

Intermediate resistance to nematode *Meloidogyne paranaensis* in Híbrido de Timor coffee genotypes

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Abstract

The aim of this study was to prove that Híbrido de Timor (HdT) accessions are resistance sources to *Meloidogyne paranaensis*. Two experiments were carried out in a greenhouse at IDR-Paraná (Londrina, Paraná State, Brazil). Open pollinated fruits were harvested from 10 accessions (HdT UFV 380-05, HdT UFV 408-10, HdT UFV 408-11, HdT UFV 408-28, HdT UFV 428-04, HdT UFV 439-14, HdT UFV 443-08, HdT UFV 445-70, HdT UFV 446-138, HdT UFV 448-75) from the EPAMIG/ UFV germplasm bank. Seeds of these accessions were sown to obtain seedlings to test the resistance to *M. paranaensis*. For each experiment, a completely randomized design was used with 11 treatments, eight replications and one plant per plot. Cultivar Mundo Novo IAC 376-4 was the susceptible check. Seedlings with three to four pairs of leaves were transplanted into plastic cups with a capacity of 700 mL and 1200 eggs. The J_2 of *M. paranaensis* (IP) were inoculated after one month. The assessments were performed 134 days after inoculation, when the data of the number of eggs and J_2 per gram of roots and the final population of nematodes (FP) were obtained. The reproduction factor (RF) was calculated using the formula: $RF = IP / FP$. To classify the resistance levels of the genotypes, the reproduction factor reduction (RFR) was used. It classified the levels from highly resistant to highly susceptible. Different levels of intermediate resistance between the accesses were observed, highlighting HdT UFV 408-28 that presented moderate resistance to *M. paranaensis*. HdT UFV 408-28 showed a high percentage of plants with intermediate resistance, which can be used in breeding programs aimed at resistance to *M. paranaensis*.

Keywords: *Coffea arabica*, breeding, root knot nematodes, Sarchimor.

Abbreviations: HdT_Híbrido de Timor, IDR-Paraná_Instituto de Desenvolvimento Rural do Paraná – IAPAR-EMATER, IP_initial population, FP_final population, RF_reproduction factor, J2_second stage juveniles, NGR_nematodes per gram of roots, RL_resistance levels, RFR_reproduction factor reduction, HS_highly susceptible, S_susceptible, MS_moderately susceptible, MR_moderately resistant, R_resistant, HR_highly resistant.

Introduction

The Brazilian coffee farmers suffer significant economic losses due to the parasitism of the phytonematode *Meloidogyne paranaensis*, which is a very aggressive species. This disease usually causes foliar necrosis, reduced growth, leaf fall, general plant decline and in some cases the death of plant. In general, there is a reduction in the productivity of Arabica coffee in infested areas, and a limitation of the implantation of new coffee plantations in these areas (Gonçalves and Silvarolla, 2007).

M. paranaensis is widespread in coffee plantations (Carneiro et al., 2000) of Paraná and São Paulo (Carneiro et al., 2005), but is becoming a serious problem in important regions of the state of Minas Gerais (Castro et al., 2008; Salgado et al., 2015). This species was also identified by attacking coffee

trees in the states of Goiás (Silva et al., 2009) and Espírito Santo (Barros et al., 2011).

Although it is less widespread in Brazil, when compared to *M. exigua*, *M. paranaensis* is becoming a serious threat to Brazilian coffee plantations, because it is very aggressive and rapidly disseminating to several important coffee regions, such as southern Minas Gerais and Triângulo Mineiro, and there are few resistant cultivars. This nematode also affects plantations in Guatemala (Carneiro et al., 2004) and Mexico (Lopez-Lima et al., 2015).

In infested areas, integrated control measures are necessary, such as genetic, biological, chemical and cultural controls (Gonçalves and Silvarolla, 2007). Genetic management using resistant cultivars is considered the best alternative for cultivation in these infested areas, because it

is an efficient control method with no environmental damages.

The use of grafted seedlings on the resistant rootstock *Coffea canephora* cv. Apoatã IAC 2258 has high control efficiency and low cost compared to other control methods. The only *C. arabica* cultivars with high resistance to *M. paranaensis* are IPR 100 (Sera et al., 2017) and IPR 106 (Ito et al., 2008), which do not require rootstock and does not increase any costs for using resistant seedlings.

There are few sources of resistance to *M. paranaensis* identified in *Coffea* spp. when compared with resistance sources to *M. exigua*. This is one of the reasons why there are few Arabica cultivars with high resistance to *M. paranaensis*. High resistance to *M. paranaensis* was identified in *C. canephora* Pierre ex Froehner (Sera et al., 2006; Andreazi et al., 2015) and in wild *C. arabica* accessions from Ethiopia (Fatobene et al., 2017).

Arabica coffee genotypes with introgression of *C. canephora* as the Icatu derivatives has high resistance (Shigueoka et al., 2016a). Most studies in *Coffea* spp. report high levels of resistance to *M. paranaensis* (Lima et al., 2015; Shigueoka et al., 2017), but some studies have reported intermediate resistance to this nematode in Híbrido de Timor (HdT) derivatives such as Sarchimor (Muniz et al., 2009; Shigueoka et al., 2016b), which also is a *C. arabica* with introgression of *C. canephora*.

There are still no results of studies that prove that this intermediate resistance was originated from HdT. In one study we observed that the genotype HdT UFV 408-01 presented resistance to *M. paranaensis* under field conditions in the state of Minas Gerais (Salgado et al., 2014). Therefore, it is important to test HdT genotypes in a controlled environment. Thus, the aim of this study was to prove that HdT accessions are resistance sources to *Meloidogyne paranaensis*.

Results

Reproduction factor and nematodes per gram of roots

The interaction genotypes x experiments was significant for RF and NGR, and several genotypes differed between experiments (Table 1).

In experiment 1 (Exp. 1), a high RF for the susceptible check was observed, indicating high nematode reproduction in the experiment. The HdT UFV 408-28, UFV 439-14, UFV 445-70 and UFV 443-08 presented lower RF, and differed from the susceptible check Mundo Novo. In experiment 2 (Exp. 2), only the HdT UFV 408-28 genotype differed statistically from the Mundo Novo check (Table 1).

The NGR of genotypes presented similar results to those of RF in the two experiments. In Exp. 1 the genotypes HdT UFV 408-28, UFV 439-14 and UFV 445-70 differed statistically from the check for NGR. The exception was the genotype UFV 443-08 that presented RF statistically different from the check, but did not differ in NGR. In Exp. 2, only the HdT UFV 408-28 genotype differed from the susceptible check Mundo Novo for NGR variable (Table 1).

Reproduction factor reduction and resistance levels

Some variations of resistance levels were observed between the two experiments. In both experiments, HdT UFV 408-28 was classified as MR, whereas HdT UFV 439-14 and UFV 445-70 were classified as MS in Exp. 1, but they were HS in Exp. 2. HdT UFV 443-08 was classified as S in Exp. 1 and HS in Exp. 2 (Table 2).

HdT UFV 408-28 stood out in all evaluations for both RF and NGR differing statistically from the Mundo Novo, presenting the highest resistance level.

Discussion

Other studies observed that Sarchimor genotypes, which was originated from the crossing Villa Sarchí x HdT C1FC 832/2, presented partial or intermediate resistance to *M. paranaensis*. In one study, we observed that Tupi IAC 1669-33, Tupi Amarelo IAC 5111, Sarchimor IAC 4361 and Obatã IAC 1669-20, all Sarchimor derivatives, presented RF of 8.8, 13.0, 11.3 and 13.9, respectively, whereas the susceptible check Catuaí Vermelho IAC 144 was statistically different and obtained RF of 19.3 (Muniz et al., 2009). In another study, the Catuaí Vermelho IAC 99 had RF of 73.76, while progenies of the genotype IAPAR 88480-8, derived from Sarchimor IAC 1669-33, differed statistically from Catuaí and showed different levels of intermediate resistance with RFs ranging from 3.80 to 27.59 (Shigueoka et al., 2016b).

In an experiment under field conditions, intermediate resistance to *M. paranaensis* was reported in a genotype of the Híbrido de Timor named HdT UFV 408-01 (Salgado et al., 2014), which was derived from the same plant HdT UFV 408 of the access HdT UFV 408-28 of our study. In a controlled condition, it was observed that one HdT UFV 408-01 genotype was resistant and one was susceptible to *M. paranaensis* (Peres et al., 2017). Albuquerque et al. (2010) reported that HdT UFV 408-28 was susceptible to *M. paranaensis* as the susceptible check Catuaí Vermelho IAC 15. However, in our study that same access was MR. This may have happened because the HdT UFV 408-28 may had intermediate resistance in heterozygous condition. However, the individual plant seeds were different (Albuquerque et al., 2010) from those used in our study.

The mother plant of HdT UFV 408-28 in our study, originated 62.5% of plants with RF between 1.01 and 10.00 and 25% of plants with RF between 10.01 and 25.00, in both experiments. However, HdT UFV 408-28 presented 12.5% of plants with RF between 25.01 and 50.00 in Exp. 1, in addition to 12.5% of plants with RF greater than 50.00 in Exp. 2 (Table 3). Therefore, the mother plant of HdT UFV 408-28 in our study has intermediate resistance to *M. paranaensis*, but also has susceptible segregating plants. In the next self-pollination generation, it will be possible to select individual plants that may have higher percentages of plants with intermediate resistance. On the other hand, HdT UFV 408-10 and HdT UFV 408-11 have shown to be HS. Salgado et al. (2014) also observed that some accessions of HdT UFV 408 did not perform well in areas infested with *M. paranaensis*. Therefore, individual plants of these HdT UFV 408 accessions must be self-pollinated to identify homozygous resistance lines.

HdT and its derivatives are well-known sources of high resistance of the vertical or qualitative type to *M. exigua* nematode (Noir, 2003; Alpizar et al., 2007). In our study it has been proven that HdT is a source of resistance to *M. paranaensis*, which may be of a quantitative type.

According to Borém et al. (2017), horizontal or quantitative resistance is controlled by many *minor genes* (polygenic) and has moderate or partial resistance levels. The values of RF found in HdT UFV 408-28 were high, but much lower than that of the susceptible check, indicating that this genotype have quantitative resistance due to the action of minor genes.

Table 1. Reproduction factor (RF) and nematode per gram of roots (NGR) of *Meloidogyne paranaensis* in Híbrido de Timor (HdT) accessions, evaluated in two experiments (Exp. 1 and Exp. 2) in a greenhouse, Londrina, PR, Brazil.

Accessions	RF		NGR	
	Exp. 1 ⁽¹⁾	Exp. 2 ⁽¹⁾	Exp. 1 ⁽¹⁾	Exp. 2 ⁽¹⁾
HdT UFV 408-10	99.56 Aa	26.13 Ba	8166.90 Aa	3571.14 Ba
HdT UFV 446-138	85.98 Aa	35.94 Ba	7631.90 Aa	3432.36 Ba
HdT UFV 408-11	77.19 Aa	44.10 Ba	8510.12 Aa	4919.26 Ba
HdT UFV 428-04	56.77 Ab	25.99 Ba	7167.64 Aa	3175.82 Ba
Mundo Novo	56.20 Ab	33.46 Ba	5492.14 Ab	3231.86 Ba
HdT UFV 448-75	55.36 Ab	41.75 Aa	4217.96 Ab	4107.00 Aa
HdT UFV 380-05	51.01 Ab	35.98 Aa	5122.22 Ab	3175.35 Ba
HdT UFV 443-08	41.41 Bc	60.73 Aa	4178.34 Ab	3483.86 Aa
HdT UFV 445-70	27.29 Ac	37.33 Aa	2826.57 Ac	4267.58 Aa
HdT UFV 439-14	27.11 Ac	45.41 Aa	2807.90 Ac	3831.49 Aa
HdT UFV 408-28	10.09 Ad	8.20 Ab	1348.43 Ac	925.51 Ab
General mean	53.45	35.91	5224.56	3465.57
CV(%)	25.94	26.67	42.46	23.06

⁽¹⁾ Means followed by the same capital letter in the row and lowercase in the column did not differ by the Scott-Knott mean clustering test at 5%. The RF data were transformed by \sqrt{x} .

Table 2. Resistance levels (RL) and reproduction factor reduction (RFR) of *Meloidogyne paranaensis* in Híbrido de Timor accessions evaluated in two experiments (Exp. 1 and Exp. 2) in a greenhouse, Londrina, PR, Brazil.

Accessions	%RFR/ Exp. 1	RL/ Exp. 1 ⁽¹⁾	%RFR/ Exp. 2	RL/ Exp. 2 ⁽¹⁾
HT UFV 408-28	82.04	MR	75.49	MR
HT UFV 439-14	51.75	MS	-35.71	HS
HT UFV 445-70	51.44	MS	-11.57	HS
HT UFV 443-08	26.31	S	-81.50	HS
HT UFV 380-05	9.24	HS	-7.53	HS
HT UFV 448-75	1.48	HS	-24.77	HS
Mundo Novo	0.00	HS	0.00	HS
HT UFV 428-04	-1.01	HS	22.32	HS
HT UFV 408-11	-37.34	HS	-31.80	HS
HT UFV 446-138	-52.99	HS	-7.41	HS
HT UFV 408-10	-77.15	HS	21.91	HS

⁽¹⁾ HS = highly susceptible; S = susceptible; MS = moderately susceptible; MR = moderately resistant.

Table 3. Percentage of plants with different categories of reproduction factor evaluated in two experiments (Exp. 1 and Exp. 2) in a greenhouse (Londrina, PR, Brazil).

Accession	Categories of reproduction factor									
	≤ 1.00		1.01 – 10.00		10.01 – 25.00		25.01 – 50.00		> 50.00	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
HdT UFV 408-10	0%	0%	0%	0%	12.5%	50.0%	0%	50.0%	87.5%	0%
HdT UFV 446-138	0%	0%	0%	0%	0%	25.0%	12.5%	75.0%	87.5%	0%
HdT UFV 408-11	0%	0%	0%	0%	12.5%	25.0%	12.5%	25.0%	75.0%	50.0%
HdT UFV 428-04	0%	0%	0%	0%	0%	62.5%	50.0%	37.5%	50.0%	0%
Mundo Novo	0%	0%	0%	0%	0%	0%	50.0%	100.0%	50.0%	0%
HdT UFV 448-75	0%	0%	12.5%	0%	0%	12.5%	25.0%	50.0%	62.5%	37.5%
HdT UFV 380-05	0%	0%	0%	0%	12.5%	50.0%	25.0%	12.5%	62.5%	37.5%
HdT UFV 443-08	0%	0%	12.5%	0%	25.0%	0%	25.0%	50.0%	37.5%	50.0%
HdT UFV 445-70	0%	0%	0%	0%	50.0%	25.0%	50.0%	62.5%	0%	12.5%
HdT UFV 439-14	0%	0%	0%	0%	37.5%	25.0%	62.5%	37.5%	0%	37.5%
HdT UFV 408-28	0%	0%	62.5%	62.5%	25.0%	25.0%	12.5%	0%	0%	12.5%

Breeders can take advantage of this quantitative resistance of HdT UFV 408-28 and can combine with *minor genes* from coffees of other origins, and may even increase the resistance level.

According to Vale et al. (2001), plants that lack quantitative resistance are rare, and even plants considered susceptible may have minor resistance genes. It was observed that HdT

UFV 408-11, HdT UFV 446-138 and HdT UFV 408-10 presented higher and statistically different RF values than Mundo Novo (Table 1). The HdT UFV 408-10, UFV 446-138 and UFV 408-11 genotypes showed, respectively, 87.5%, 87.5% and 75% of plants with RF greater than 50 in Exp. 1, while Mundo Novo had 50% (Table 3). So this indicates that even the Mundo Novo may have minor resistance genes,

while the first three could be classified as extremely susceptible, meaning that they were more susceptible than highly susceptible check.

The horizontal resistance is non-specific to physiological races and it is more durable than the vertical resistance, since the greater amount of genes involved in the character hinders the breakdown of resistance by pathogens (Borém et al., 2017). Thus, it is possible that HdT UFV 408-28 has non-specific resistance to *M. paranaensis* races, in addition to being more durable than vertical resistance.

The HdT was originated from a spontaneous cross between the species *C. arabica* and *C. canephora*, is auto-fertile, allotetraploid ($2n = 4x = 44$ chromosomes) and possesses from *C. arabica* phenotype (Pereira et al., 2005). Most of the coffee cultivars improved for resistance to leaf rust in the world had the HdT genotypes as a source of resistance (Várzea and Marques, 2005). In addition to resistance to leaf rust, HdT coffee plants are of great importance for breeding. They also have: resistance to Coffee Berry Disease, caused by the fungus *Colletotrichum kahawae* (Van der Vossen and Walyaro, 1980; Rodrigues-Júnior et al., 2004); moderate resistance to bacterial halo blight caused by the bacteria *Pseudomonas syringae* pv. *garcae* (Mohan et al., 1978); high resistance to the nematode *Meloidogyne exigua* (Muniz et al., 2009); intermediate resistance to *M. incognita* (Albuquerque et al., 2010); specialty cup quality with chocolate, caramel, fruity and floral flavors (Sobreira et al., 2015).

Therefore, HdT accessions with intermediate resistance levels are new options to be used by breeding programs for development of new cultivars or as sources of resistance. Future cultivars with the same resistance level of HdT UFV 408-28 could be used in infested areas combined with other control methods such as biological, cultural and chemical.

Materials and methods

Plant materials

Open pollinated fruits were harvested from 10 accessions (HdT UFV 380-05, HdT UFV 408-10, HdT UFV 408-11, HdT UFV 408-28, HdT UFV 428-04, HdT UFV 439-14, HdT UFV 443-08, HdT UFV 445-70, HdT UFV 446-138, HdT UFV 448-75) from the EPAMIG/ UFV germplasm bank. These fruits produced seeds, which were sown to obtain seedlings to test the resistance to *M. paranaensis*.

It is unknown what are the self-pollination generations of these accessions. The susceptible check used was cultivar Mundo Novo IAC 376-4.

Installation and conduction of experiments

Two experiments (experiment 1 and experiment 2) were conducted in a greenhouse at the Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) in Londrina, PR, Brazil (lat 23°21'20.0"S, long 51°09'58.2" W) between October 2017 and February 2018. Two identical experiments (replicates) were evaluated to test the resistance of coffee accessions. For each experiment, the experimental design was completely randomized with 11 treatments, eight replications and one plant per plot. During the experiment period, the maximum and minimum average temperatures recorded were 32.4°C and 22.8°C, respectively.

The seedlings were obtained through sowing in germinators containing sand, being transplanted to the tubes when they reached the cotyledonary stage. The seedlings with three to four pairs of leaves were transplanted into plastic cups with

a capacity of 700 mL. The substrate was formulated containing a mixture 1:1 of soil (Clayey Latosol) and sand, previously oven sterilized at 100 °C for three hours. For each 72 liters of the soil mixture, 230 g of single superphosphate, 22 g of KCl, 24 g of urea and 72 g of dolomitic limestone were added according to the technical recommendation.

Obtainment, quantification and inoculation of nematodes

The inoculum of *M. paranaensis* was obtained from the municipality of Apucarana (Paraná State, Brazil) and registered at the Nematology Laboratory of IDR-Paraná under number 98.1. Species identification was carried out with α -esterase phenotypes (Carneiro et al., 2000), morphological approaches (Hartman and Sasser, 1985) and examination of the perineal pattern of females. To obtain the purified population, a single egg mass was multiplied in tomato cv. Santa Clara. After this multiplication, the inoculum was maintained in coffee cv. Mundo Novo IAC 376-4. For multiplication of the inoculum that was used in the experiment, about 60 days before inoculation, eggs and juveniles of second stage (J2) were extracted from coffee roots (Boneti and Ferraz, 1981) and inoculated in tomato cv. Santa Clara for inoculum multiplication. The eggs and J2 were extracted from the roots of the tomato (Boneti and Ferraz, 1981) and the suspension calibrated to 1000 eggs and J2/ mL.

In October 2017, at the two experiments and 32 days after transplanting seedlings in plastic cups, 1200 eggs and J2 of *M. paranaensis* (initial population = IP) were inoculated into three orifices approximately 1 cm deep, made with a glass rod around the the plants.

Resistance evaluation

The evaluations were carried out 134 days after the inoculation. The root systems of the plants were collected, washed in running water and weighted. Then, eggs and J2 juveniles were extracted using the Boneti and Ferraz (1981) methodology. After extraction, the final population (FP) of *M. paranaensis* was quantified by counting the number of eggs and J2 per root system using the Peters counting slide with 1 mL in optical microscope. With data of fresh root weight and nematode quantification, the number of eggs and J2 juveniles per gram of roots (NGR) was determined.

The reproduction factor (RF) was calculated using the formula: $RF = \frac{FP}{IP}$ (Oostenbrink, 1966).

Classification of resistance levels

To classify the resistance level of the genotypes, we used the reproduction factor reduction (RFR) based on the following formula (Shigueoka et al., 2017).

$RFR = \frac{(RF_{mpSusc} - RF_{mpTreat})}{RF_{mpSusc}} \times 100$, where: RF_{mpSusc} = RF mean

of the plots of the susceptible check; $RF_{mpTreat}$ = RF mean of the plots of each treatment, including of the susceptible check.

Based on RFR values, genotypes were classified according to the scale: < 25.00% = HS; 25.00 to 49.99% = S; 50.00 to 74.99% = MS; 75.00 to 89.99% = MR; 90.00 to 94.99% = R; 95.00 to 100% = HR (Shigueoka et al., 2017).

For each genotype, we calculated the RF and RFR means based on the data of the mean plots (mp). RFR values of the susceptible check was 0.00 because the RFR values of the genotypes was based on the RF values of this check (Shigueoka et al., 2017).

To verify whether the plants had homozygous or heterozygous resistance and also to check the resistance level of the lines, the percentage of plants with the following RF categories was calculated: ≤ 1.00 ; 1.01 - 10.00; 10.01 - 25.00; 25.01 - 50.00; > 50.00 .

Statistical analyses

A joint analysis of the experiments was carried out. After verification of the normality Shapiro-Wilk test and homogeneity of variances by Bartlett test, the data were submitted to the Analysis of Variance of the two experiments and the means grouped by the Scott-Knott Test at 5% significance (R Core Team, 2016). The RF data were transformed by \sqrt{x} .

Conclusion

HdT UFV 408-28 has shown to possess resistance genes to the *M. paranaensis* nematode, which can be used in crosses with plants that have other agronomic traits of interest. Because it possesses resistance genes, probably quantitative, HdT UFV 408-28 can be crossed with the objective of increasing resistance already existing in other genotypes.

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