

# Reaction of Cotton Genotypes to Meloidogyne Incognita Race 3 and Selection Ofisolates With High Reproductive Rate in Cotton

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## Research

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## Abstract

**Background:** Among the pathogens that reduce cotton productivity in Brazil, *Meloidogyne incognita* is one of the most important as it causes severe yield losses and is widespread. The most recommended methods for control of this species are the use of resistant cultivars and crop rotation systems. In Brazil, *M. incognita* races 3 and 4 have already been reported as cotton parasites but the race 3 is the most commonly found and widely disseminated. This work aimed at selecting virulent and aggressive populations of *M. incognita* race 3 for testing sources of resistance to this nematode in cotton genotypes.

**Results:** The three isolates of *M. incognita* race 3 were important for screening of resistant cotton genotypes. The isolate from Umuarama was the most aggressive followed by Moreira Sales and Iporã. The genotypes CD 05-419, CD 05-945, CD 05-1087 and CD 05-1170 showed good performance against *M. incognita* race 3 in both greenhouse and field conditions.

**Conclusions:** The cotton genotypes CD05-945, CD05-1170, CD05-1087 and CD05-419 will be selected for future work involving tests for resistance against other important cotton pathogens. Those genotypes can also be used as source of genes for resistance to nematodes in cotton breeding programs. The search for virulent and aggressive *M. incognita* isolates was very important when screening for resistance in cotton germplasm.

## Background

The herbaceous cotton (*Gossypium hirsutum* L.) is one of the most important annual crops in Brazil, due to its excellent economic profit and market competitiveness (Dohlman et al., 2019). In the crop year 2016/2017 Brazil was ranked as the third major exporter and fifth largest cotton producer (FAO, 2019). Most of the Brazilian production is concentrated in the states of Mato Grosso do Sul, Goiás, Bahia and Minas Gerais (Silva et al. 2004).

Although cotton producing areas in Brazil have reached high productivity, cotton output has varied in recent years mainly due to the incidence of several pathogens. According to Lawrence et al., 2014, the losses caused by cotton pathogens are estimated at nearly 12%. However, in areas where root-knot nematodes are present yield losses might be similar or worse than those caused by other cotton pathogens (Blasingame and Patel, 2013).

One of the major nematode species of the cotton crop is the southern root-knot nematode *Meloidogyne incognita* (Lu et al. 2014). This nematode is highly important due to its worldwide distribution and large host-range. The main symptoms caused by this nematode in cotton plants are root galls and stunting which leads to a decrease in crop yield. The losses in cotton plantations are dependent on levels of this nematode in the soil (Starr and Page, 1990).

*M. incognita* presents four physiological races but only races 3 and 4 are known to parasitize cotton worldwide (Starr and Page, 1990) with race 3 being the most widespread in commercial areas of cotton production in Brazil (Pires et al. 2008).

The difficulties associated with effective control of *M. incognita* in commercial areas of cotton cultivation are mainly related to the extension of cultivated areas and improper crop management, this latter factor being the most significant, as farmers fail to rotate crops seeking immediate return from the land. This leads to a sharp increase in the density of the nematodes which soon become epidemic (Starr et al. 2007).

In cotton growing areas the chemical control of *M. incognita* may be performed through the use of nematicides. However, these are highly toxic and harmful to the environment, and also increase the costs of cotton production (Overstreet et al. 2014). Furthermore, prolonged use of chemical molecules can foster the selection of resistant variants in the nematode population.

For this reason, the use of cotton resistant varieties is the most desirable method for controlling *M. incognita* due to its large range of host plants which makes crop rotation a difficult task. In this work, we tested the reaction of several cotton genotypes against different populations of *M. incognita* race 3, aiming to select sources of resistance to this nematode.

## Material And Methods

## Collection, establishment and identification of *M. incognita* populations

The five populations of *Meloidogyne incognita* (EST I1, race 3) used in this study were previously collected in commercial cotton planting areas in Paraná state, Brazil and kept in glasshouse at 25 °C. *Meloidogyne incognita* populations were identified by the esterase phenotype (Esbenshade and Triantaphyllou, 1990) and physiological races were determined (Pires et al. 2008), according to Hartman and Sasser (1985). Single egg masses were extracted from cotton roots and inoculated on tomato plants cv. Rutgers for nematode reproduction. Tomato plants were grown in pots of 1.5 Kg with sterile substrate (1:1 v/v soil and sand) and kept in a greenhouse with humidity control and temperature ranging from 25 to 28 °C.

### Extraction of eggs and J2 of *M. incognita* from tomato roots

Sixty days after inoculation (dai) tomato roots were separated from the shoot, washed with tap water, sectioned into small pieces and crushed in a blender with sodium hypochlorite (NaOCl) at 0.5% for 60 seconds and under low rotation (Hussey and Barker, 1973). The suspension was passed through a set of sieves of 60 and 500 mesh. Eggs and J2 retained on the 500 mesh sieve were transferred to a beaker and quantified in a Peters' slide before inoculation.

### Selection of *M. incognita* populations for resistance tests in cotton germplasm

Five populations of *M. incognita* race 3 were tested for virulence and aggressiveness on the resistant cotton varieties IAC 24 and CD 201 and the susceptible FM966. This assay aimed at selecting the most aggressive and virulent populations of *M. incognita* for testing the cotton germplasm provided. Cotton plants were inoculated with 5,000 eggs of *M. incognita* and kept in a glasshouse at 25 - 28 °C. Cotton plants were assessed at 120 days after inoculation based on the variables gall index, total eggs and reproduction factor.

### Cotton genotypes

Twenty eight cotton genotypes, developed by the Central Cooperative of Agricultural Research (Cooedetec) in collaboration with CIRAD-France, were tested against three populations of *M. incognita* (Table 3). The IAC 24 and FM966 were used as resistant and susceptible cotton cultivars, respectively. The cultivar FMT 701, tolerant to *M. incognita*, was also included in field and greenhouse trials together with all the genotypes and the other cultivars.

### Greenhouse experiment - Inoculation of *M. incognita* race 3 on cotton genotypes

Cotton genotypes (Table 1) were kept in a greenhouse at a temperature of 27°C and 60% relative humidity. Single cotton plants were grown in plastic tubes of 7 x 18 cm containing sterile substrate composed of soil and sand in the ratio 1.5:1 and fertilized with 2 grams of N-P-K 8-20-20. Plants with two true leaves were inoculated with 3 mL of solution containing 5,000 eggs and juveniles of *M. incognita* race 3. The quantification of eggs and juveniles was determined in a Peters' slide using a light microscope. Water was sprayed on cotton plants twice a day during the experiment. The experimental design was completely randomized with 31 treatments (cotton genotypes and varieties) and ten replicates. Evaluation of greenhouse experiment Cotton genotypes were evaluated at 120 days after inoculation with *M. incognita* through the following variables: gall index, total eggs and reproduction factor (RF) (Shepherd 1979). Data obtained for eggs were transformed to log X + 1. Reproduction Factor was calculated using the equation RF=Fp/Ip, where Fp means final population and Ip means initial population. Cotton genotypes with RF values<1.0 were classified as resistant (R), Moderately resistant (MR) when 1≥RF<2, Moderately susceptible (MS) with 2≥FR<3 and susceptible (S) when RF>3. This classification followed Khan et al. (2016).

### Field experiment

Cotton genotypes were cultivated in a commercial area of 120.75 m<sup>2</sup> infested with *M. incognita* race 3 where cotton had been cultivated for three years without rotation. The area is located in the municipality of Moreira Sales, in the Northwest of Paraná State. The experimental design adopted was randomized blocks with 31 treatments (cotton genotypes and varieties) and 10 replicates. Seeds of each treatment (table 1) were sowed with plant spacing and row spacing of 50 cm. The cultivars IAC-24 and Fiber Max 966 were used as patterns of resistance and susceptibility, respectively, and arranged on the edges of the blocks. The population of *M. incognita* found in Moreira Sales was also collected and tested in the greenhouse experiments. Data were submitted to variance

analysis followed by a grouping analysis based on the Scott Knott methodology at 5%. All the analyses were performed using the Statistic Package Genes (Cruz, 2006).

#### Quantification of J2 in soil samples and physical and chemical analyses of soil

Soil samples were collected for chemical, physical and nematological analyses. Soil components revealed silt, sand and clay contents of 4%, 88% and 8%, respectively. Soil chemical contents were used for fertilizer recommendation. For quantification of J2 in soil in the experimentation area, soil samples were collected at 0-20 cm depth before seeding, 60 days after seeding and 120 days after seeding (Figure 1).

#### Extraction of nematodes

Nematodes were extracted from 100 cc of soil following Jenkins (1964) and from cotton roots according to Coolen and D'Herde (1972). Sieves of 48 and 400 mesh were used for separation of nematodes. Eggs and J2 of *M. incognita* were quantified under a light microscope using a Peters' slide.

#### Evaluation

For the field trial the assessment was conducted at 126 days after seeding. Cotton plants were uprooted and the root system rated according to the scale proposed by Khan et al. (2016) (Table 2).

## Results

#### Greenhouse Experiments

The greenhouse experiments revealed that the three populations of *M. incognita* race 3 were important in the selection of resistant cotton genotypes. In this sense, the population of Umuarama (UMU) was most virulent to 68% of the cotton genotypes, followed by Moreira Sales (MS) to 26% and Iporã (IPO) 6%.

The cultivar IAC 24 behaved as resistant against the populations of MS (mean of RF=0.67) and IPO (mean of RF=0.38) and moderately resistant against the population of UMU (mean of RF=1.75). Therefore, UMU was the only population presenting virulence to that resistant cultivar. The susceptibility of cv. Fiber Max 966 was confirmed by the high reproductive rates of the three populations of *M. incognita* race 3 (Table 3).

The RF obtained for the 31 tested genotypes considering the evaluation performed at 120 dai for the three isolates, made possible their ranking into different categories based on their reaction to *M. incognita*. Genotypes CD05-1222, CD05-419, CD05-1087, CD05-1323 and CD05-1170 were classified as R (Table 4), while the remaining was classified either as MR, or MS or S (Table 3).

The genotypes CD05-419, CD05-1087, CD05-1170, CD05-1222, CD05-1323 and CD05-945 presented RFs below 1.0 against the three populations of *M. incognita* race 3 and were classified as resistant and selected for future work. Those genotypes whose RF from any of the *M. incognita* populations was higher than 1.0 were discarded.

#### Field Experiment

The results obtained in the field showed that the evaluation by rating scale (Table 2) allowed the separation of the genotypes into 4 groups, according to the Scott Knott test at 5% probability (Table 4). According to the evaluation criterion adopted (gall index), the cotton genotypes CD05-419 (GI= 1.90), CD05-1087 (GI= 1.70), CD05-1170 (GI= 1.70) and CD05-945 (GI= 1.60) were classified as resistant due to gall indexes less than 2.0. The other genotypes were classified as moderately resistant, moderately susceptible and susceptible. The patterns of susceptibility and resistance, Fiber Max 966 and IAC-24, respectively, presented gall indexes 4.40 and 2.20 and were classified as susceptible and moderately resistant (Table 4).

Some cotton genotypes reached different status when data from the field were compared with data from greenhouse. The genotypes CD05-1222 and CD05-1323 were classified as resistant in the greenhouse assay (mean of GI= 5 with RF= 0.37) and moderately resistant in the field (mean of GI= 2.0). In this case, these genotypes were classified as moderately resistant. Despite these differences, some genotypes such as CD05-419 and CD05-1170, maintained the same performance in both conditions.

## Discussion

This work, which presents results of the screening of cotton genotypes for resistance to *M. incognita* may help direct management practices, as it may indicate appropriate cotton genotypes for growing in areas infested with the nematode *M. incognita*.

In screening assays for nematode resistance, one needs to consider the dynamics of the *M. incognita* population in the soil. This is because a below-threshold level of nematodes will mask the susceptibility of some genotypes. Therefore, the amount of J2 needs to be closely monitored during the period of experimentation. In the field experiment, the amount of J2 increased over time as shown in Figure 1, reaching levels which ensure that most of the cotton plants were indeed infected.

The five isolates of *M. incognita* race 3, representative for the Northwest region of Paraná state, Brazil, were previously tested for virulence on two resistant Brazilian cotton cultivars (CD 201 and IAC 24) and for aggressiveness on the susceptible cultivar FM 966. Based on this test, the UMU isolate was selected for its virulence to the cultivar IAC 24 and CD 201 and the other two isolates, MS and IPO, for their aggressiveness in the susceptible cultivar FM 966 (Table 2).

The cultivation of resistant plants in infested areas with root-knot nematodes reduces the population level of the nematode contributing to a good crop yield. However, prolonged use of cotton cultivars with the same resistance gene(s) contribute(s) to the increases in populations of virulent nematode (Roberts, 1995).

Differences in the virulence of *M. incognita* isolates on resistant cotton genotypes have been demonstrated by Ogallo et al. (1997), Zhou et al. (2000), Anwar and McKenry (2007) and Silva et al. (2014). Different strategies have been used for assessing resistance to *M. incognita* in cotton germplasm collections. Some authors reported the use of single *M. incognita* isolates to assess cotton genotypes (Davis and May, 2003; Carneiro et al., 2005).

The use of inoculum from virulent populations of *M. incognita* with increased reproduction on resistant cotton was reported by Ogallo et al. (1997) when testing cotton germplasm for resistance. Nevertheless, this strategy may complicate the selection of cotton genotypes expressing oligogenic resistance (Zhou et al., 2000).

The populations of *M. incognita* tested in this study were collected in commercial areas where non-resistant cotton varieties had been cultivated. The use of the three *M. incognita* isolates, previously tested for aggressiveness and virulence, was very valuable in the selection of resistant cotton genotypes.

Indeed, differences in the reaction of some cotton genotypes were found when evaluated under greenhouse and field conditions. The genotypes CD05-1222 and CD05-1323, for example, were classified as resistant in the greenhouse based on the RFs below 1.0 whereas in the field the same genotypes behaved as moderately resistant (GI=2.0). A non-specified difference in behavior has already been reported by Ogallo et al. (1997) for one genotype also tested under both these conditions.

Field trials allow a better understanding of the parasite-host-environment interaction, being dependent on the concentration and distribution of the inoculum and on climatic variations (humidity and temperature) during the period of the tests. In the case of the genotypes CD05-1222 and CD05-1323, it is more likely to be due to a case of environmental interaction since there was satisfactory inoculum in the field, as shown in Figure 1, with an increase of *M. incognita* population during cotton cultivation. Moreover, the MS isolate tested in the greenhouse experiments was collected in the same area where the field experiment was run and showed good performance when inoculated onto the standard cotton cultivars for resistance and susceptibility (Table 1).

## Conclusions

The results obtained in this work allowed the selection of four cotton genotypes (CD05-945, CD05-1170, CD05-1087 and CD05-419), through greenhouse and field experiments, for which *M. incognita* presented a low reproductive rate. Additionally, the selection for virulence and aggressiveness in *M. incognita* isolates, before performing germplasm screening for resistance, was very important and helpful.

## Declarations

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**Authors' contributions:** Cleber Furlanetto: planning, supervision and statistical data analysis. Also responsible for writing this manuscript. Ely Pires: master student and main executor of this research, having also assisted with the statistical analysis; Vanessa A. Antes, Gisele P. Domiciano, Caio Felipe de Barros Souza and Edriana Araújo de Lima: contributed equally to the development of this research, having provided critical feedback for this manuscript.

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**Competing interests:** The authors declare that no competing interests exist.

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## Tables

**Table 1.** Virulence and aggressiveness of *Meloidogyne incognita* race 3 populations on cotton varieties for germplasm assessment

Varieties	Populations of <i>Meloidogyne incognita</i>														
	MS			IPO			UMU			PLV			SLU		
	GI	Eggs	RF	GI	Eggs	RF	GI	Eggs	RF	GI	Eggs	RF	GI	Eggs	RF
Fiber Max 966 <sup>a</sup>	5	25052	5	4,5	20008	4	5	25000	5	3.6	6500	1.3	4.2	11022	2.2
IAC-24 <sup>b</sup>	2,3	3750	0,75	3,2	2750	0,55	5	10022	2	2.6	3612	1.2	3.1	1900	0.38
CD 2013 <sup>c</sup>	2,5	5102	1	3,4	4600	0,92	5	7505	1,5	2.8	5050	1	3	4000	0.8

GI = Gall Index (TAYLOR&SASSER, 1978) - 0 = No galls or egg masses ; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 1-100 e5>100;PLV = population from Pérola/PR; IPO = population from Iporã/PR; UMU = population from Umuarama/PR; MS = population from Moreira Sales/PR; SLU = population from Santa Lúcia/PR; Mean values represent five plants/treatment;a= susceptible cultivar; b and c= Resistant cultivars.

Table2. Gall index scale used to assessing cotton genotypes against *M. incognita* in the field experiment (Khan et al.,2016).

Notes	Galls	Ranking*
1	0 – 3	R
2	4 – 10	MR
3	11 – 30	MS
4	31 – 100	S
5	+ de 100	HS

\*R= resistant; MR= moderately resistant; MS= moderately susceptible; S= susceptible; HS=highly susceptible.

**Table 3.** Mean of galls and Reproduction Factor (RF) of *Meloidogyne incognita* race 3 on cotton genotypes in greenhouse experiments.

Plant genotypes*	Nematode populations						Nematode populations						
	MS		IPO		UMU		Plant Genotypes*	MS		IPO		UMU	
	Galls	RF	Galls	RF	Galls	RF		Galls	RF	Galls	RF	Galls	RF
FMT 701	197.5	0.45	454	0.18	344.5	2.08	CD05-206	370	2.53	250	1.45	496	2.51
CD 406	384.5	2.16	557.5	1.55	239.5	2.68	CD05-243	669.5	5.01	387	1.94	428.5	4.72
CD 408	568.5	2.04	598	1.15	487.5	6.02	CD05-419	243	0.35	218	0.55	118.5	0.54
CD 409	300	1.05	228.5	1.17	439	3.64	CD05-485	179	1.2	200	0.36	156	0.4
CD 410	183	1.6	511	1.1	526.5	4.24	CD05-700	487.5	2.32	408.5	0.71	240.5	0.62
CD02-621	281.5	1.1	432	1.05	328	1.27	CD05-865	513	2.18	241.5	1.94	260.5	1.94
CD02-1637	184.5	1.41	227.5	1.29	353	1.77	CD05-945	154.5	0.21	238	0.59	151	1.39
CD03-5198	288	2.76	219.5	5.54	393.5	4.05	CD05-1039	316	2.27	359.5	1.53	316.5	2.85
CD04-4939	318	1.36	329.5	1.46	283	6.86	CD05-1087	47.5	1.12	102	0.04	218	0.69
CD04-3361	269	2.64	269.5	2.49	394	2.71	CD05-1170	70.5	0.09	175	0.35	192	0.69
CD04-3040	120	0.79	305	0.88	247	4.07	CD05-1222	244	0.19	201	0.16	138	0.57
CD04-3278	341	1.99	223.5	0.61	259.5	3.78	CD05-1323	317.5	0.34	344.5	0.3	246	0.47
CD04-3816	320	1.07	213	0.28	372	1.72	CD04-5281	432	1.63	314	0.76	208	1.67
CD04-4721	324.5	0.79	236.5	0.64	309	4.32	CD04-2990	411	1.65	423.5	1.42	469.5	2.1
CD04-5081	202.5	0.6	326.5	1.2	185	0.74	Fiber Max 966	572	5.64	503.05	5.1	445	5.79
IAC-24	267.5	0.67	180.5	0.38	348.5	1.75							

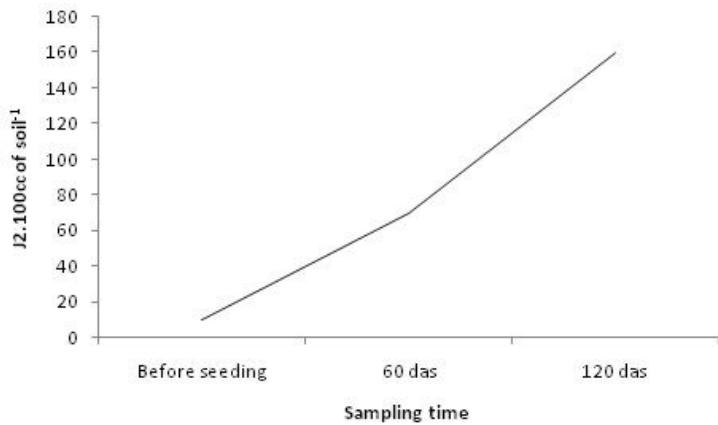
\*Data obtained from two experiments in greenhouse kept at 25-28 °C; MS=population of *M. incognita* from Moreira Sales; IPO=Population of *M. incognita* from Iporã; UMU=population of *M. incognita* from Umuarama.

**Table4.** Host status of cotton genotypes to *M. incognita* evaluated under greenhouse and field conditions.

Genotypes	Host Status*	Field Score	Host Status**	Genotypes	Host Status*	Field Score	Host Status**
CD 05-945	R	1.60 a <sup>#</sup>	R	CD 04-5281	MR	2.40 b	MR
CD 05-1087	R	1.70 a	R	CD 409	S	2.60 b	MR
CD 05-1170	R	1.70 a	R	CD 02-621	MR	2.60 b	MR
CD 05-419	R	1.90 a	R	CD 05-700	MS	2.70 b	MR
CD 05-1222	R	2.00 a	MR	CD 02-1637	MS	2.80 c	MR
CD 05-1323	R	2.00 a	MR	CD 410	S	2.90 c	MR
CD 05-485	R	2.00 a	MR	FMT 701	MR	3.00 c	MS
CD 04-3816	MR	2.10 a	MR	CD 05-1039	MS	3.10 c	MS
CD 04-4939	MR	b 2.20	MR	CD 408	S	3.20 c	MS
IAC - 24	MR	b 2.20	MR	CD 406	MS	3.60 d	MS
CD 04-3361	MR	b 2.20	MR	CD 03-5198	S	3.70 d	MS
CD 04-3278	MR	b 2.20	MR	CD 05-862	MS	3.70 d	MS
CD 04-3040	MR	b 2.30	MR	CD 05-243	S	3.80 d	MS
CD 04-4721	MR	b 2.40	MR	CD 05- 206	MS	3.90 d	MS
CD 04-5081	MR	2.40 b	MR	Fiber Max 966	S	4.40 e	S

\*Column showing host status of cotton genotypes to *M. incognita* in the greenhouse experiments; \*\*Column containing the ranking of cotton genotypes to *M. incognita* in the field experiment; # Means followed by the same letter do not differ by the Scott Knott test at 5% probability; \*\*\*Ranking adapted from Taylor and Sasser (1978).

## Figures



**Figure 1**

Dynamics of *M. incognita* population in the field experiment in Moreira Sales/PR through quantification of J2 in soil before seeding, 60 and 120 days after seeding.