

Coffea arabica cultivars with resistance to nematode *Meloidogyne exigua* originated from Costa Rica

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Abstract

The nematode *Meloidogyne exigua* causes moderate losses in almost all coffee plantations in Costa Rica. The objective of this research was to evaluate the resistance of *Coffea arabica* cultivars developed in Brazil to *M. exigua* native from Costa Rica. The trial was carried out in Heredia, Costa Rica. A randomized complete block design with five replications was used to evaluate the cultivars Caturra, Catuaí Vermelho IAC 99, IAPAR 59, IPR 98, IPR 99, IPR 100, IPR 102, IPR 103, IPR 106 and IPR 107. The plants were grown in pots and inoculated with 2500 eggs + J₂ 117 d after the transplant. The evaluation was performed 209 d after the inoculation considering the number of eggs + J₂ in 100 g of roots and the estimation of gall index (GI) of each plant. The reduction in the reproduction factor showed resistance for the cultivars IPR 99, IPR 100, IPR 102, IPR 103 and IPR 107. This represents a great possibility to face *M. exigua* of Costa Rica in a sustainable way with varieties developed in Brazil.

Keywords: Coffee, genetic breeding, root-knot nematode, IPR.

Abbreviations: Instituto Agronômico de Paraná_IAPAR; Centro de Investigaciones en Café _ICAFFE; IP_initial population; FP_final population; gall index_GI; RF_reproduction factor; J2_second stage juveniles; RRF_reduction in the reproduction factor; HS_highly susceptible; S_susceptible; MS_moderately susceptible; MR_moderately resistant; R_resistant; HR_highly resistant; TH_Timor Hybrid.

Introduction

In Costa Rica, around 84000 ha is cultivated with coffee (INEC, 2015), in 8 zones with different characteristics of climate, altitude and the soils. It is estimated that 80% of the cultivars used correspond to Caturra and Catuaí, which practically all the genetic materials are susceptible to nematodes of the *Meloidogyne* Chitwood genus. In the plantations, the presence of *Meloidogyne exigua* (Salas and Ehandi, 1961; ICAFFE, 1998), *M. arabicida* (López and Salazar, 1989; ICAFFE, 1998), *M. javanica*, *M. incognita*, *M. enterolobii* (Hernández, 2007) and *M. lopezi* (Humphreys et al., 2014) has been reported.

In almost all coffee plantations in Costa Rica, there are moderate populations of the genus *Pratylenchus*, while in some isolated places high population of *M. arabicida* and *M. exigua* has been reported throughout the country (Rojas, 2010), constituting the most important species. The distribution of *M. exigua* in coffee plantations is also wide in Brazil (Gonçalves and Silvarolla, 2007), Colombia (Leguizamón and López, 1972; Baeza et al., 1978) and Central America (Hernández, 2007).

In plantations under five yr of age in Rio de Janeiro, Brazil, the loss at the field level could reach 13%, with population levels of *M. exigua* between 10 and 15 J₂ / 100 cm³ of the soil (Barbosa et al., 2004). In Costa Rica, Bertrand et al. (1998) estimated that *M. exigua* can reduce production by up to 15%. At the greenhouse level, Rojas and Salazar (2013) reported that the pathogen could affect all the growth variables from an initial inoculum of 0.125 eggs + J₂ / cm³ of substrate.

Chemical control with nematicides has not been satisfactory (Jaehn et al., 1984; Alpizar et al., 2006; Marcolan et al., 2009) and genetic resistance seems to be the best way to control the root-knot nematodes (Villain et al., 1999; Alpizar et al., 2006; Ito et al., 2008). It has been determined that the resistance to *M. exigua* in several lines of *C. arabica* derived from the Timor Hybrid comes from *Coffea canephora* (Bertrand et al., 2001) and that it is controlled by a major gene called *Mex-1* (Noir et al., 2003).

Some of the cultivars developed by the IAPAR have shown resistance to *Meloidogyne* spp. (Salgado et al., 2005; Ito et al., 2008; Silva et al., 2010; Dias-Arieira et al., 2012; Sera et

al., 2017) and represent an important tool to face this plague in a sustainable way. The cultivars of IAPAR are derived from Sarchimor and Icatu, which are sources of resistance to *M. exigua* (Bertrand et al., 1997; Gonçalves and Pereira, 1998; Silvarolla et al., 1998; Salgado et al., 2005). Of the IAPAR cultivars, only IAPAR 59 (Salgado et al., 2005) and IPR 100 (Rezende et al., 2017) were identified as resistant to *M. exigua* populations in Brazil. So far, only IAPAR 59 was tested for resistance to *M. exigua* from Costa Rica (Bertrand et al. 1998, Anthony et al. 2005, Alpizar et al. 2006). The objective of this research was to evaluate the resistance of *Coffea arabica* cultivars developed in Brazil to *M. exigua* native from Costa Rica.

Results and discussion

Gall index

The average of gall index (GI) clearly separated four groups of cultivars (Fig. 1). In the susceptible cultivars Caturra and Catuaí Vermelho IAC 99, *M. exigua* managed to develop galls in more than 50% of the root system. In field conditions, high level of galls would quickly affect the development of the root system, the productivity and the plant's disposition to resist biotic and abiotic conditions that arise in the production system. The cultivar IPR 106 showed galls in more than 25% of roots, which will cause deterioration of the plant in the long term. Under field conditions, roots with galls tend to rot and; therefore, the root system and its capacity to absorb water and nutrients are reduced. The cultivars IPR 98, IPR 99, IPR 100, IPR 102, IPR 103, IPR 107 and IAPAR 59 standing out due to their low level of galls (less than 25%). These seven cultivars developed by IAPAR showed very good resistance to the development of galls despite the good initial inoculum and the months that these young plants were exposed to the nematode.

The GI obtained in each of the evaluated plants showed the segregation or genetic homogeneity of the cultivars for this variable (Fig 2). The susceptible cultivars Caturra and Catuaí Vermelho IAC 99 showed around 90% and 70% of the plants with GI of 4 and 5, respectively (galls in more than 50% of roots). The IPR 106 showed low resistance level to gall formation and high segregation, since it presented relatively high percentages of plants in all grades of the GI. On the other hand, cultivars IPR 99, IPR 107, IPR 102 and IPR 103 showed more than 90% of the plants with GI of 1, indicating their good uniformity and high resistance to gall formation by Costa Rican *M. exigua*.

Population density

The population density variable of the nematode in the roots showed three clearly separated groups of cultivars (Fig 3, Table 1), with the IPR 98, IPR 99, IPR 100, IPR 102, IPR 103, IPR 107 and IAPAR 59 standing out due to low population of nematodes. In the roots of these cultivars, the nematode densities were oscillated between 3600 and 17600 J_2 / 100 g of root, compared to densities between 210000 and 230800 J_2 / 100 g of root of Catuaí Vermelho IAC 99 and Caturra, respectively. The IPR 106 was presented with an average population density of 111200 J_2 / 100 g of root, showing a good relationship with the GI.

Only during the development of the nursery, populations of *M. exigua* higher than 200000 J_2 / 100 g of root can cause losses of 50% of the aerial weight of the plants (Rojas and Salazar, 2013).

Reproduction factor

In congruence with the population density, three groups were statistically separated for RF: Caturra and Catuaí Vermelho IAC 99 with 92 and 84, respectively; IPR 106 with 44 and the other cultivars with less than 7 (Table 1). The results of RF for the susceptible cultivars of this trial are similar (Carneiro et al., 2008) or higher (Salgado et al., 2005; Silva et al., 2007; Muniz et al., 2009) than those reported in other trials for susceptible cultivars, indicating optimal conditions to obtain reliable results. Anthony et al. (2005) in a similar trial reported that the nematode extracted from the same site (CICAFE) on IAPAR 59 caused hypersensitivity reaction in the radical cells affected by the nematode, abnormal nematodes, lower reproductive rate and reduction in penetration, compared to Caturra.

Cultivars IPR 98, IPR 99 and IPR 107 would have inherited the *Mex-1* gene from TH 832/2, the same as IAPAR 59, while cultivars IPR 102 and IPR 103 would have inherited the resistance of *C. canephora* from of the Icatu. The resistance of the IPR 100 might have come from the *C. liberica*. However, the source of resistance in the BA-10 and the responsible genes is unknown.

In relation to Caturra, the RRF for Catuaí Vermelho IAC 99 was 9% and for the IPR 106 it was 52%. Meanwhile, for the other cultivars it was greater than 92%. The RRF of the IAPAR 59 of this trial is the same as that reported by Salgado et al. (2005), showing the stability of this genotype.

The high reproduction rate of *M. exigua* in the susceptible cultivars grown in Costa Rica is impacting the productivity and economic development of coffee farmers. Again, the use of varieties of the IAPAR with low reproductive rate of the nematode will represent a great alternative for the country.

Resistance

Based on the RRF values (Shigueoka et al., 2017), the cultivars Caturra, Catuaí Vermelho IAC 99 and IPR 106 were classified as S to Costa Rican *M. exigua*. The IAPAR 59 and the IPR 98 were classified as R; while the rest of the cultivars evaluated (IPR 99, IPR 100, IPR 102, IPR 103 and IPR 107) were classified as HR (Table 1). The results of this test corroborate the susceptibility of Caturra and Catuaí Vermelho IAC 99 to *M. exigua*, as well as the resistance of IAPAR 59 to this nematode. The resistance of IAPAR 59 to *M. exigua* has also been verified by Alpizar et al. (2006) and Bertrand et al. (1998) in Costa Rica, by Dias et al. (2009) and Salgado et al. (2002, 2005) in Brazil.

Despite the resistance observed in IAPAR 59, the FR was > 1.0, unlike the study by Muniz et al. (2009), in which this cultivar presented FR < 1.0 for two populations of *M. exigua*. The other cultivars classified as R or HR also showed FR > 1.0. This indicates that the population of *M. exigua* of Costa Rica used in our study may be more aggressive for those cultivars with resistance than the study by Muniz et al.

Table 1. Classification of resistance levels of coffee cultivars to *M. exigua*. Heredia, Costa Rica. 2016.

Cultivar	Population density (J ₂ /100 g root) [†]	Reproduction factor [†]	Reduction in reproduction factor (%)	Classification
Caturra	230800 a	62.32 a	0.00	Susceptible
Catuaí	210000 a	84.00 a	9.01	Susceptible
IPR 106	111200 b	44.48 b	51.82	Susceptible
IPR 98	17600 c	7.04 c	92.37	Resistant
IAPAR 59	14400 c	5.76 c	93.76	Resistant
IPR 107	6800 c	2.72 c	97.05	Highly resistant
IPR 102	6000 c	2.40 c	97.40	Highly resistant
IPR 100	4200 c	1.68 c	98.18	Highly resistant
IPR 99	3600 c	1.44 c	98.44	Highly resistant
IPR 103	1800 c	1.92 c	97.92	Highly resistant

[†] Within columns, means followed by the same letter are not significantly different according to Fisher LSD test ($\alpha = 0.05$).

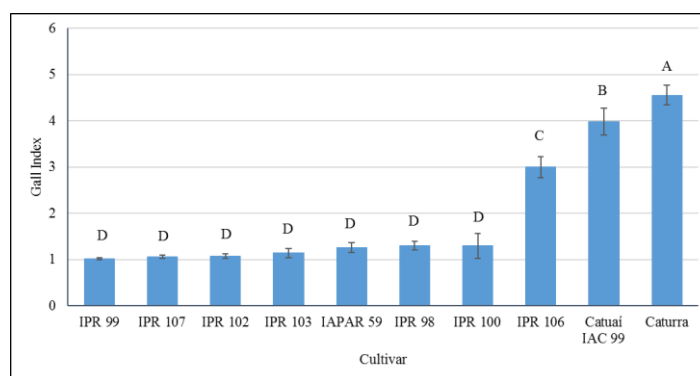


Fig 1. Gall index (GI) caused by *M. exigua* according to the cultivar evaluated. Different lowercase letters above the bar indicate significant at $P < 0.05$.

Table 2. Arabica coffee cultivars evaluated against *Meloidogyne exigua*. Heredia, Costa Rica. 2016.

Cultivar	Origin
Caturra (susceptible control)	Natural mutation of Bourbon
Catuaí Vermelho IAC 99	Caturra 476-11 x Mundo Novo IAC 374-19
IAPAR 59	Villa Sarchí 971/10 x TH 832/2
IPR 98	Villa Sarchí 971/10 x TH 832/2
IPR 99	Villa Sarchí 971/10 x TH 832/2
IPR 100	Catuaí x (Catuaí x BA 10)
IPR 102	Catuaí x Icatu
IPR 103	Catuaí x Icatu
IPR 106	Icatu
IPR 107	(IAPAR 59 x Mundo Novo IAC 376-4)

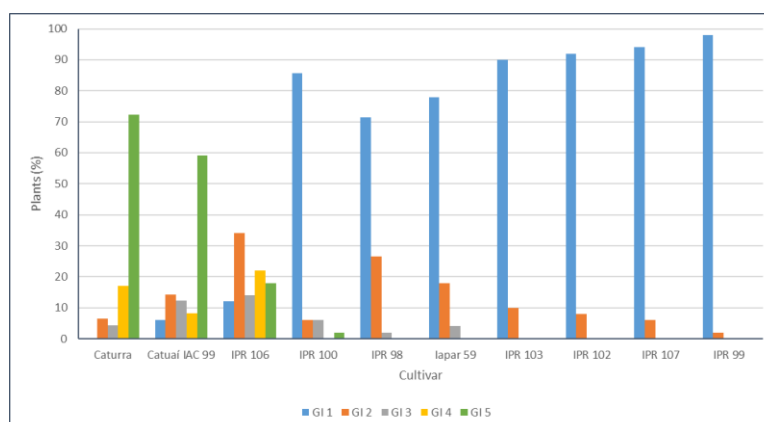
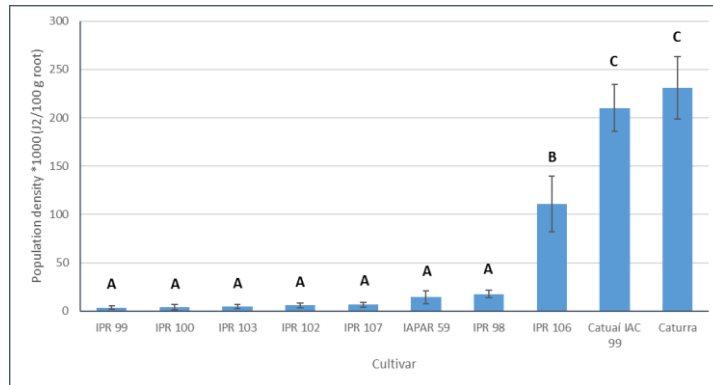


Fig 2. Percentage of plants with different Gall Index (GI) caused by *M. exigua* according to the cultivar evaluated.

Table 3. Qualitative scale used to determine the gall index.

Gall Index	Percentage of the root system with galls
1	0%
2	1-25%
3	26-50%
4	51-75%
5	76-100%

**Fig 3.** Population density of *M. exigua* according to the cultivar evaluated. Different lowercase letters above the bar indicate significant at $P < 0.05$.

(2009). According to Salgado et al. (2005), this resistance is a post-infectious type due to the presence of eggs and galls in the roots. In addition, this presence would be responsible for triggering the coffee tree defense process through the interaction between substances produced by the nematode and the plant cell from the beginning of the penetration. Muniz et al. (2009) reported that a population of *M. exigua* from Rio de Janeiro reached a reproduction factor of 165.7, surpassing the resistance conferred by the *Mex-1* gene. Another hypothesis of our study is that the qualitative resistance of IAPAR 59 and IPR 98 was broken down by this population of *M. exigua*, but the quantitative resistance is acting to decrease the nematode reproduction.

Regarding the resistance reaction of a Catimor to *M. exigua* at cellular level, Rodrigues et al. (2000) reported that the nematode may induce chromatin condensation, alteration of carbohydrate metabolism, development of lysosomes and autophagic vacuoles, causing necrosis of damaged root cells as a possible hypersensitivity response. All these led to cell death six days after infection.

The resistance of the IPR 100 was also tested in this research. This cultivar was recently reported in Brazil as resistant to *M. exigua* (Rezende et al., 2017), to *M. paranaensis* (Ito et al., 2008; Andraezi et al., 2015; Sera et al., 2017) and *M. incognita* races 1 (Kanayama et al., 2009) and 2 (Ito et al., 2008). This simultaneous resistance to several nematodes is of extreme importance, as it is common to find mixtures of *Meloidogyne* species and races in coffee crops.

The IPR 106 was reported as resistant to *M. paranaensis* and *M. incognita* race 2 (Ito et al., 2008) but susceptible to *M. exigua* from Costa Rica. This result is very important for the selection of cultivars to be grown in a region contaminated with the *Meloidogyne* species and shows genetic differences between the cultivars IPR 102 and IPR 103, despite coming from the same crossing (Catuaí x Icatu). Pereira et al. (2012) evaluated genotypes resulting from the crossing of Icatu or Catuaí with the TH or Catimor against *M. exigua* and

reported them as mostly susceptible. Therefore, it is possible that IPR 106 has resistance genes to *M. paranaensis* and *M. incognita*, but not to *M. exigua*, probably due to host-pathogen interactions. This observation had already been made by Hernández et al. (2004), Carneiro et al. (2008) and Muniz et al. (2009). The resistance found in cultivars IAPAR 59, IPR 98 and IPR 99, was probably originated from the TH 832/2, whereas from IPR 107 from the parent IAPAR 59, since Mundo Novo is susceptible. Icatu can be the source of resistance of cultivars IPR 102, IPR 103 and IPR 106, while IPR 100 can be coffee BA-10. Other studies also report that derivatives of Timor Hybrid (Bertrand et al., 1997; Gonçalves and Pereira, 1998) and Icatu (Silvarolla et al., 1998) are sources of resistance to *M. exigua*. In Brazil, the cultivars identified as resistant to *M. exigua* are IAPAR 59 (Muniz et al., 2009), IAC 125 RN, MGS Catigua 3, Catucaiam 78515, Catuaí 785-15 and Acauã (Pereira and Baião, 2015). This study demonstrated the resistance of IPR 98, IPR 99, IPR 102, IPR 103 and IPR 107 to *M. exigua* native from Costa Rica, which will be new planting options for areas infested with this nematode.

Materials and methods

Study site

The trial was established outdoor at the Centro de Investigaciones en Café (CICAFE), Heredia, Costa Rica (lat. 10°2'2.88168"N, long. 84°8'16.12284"W). The site is located at 1180 m asl and has an average annual temperature and rainfall of 21.5 °C and 2650 mm, respectively.

Plant materials

Eight Arabica coffee cultivars developed by the IAPAR were evaluated. The susceptible cultivars Caturra and Catuaí Vermelho IAC 99 were also included (Table 2).

Experimental design and conduction of study

We used a randomized complete blocks design with 10 treatments and 5 replications with 10 plants. The Caturra cultivar was the susceptible control.

The seeds of each cultivar were placed to germinate on Peat moss substrate. On Sept. 25, 2015, the seedlings in the cotyledonal state were individually transplanted in polyethylene bags with 1.3 L of substrate composed of soil (50%), organic fertilizer (25%) and rice husk (25%). The bags were placed under a double row shade mesh, with 15 cm. The plants were maintained with the necessary care of irrigation, nutrition and disease control to develop properly. The inoculum of *M. exigua* was extracted from a lot naturally infested at CICAFAE, where the species had been identified with enzymatic (Hernández, 2007), morphological and molecular (Rojas, 2010) techniques. The inoculation was carried out on Jan. 20, 2016 (117 d after transplanting) with 2500 eggs + J₂ per plant (initial population = IP), from a liquor solution of roots with galls and sifted through a set of 100 and 500 mesh superimposed screens.

Traits measured and statistical analysis

The evaluation of the final population (FP) was carried out on Aug. 17, 2016 (209 d after the inoculation), considering the number of eggs + J₂ in 100 g of roots of 10 plants per replication and the estimation of gall index (GI) of each plant (Table 3). The statistical program InfoStat (version 1.0, 2001) was used for the ANOVA and comparison of means with the Fisher LSD test ($\alpha = 0.05$), from homogeneous variances. With the population density data, the reproduction factor (RF) was calculated using the formula: $RF = FP / IP$ (Oostenbrink, 1966). To classify the resistance levels, the reduction in the reproduction factor (RRF) of *M. exigua* of each cultivar was used in relation to the susceptible control Caturra, with the formula $RRF = RF \text{ of the susceptible control} - RF \text{ of the cultivar} / RF \text{ of the susceptible control} \times 100$ (Moura and Regis, 1987). Based on the RRF values, the cultivars were classified according to the criteria of Shigueoka et al. (2017), being: < 25.00% = highly susceptible (HS); 25.00 to 49.99% = susceptible (S); 50.00 to 74.99% = moderately susceptible (MS); 75.00 to 89.99% = moderately resistant (MR); 90.00 to 94.99% = resistant (R); 95.00 to 100% = highly resistant (HR).

Conclusion

Under the conditions in which this research was developed, the cultivars IAPAR 59 and IPR 98 were resistant to *Meloidogyne exigua*, native of Costa Rica. The IPR 99, IPR 100, IPR 102, IPR 103 and IPR 107 were stood out for their high resistance to this nematode.

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