification of the nematode populations prevalent in the areas of cotton production, and the characterization of intraspecific genetic diversity among *M. incognita* populations is important. Within *M. incognita* species there are physiological races; however, only races 3 and 4 parasitize cotton, with race 3 found most often in commercial production areas (Inomoto, 2001). Although the occurrence of races is recognized in *M. incognita*, Moens *et al.* (2009) recommended discontinuation of this terminology, because a small variation among populations of the same species is measured and the range of hosts is very large. The analysis of the aggressiveness and virulence of the nematode populations to a given crop would be the most appropriate strategies.

Parthenogenetic *Meloidogyne* species such as *M. arenaria*, *M. incognita* and *M. javanica* have a genetic variability that allows rapid adaptation to unfavourable conditions, such as adaptation to resistant host plants (Trudgill & Blok, 2001). Reports of resistance breakdown in natural nematode populations demonstrates the ability of the pathogen to develop mechanisms of adaptation to resistance genes in the case of continuous use of the same source of resistance (Castagnone-Sereno, 2002). The selection of *M. incognita* virulent populations after successive resistant cotton plantations has occurred in California (Ogallo *et al.*, 1997) and Texas (Zhou *et al.*, 2000).

With the aim of contributing to breeding programmes for resistance of cotton to *M. incognita*, the objective of this study was to characterize the intraspecific genetic diversity and aggressiveness/virulence of *M. incognita* populations prevailing in cotton production areas in the western region of Bahia state, Brazil. The development and use of cotton cultivars resistant to this RKN species could reduce quantitative and qualitative losses of cotton fibre, in addition to representing an important management strategy in infested areas.

Materials and methods

Characterization and identification of RKN species and races

Ten populations of *M. incognita* were collected in infested cotton (*Gossypium* spp.) farms from the western part of the state of Bahia (Table 1) and multiplied in tomato (*Solanum lycopersicum* 'Santa Clara') plants. After 4 months, females were removed from the tomato roots, then identified using esterase (EST) profiles, according to the protocol described by Carneiro & Almeida (2001), and the races of *M. incognita* were determined according to Hartman & Sasser (1985).

Extraction of eggs and genomic DNA

The extraction of eggs from each population was done according to Carneiro *et al.* (2004). Total genomic DNA was extracted according to the method described by Randig *et al.* (2002), quantified and then stored at −20 °C.

Identification of the *Meloidogyne* species by SCAR markers

Confirmation of the identification and purity of the inoculum was done using the SCAR species-specific markers *M. incognita* Inck14 (Randig *et al.*, 2002) and *M. javanica* Jav (Ziljstra *et al.*, 2000). The latter was included due to the high incidence of *M. javanica* in the region, especially in areas where soybean is grown in succession to cotton.

Characterization of *M. incognita* genetic diversity by RAPD markers

The genetic diversity of Brazilian *M. incognita* populations was compared using populations from Paraná (PR) and Mato Grosso do Sul (MS) states, studied by Silva *et al.* (2014), and a population of *M. enterolobii* was included as an out-group (Table 1). RAPD reactions occurred in a volume of 13 µL containing 9 ng genomic DNA under the conditions described by Randig *et al.* (2002). Forty random 10-mer oligonucleotide primers (Operon Technologies; A12, AB1, AB06, AD03, AG04, AU13, C7, C9, F06, G2, G4, G13, J20, K10, K19, L19, M20, N7, N10, P02, P1, P6, Q10, R3, R4, R7, S20, T6, U05, V2, V7, V17, W05, W6, W15, X20, Y05, Y16, Z4, Z17) were used in the analysis. The amplification products were separated by 1.5% agarose gel electrophoresis following the methodology of Randig *et al.* (2002), with reactions done in duplicate.

Table 1 *Meloidogyne incognita* populations and *M. enterolobii* (M. ent, out-group), their origin (Brazilian municipalities and states), races and esterase phenotypes (EST).

Population code	Origin	Race	EST
1	Luís Eduardo Magalhães, BA	3	I2
2	Luís Eduardo Magalhães, BA	3	I2
3	Luís Eduardo Magalhães, BA	3	I2
4	São Desiderio, BA	3	I2
5	Barreiras, BA	3	I2
6	São Desiderio, BA	3	I2
7	São Desiderio, BA	4	I2
8	Barreiras, BA	3	I2
9	Luís Eduardo Magalhães, BA	3	I2
10	Barreiras, BA	3	I2
11	Londrina, PR	3	I1
12	Umuarama, PR	3	I2
13	Dourados, MS	3	I2
M. ent	Petrolina, PE	–	VS1-S1

BA, Bahia; PR, Paraná; MS, Mato Grosso do Sul; PE, Pernambuco.

Characterization of *M. incognita* genetic diversity by AFLP markers

Approximately 1 μL of genomic DNA was digested by the restriction enzyme *Eco*RI; adaptors were attached to the ends of the fragments in a final volume of 20 μL and incubated overnight at 37 °C following the recommendations of Suazo & Hall (1999). The digestion-ligation reactions were diluted with Tris-EDTA buffer to a final volume of 200 μL and stored at −20 °C. A series of thirteen 19-mer primers (Integrated DNA Technologies) were used, consisting of *Eco*RI adapter core sequence 5′-GACTGCGTACCAATTCAGT-3′ plus the selective nucleotides AGT, ACT, ATT, GCG, CAG, TGG, CCT, ACC, GCC, CGA, CAT, CTC and CCG. The amplified fragments were separated by electrophoresis on high resolution 1.5% gel agarose-synergel (Diversified Biotech Synergel) as described by Semblat *et al.* (1998).

Phylogenetic analysis

For each marker type, the amplified fragments were recorded as present or absent, and were converted into a binary matrix. Phylogenetic reconstruction was performed using the unweighted pair group method with arithmetic mean (UPGMA), implemented in PAUP v. 4b10 (Swofford, 2002). The stability of the dendrogram nodes was tested by 1000 bootstrap replicates. The percentage of polymorphisms was calculated based on the presence of polymorphic and monomorphic bands in the binary matrices using the formula: $P = P/(P + M) \times 100$, where P = polymorphic bands and M = monomorphic bands.

Aggressiveness/virulence of *M. incognita* populations on cotton accessions

Gossypium *accessions*

The accessions of *G. hirsutum* and *G. barbadense* were obtained from Embrapa Cotton's germplasm collection (Table 2). These accessions were previously studied and shown to be moderately to highly resistant to populations of *M. incognita* races 3 and 4 (Mota *et al.*, 2013; Silva *et al.*, 2014). *Gossypium hirsutum* 'FiberMax 966' was used as a susceptible control, while *G. hirsutum* breeding line M-315 RNR was used as a resistant control.

Nematode inoculum

Six of the 10 populations collected in the state of Bahia were selected for the study of aggressiveness/virulence, based on the information of genetic variability and geographic distribution. Prior to inoculation, the populations were multiplied on tomato cv. Santa Clara for 3 months under greenhouse conditions. Eggs were extracted from infected roots using 0.5% NaOCl, according to Hussey & Barker (1973), using a blender instead of manual agitation.

Evaluation of nematode resistance in greenhouse conditions

Seven plants of each cotton accession were grown in pots (20 × 15 cm) filled with a mixture of autoclaved soil and Bioplant compost (1:1) and maintained at 25–30 °C under greenhouse conditions. Twenty-five days after seedling emergence, pots were inoculated with 5000 eggs of *M. incognita*. Plants were arranged in a randomized block design, and watered and fertilized as needed. Three months after inoculation, the root systems were rinsed under tap water and weighed, stained with phloxine B and evaluated for gall (GI) and egg mass (EMI), categorized as 1: 1–2 galls or egg masses; 2: 3–10 galls or egg masses; 3: 11–30 galls or egg masses; 4: 31–100 galls or egg masses; and 5: >100 galls or egg masses per root system (Hartman & Sasser, 1985). Eggs were extracted using the same methodology, and 1% NaOCl. The reproduction factor (RF) was calculated as RF = FP/IP, where FP = final population and IP = initial population (5000 eggs). The average RF was transformed as log $(x + 1)$, submitted to analysis of variance and the means grouped using Scott–Knot's test ($P < 0.05$).

Table 2 *Gossypium* spp. accessions.

Accession name	Species	Origin – accession number
CIR1348	*G. barbadense* race barbadense	Peru – wild accession; CIRAD CIR1348
Clevewilt 6	*G. hirsutum*	USA – obsolete cultivar with the resistance locus qMi-C11 to RKN
Wild Mexican Jack Jones	*G. hirsutum*	Mexico – wild accession, with the resistance locus qMi-C14 to RKN. NPGS PI 593649
LA 887	*G. hirsutum*	USA – obsolete cultivar with resistance to RKN
M-315 RNR	*G. hirsutum*	USA – breeding line with the resistance loci qMi-C11 and qMi-C14 to RKN
FiberMax 966	*G. hirsutum*	Australia – commercial variety susceptible to RKN

CIRAD, French Agricultural Research Centre for International Development; NPGS, National Plant Germplasm System; RKN, root knot nematodes.

Results

Characterization/identification of RKN species and races

All populations from western Bahia exhibited *M. incognita* phenotype EST I2 with two bands, a major band (Rm 1.1) and a minor band (Rm 1.2; Fig. 1a, Table 1). The specific SCAR markers of *M. incognita* (Inck14) and *M. javanica* (Jav) confirmed the identification and purity of *M. incognita* populations (Fig. 1b). The 10 *M. incognita* populations from Bahia varied in their response to resistant tobacco NC95; population 7 from São Desiderio reproduced on tobacco and was classified as belonging to race 4, whereas populations 1, 2, 3, 4, 5, 6, 8, 9 and 10 did not, and were assigned to race 3 (Table 1).

Genetic diversity

The number of reproducible amplified fragments varied from 10 to 30 per primer and their size ranged from 200 to 4000 bp; a total of 820 amplified fragments were scored for both RAPD and AFLP markers (Fig. 2a,b) and 361 were polymorphic. The cluster analysis, using RAPD and AFLP markers, revealed that 44% of polymorphic fragments were

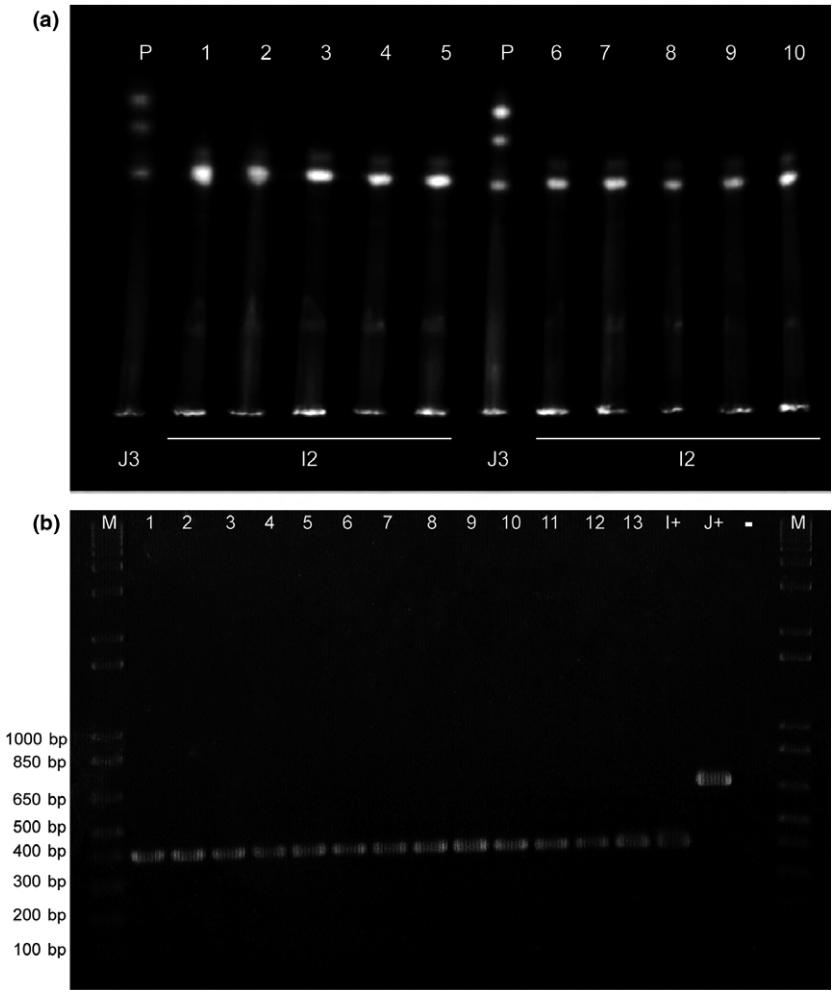

Figure 1 (a) Esterase phenotypes of *Meloidogyne incognita* (EST I2) 1–10: isolates from Bahia. P: *M. javanica* pattern (EST J3) included as reference. (b) PCR amplification patterns of *Meloidogyne* spp. generated with specific SCAR primers inc-K14-F/R (Randig *et al.*, 2002). 1–10: isolates from Bahia; 11 and 12: isolates from Paraná; 13: isolate from Mato Grosso do Sul; (I+, J+): positive controls, *M. incognita* and *M. javanica*, respectively; (−): DNA negative control; M: 1 kb Plus DNA ladder (Invitrogen); bp: base pairs.

among the 13 populations. The 10 populations of *M. incognita* from Bahia presented a diversity of 33.7% when compared to each other and 25% when population 8 from Barreiras, the most polymorphic, was excluded. This polymorphism increased when populations from other Brazilian states Paraná and Mato Grosso do Sul were included (Table 3). The dendrogram resulting from the concatenation of the RAPD and AFLP datasets is shown in Figure 3. All populations of *M. incognita* clustered together with 89% bootstrap support; however, population 8 from Bahia and 13 from Mato Grosso do Sul proved to be the most genetically distinct. The populations from Paraná (11 and 12) were the closest, with 100% bootstrap support.

Aggressiveness and virulence of *M. incognita* populations on cotton accessions

Aggressiveness and virulence were evaluated using the criteria of resistance and susceptibility: GI, EMI, number of eggs per g of roots and RF. All nematode populations showed reduced RF (<0.7) on the resistant accessions M-315 RNR and CIR1348 (with two resistance QTLs; Table 4). Gall and egg mass formation were also partially suppressed on these cotton accessions (Table 5). The other three cultivars (Wild Mexican Jack Jones, LA 887 and Clevewilt 6) with a single resistance gene (moderate resistance) were also resistant using the Hussey & Janssen (2002) concept, which predicts reproduction for the resistant accession as <10% of the susceptible accession. Considering this concept, none of the populations from Bahia was virulent to the five cotton cultivars with different resistance genes (Tables 4, 5 & 6). In contrast, the susceptible control FiberMax 966 exhibited high gall and egg mass numbers, number of eggs per g of roots and high RF for all the populations (Tables 4, 5 & 6), with two of the populations (8 and 10) standing out from the others as highly aggressive, reaching a RF of 539.3 and 218.0

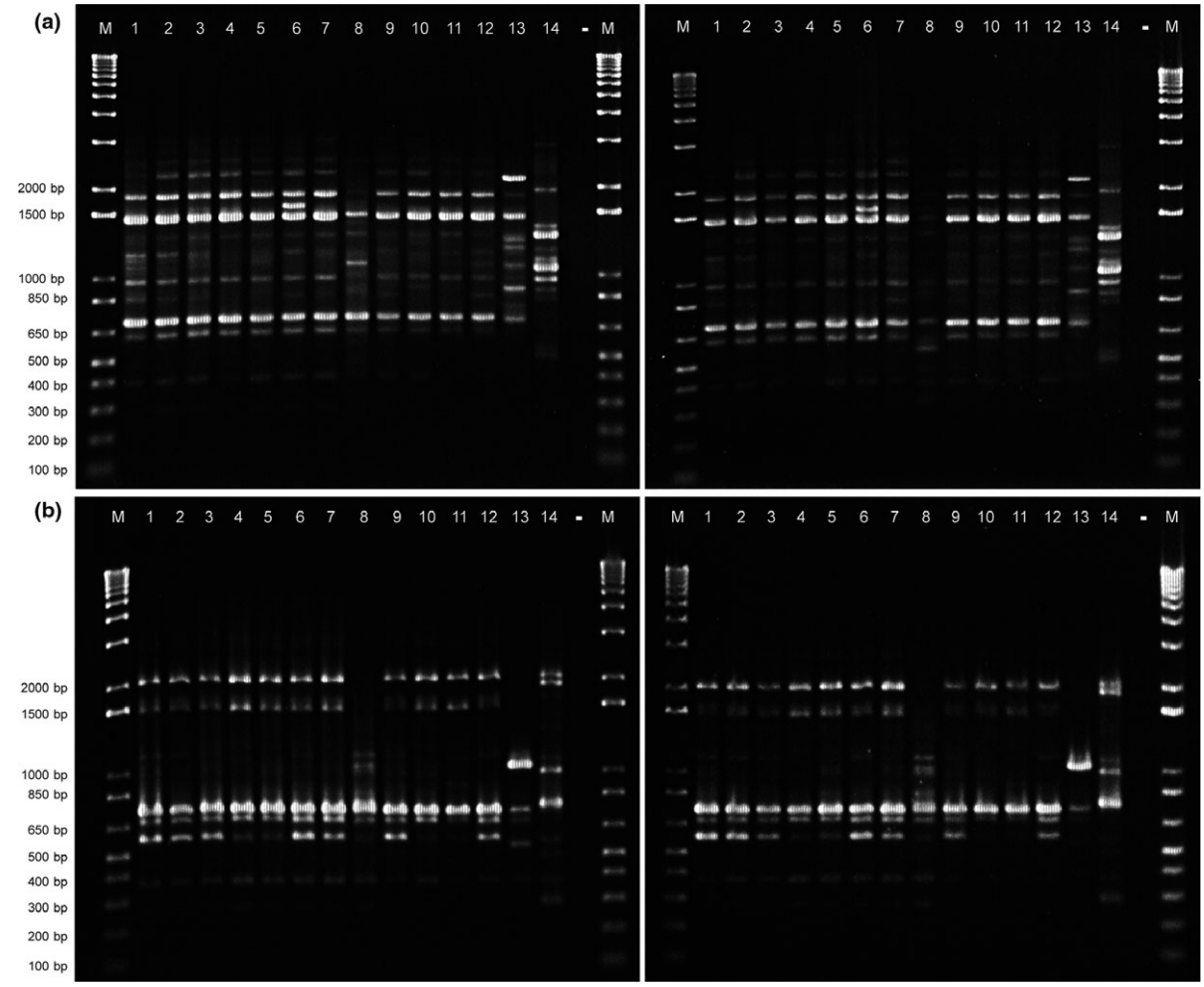

Figure 2 Genetic diversity of *Meloidogyne incognita* analysed with primers RAPD Z4 (a) and AFLP 18 (b); left and right panels show duplicates of the same RAPD/AFLP primer. 1–10: isolates from Bahia; 11 and 12: isolates from Paraná; 13: isolate from Mato Grosso do Sul; 14: *M. enterolobii* (out-group); (−): DNA negative control; M: 1 kb Plus DNA Ladder (Invitrogen); bp: base pairs.

Table 3 Percentage of polymorphisms detected in *Meloidogyne incognita*[a] at population level.

	RAPD fragments		AFLP fragments		RAPD + AFLP fragments	
M. incognita populations	Amplified	Polymorphic (%)	Amplified	Polymorphic (%)	Amplified	Polymorphic (%)
1–10; 11; 12; 13	621	289 (46.5)	199	72 (36.2)	820	361 (44.0)
1–10 (excluding 8); 11; 12; 13	603	235 (39.0)	185	33 (17.8)	788	268 (34.0)
1–10	590	217 (36.8)	182	43 (23.6)	772	260 (33.7)
1–10 (excluding 8)	583	172 (29.5)	182	22 (12.1)	765	194 (25.4)

[a]Population data in Table 1.

(means), respectively (Table 4). Comparing these results with the analysis of genetic variability (Fig. 3), the most aggressive population (8) was also the most genetically divergent for the RAPD and AFLP markers.

The correlation analysis between the evaluation parameters, performed using the Pearson coefficient (Table 7), showed a general significant positive correlation between GI, EMI, RF and eggs per g of roots; but for the resistant accessions CIR1348 and M-315 RNR there was a low correlation between GI/EMI, GI/eggs per g of roots, GI/RF, EMI/eggs per g of roots and EMI/RF. In accessions LA 887, Clevewilt 6 and Wild Mexican Jack Jones, a higher correlation was observed between EMI/RF than between GI/RF, and for all accessions RF/egg per g of roots had the highest correlation.

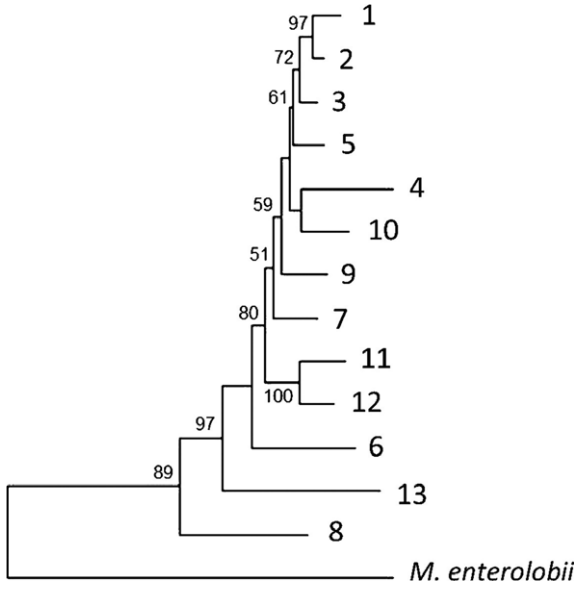

Figure 3 Phylogenetic tree of *Meloidogyne incognita* populations from Bahia (1–10); Paraná (11 and 12) and Mato Grosso do Sul (13) from RAPD and AFLP markers.

Table 4 Reproduction factor (RF) of six *Meloidogyne incognita* populations from Bahia state, Brazil, in cotton accessions with different levels of resistance.

Cotton accession[a]	Population[b]					
	1	4	6	7	8	10
FiberMax 966	45.2 a	67.9 a	84.5 a	74.8 a	539.3 a	218 a
Wild Mexican Jack Jones	1.0 b	2.7 b	4.0 b	5.0 b	2.6 b	4.1 b
LA 887	0.3 b	1.0 c	0.8 c	1.8 c	1.4 b	0.8 c
Clevewilt 6	0.3 b	0.1 d	0.1 d	0.1 d	1.3 b	7.2 b
CIR1348	0.1 b	0.0 d	0.7 c	0.1 d	0.1 c	0.7 c
M-315 RNR	0.0 b	0.1 d	0.0 d	0.0 d	0.0 c	0.0 d

Coefficient of variation (%) = 40.7.

[a]Cotton accessions described in Table 2.

[b]*Meloidogyne incognita* population data in Table 1.

Mean values (7 plants per accession) are transformed as log ($x + 1$). Means followed by different letters within columns are significantly different ($P < 0.05$) according to Scott–Knot's test.

Discussion

This study evaluated the genetic variability and aggressiveness/virulence of 10 populations of *M. incognita* from Bahia state, Brazil and three populations from Paraná and Mato Grosso do Sul states, previously studied by Silva *et al.* (2014). Six populations from Bahia state were evaluated on different cotton cultivars that harbour resistance genes to RKN. Similar results have been reported for *M. incognita* on cotton from different Brazilian states (Silva *et al.*, 2014) and for different populations from different crops (Santos *et al.*, 2012). Despite the existence of three esterase profiles for *M. incognita* (EST I1, I2 and S2) and a low genetic variability reported by Santos *et al.* (2012), only one phenotype (EST I2) was detected in all the populations, but high genetic diversity (44%) was found, mainly due to population 8, which differed significantly from the others. Removing this population from the analysis of variability, the genetic diversity was only 25%. *Meloidogyne incognita* is known to have low genetic variability due its parthenogenetic reproduction and similarities in the chromosome number (Santos *et al.*, 2012). Phylogenetically, all *M. incognita* Brazilian populations clustered together with 89% bootstrap support. In addition, the populations from Paraná remained together with 100% bootstrap support, but no other geographical relationship among populations of *M. incognita* from cotton was found. Similar results were also reported for other *M. incognita* isolates (Randig *et al.*, 2002; Carneiro *et al.*, 2004; Santos *et al.*, 2012; Silva *et al.*, 2014).

The identification of races in RKN is important not only for the characterization of resistance, but also for the development of management programmes in infested areas (Fassuliotis, 1985). The prevalence of race 3 in comparison to race 4 in cotton was reported for the first time by Silva *et al.* (2014), and the present results confirm that RKN populations from cotton, virulent to resistant tobacco NC 95 (race 4), are less frequent. Despite the existence of two races in these populations, which is important for the establishment of management strategies, Moens *et al.* (2009) recommended the discontinuation of this terminology. Indeed, this concept has never been universally accepted because it measures a very restricted portion of the potential variation in parasitic variability. In the present study, no relationship was observed among host races and genetic polymorphism or phylogeny. These findings suggest that race 4 (population 7) has low genetic polymorphism, which is in agreement with previous observations (Carneiro & Cofcewicz, 2008). Recently, it was shown that the genomes of apomictic *Meloidogyne* are made of duplicated regions, with functional divergence between gene copies, and which are rich in transposable elements, which might be responsible for their genomic plasticity and adaptation to the environment (Blanc-Mathieu *et al.*, 2017).

Population 8 was the most divergent and also highly aggressive to the susceptible cotton FiberMax 966. This correlation between aggressiveness and genetic variability was not observed in the other populations, as another aggressive population (10) did not present high genetic divergence. Previous studies have also failed to establish this correlation (Silva *et al.*, 2014; Mattos *et al.*, 2016). Aggressiveness reflects the ability of nematodes to reproduce on a susceptible host, as measured by the RF, whereas virulence is their ability to reproduce on resistant hosts (Hussey & Janssen, 2002). Therefore, in this study, no *M. incognita* populations were virulent for cotton cultivars bearing resistance genes. According to Castagnone-Sereno (2002), genomic polymorphisms are independent of virulence, and are probably the result of independent mutational events.

Table 5 Gall index (GI) and egg mass index (EMI) of six *Meloidogyne incognita* populations from Bahia state, Brazil, on selected cotton accessions.

	Population[b]											
	1		4		6		7		8		10	
Cotton accession[a]	GI[c]	EMI[c]	GI	EMI	GI	EMI	GI	EMI	GI	EMI	GI	EMI
FiberMax 966	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Wild Mexican Jack Jones	1.8	3.6	3.8	4.5	2.0	4.5	4.5	3.5	3.6	3.0	3.8	3.2
LA 887	3.3	4.7	4.0	4.2	4.8	4.4	4.4	2.8	4.7	2.7	4.0	1.4
Clevewilt 6	0.7	1.6	1.6	1.2	1.3	4.7	4.7	0.4	1.6	0.4	1.6	0.4
CIR1348	0.1	0.8	1.3	0.0	0.8	0.4	0.4	0.0	0.8	0.0	1.3	1.3
M-315 RNR	0.0	0.4	0.0	0.3	0.0	0.1	0.1	0.0	0.4	0.0	0.0	0.0

[a]Cotton accession data in Table 2.
[b]*Meloidogyne incognita* population data in Table 1.
[c]Mean values (7 plants per accession) of GI or EMI. 0: no gall or egg-mass, 1: 1–2 galls or egg-masses, 2: 3–10 galls or egg-masses, 3: 11–30 galls or egg-masses, 4: 31–100 galls or egg-masses, and 5: >100 galls or egg-masses per root system (Hartman & Sasser, 1985).

Table 6 Eggs per g of roots of six *Meloidogyne incognita* populations from Bahia state, Brazil, in cotton accessions with different levels of resistance.

	Population[b]					
Cotton accession[a]	1	4	6	7	8	10
FiberMax 966	3844.9 a	5264.0 a	7494.5 a	4985.5 a	33104.5 a	13563.4 a
Wild Mexican Jack Jones	52.7 b	156.1 b	236.4 b	270.8 b	82.1 b	203.1 c
LA 887	19.1 b	46.9 b	47.7 c	100.6 b	75.0 b	57.0 d
Clevewilt 6	12.1 b	2.8 c	4.1 d	2.9 c	35.4 b	560.8 b
CIR1348	3.9 c	0.8 c	35.7 c	6.9 c	4.8 c	38.7 d
M-315 RNR	1.5 c	2.8 c	1.1 d	0.0 d	0.7 c	0.4 e

Coefficient of variation (%) = 29.7.
[a]Cotton accession data in Table 2.
[b]*Meloidogyne incognita* population data in Table 1.
Mean values (7 plants per accession) are transformed as log ($x + 1$). Means followed by different letters within columns are significantly different ($P < 0.05$) according to Scott–Knot's test.

Table 7 Correlation coefficients between the evaluation parameters for the six cotton accessions.

Cotton accession[a]	Statistic	GI/EMI	GI/eggs per g of roots	GI/RF	EMI/eggs per g of roots	EMI/RF	RF/eggs per g of roots
LA 887	*r*[b]	0.6719	0.3768	0.3994	0.6081	0.6416	0.9693
	P[c]	<0.0001	0.0139	0.0088	<0.0001	<0.0001	<0.0001
Clevewilt 6	*r*	0.8792	0.6547	0.6795	0.7261	0.7645	0.9869
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wild Mexican Jack Jones	*r*	0.8054	0.5501	0.512	0.7428	0.7729	0.9799
	P	<0.0001	0.0002	0.0005	<0.0001	<0.0001	<0.0001
CIR1348	*r*	0.3804	0.252	0.1925	0.2276	0.0524	0.9727
	P	0.0130	0.1068	0.2220	0.3862	0.2419	<0.0001
M-315 RNR	*r*	=	0.4523	0.4504	=	=	0.8834
	P		0.0028	0.0026			<0.0001
FiberMax 966	*r*	=	=	=	=	=	0.9082
	P						<0.0001

GI, gall index; EMI, egg mass index; RF, reproduction factor; =, indicates standard deviation of 0.
[a]Cotton accession data in Table 2.
[b]Pearson coefficient |r| under H_0:rho = 0.
[c]Significance level.

All cotton accessions were resistant to the populations of *M. incognita* from Bahia, according to the concepts of Hussey & Janssen (2002), where RF below 10% in relation to the susceptible control is considered resistant. These results demonstrate a strong effect of available resistance genes against populations prevalent on cotton

farms in Bahia. The presence of one QTL associated with RKN resistance (Wild Mexican Jack Jones, Clevewilt 6 and LA 887) is sufficient to drastically reduce the nematode population, whereas the combination of two QTLs (CIR1348 and M315) leads almost to immunity, even considering highly aggressive populations (8 and 10). This information corroborates the possibility of using available elite strains possessing only one resistance QTL in the management of *M. incognita* in the west of Bahia in the short term. However, although there was a high population reduction, it is important to note that accessions with only one resistance gene allow low reproduction of the nematode. Unrestricted use of it in the long term can lead to the emergence of virulent populations. Resistance based on a few genes may impose a selection pressure on nematode populations and hasten the selection of virulent isolates, as has been observed in other crops (Janssen *et al.*, 1990). Parthenogenetic species of RKN have a highly adaptive responsiveness to the environment, and their ability to overcome resistance genes has been demonstrated (Roberts, 1995; Castagnone-Sereno, 2002).

A low correlation was found between the number of eggs per g of roots and the GI, showing that, depending on the cultivar, the values may not be correlated. This means that the assessment of cultivars regarding resistance to RKN based only on GI can lead to errors, which can be explained by the fact that in several cases the resistance response is late, allowing gall development but preventing the formation of egg masses (Mota *et al.*, 2013). The findings here reinforce the need to use combined parameters to evaluate the RKN resistance.

The high level of resistance to *M. incognita* found in the cotton breeding line M-315 RNR and in other lines derived from the same source (Auburn 634 RNR), has not been transferred to superior varieties. This resistance is inherited from two major genes, presumably one from Clevewilt 6 and the other from Wild Mexican Jack Jones (McPherson *et al.*, 2004; Starr *et al.*, 2010). Clevewilt 6 has one recessive resistance gene that confers moderate resistance to *M. incognita* (McPherson *et al.*, 2004), and it is also believed to be the source of resistance in LA 887 (Jones *et al.*, 1990). The same resistance allele is present in some of the cultivars in Brazil (P. Barroso, Embrapa Territorial Intelligence, Brazil, personal communication), pointing to the need for more efficient resistance gene combinations. All the populations tested were avirulent to M-315 RNR, and all these harbour a second gene in addition to that originating from Clevewilt-6. The resistance present in Wild Mexican Jack Jones has never been deployed in varieties cultivated in Brazil. Interestingly, this accession showed a high level of resistance to all populations, even to the most aggressive ones. The other accessions that showed high levels of resistance to all populations in this study were LA 887 and CIR1348, and they also constitute potential sources of resistance that have, to the best of the authors' knowledge, never been deployed in commercial cultivars in Brazil. In the present study, it has been shown that these sources of resistance could have a large adaptability. Further studies are underway to find out whether the resistance gene(s) and allele(s) in LA 887 and CIR1348 are different from those present in Auburn 634 RNR and in the derived germplasm. The characterization of new *M. incognita* populations from Bahia and identification of novel sources of resistance that can be pyramided and/or rotated is an important goal towards the effective and durable management of RKN on cotton farms.

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