

Research Note

Arabica coffee accessions originated from Ethiopia with resistance to nematode *Meloidogyne paranaensis*

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Abstract

The aim of this study was to evaluate the resistance of Ethiopian *Coffea arabica* from IAPAR's germplasm bank to *Meloidogyne paranaensis*. Two experiments were carried out in a greenhouse in IAPAR, Londrina, Paraná State, Brazil. A completely randomized design was used with 15 treatments, eight replications and one plant per plot. 14 accessions of *C. arabica* from Ethiopia were evaluated and the cultivar Mundo Novo IAC 376-4 was used as a susceptible control. Seedlings with three to four pairs of leaves were transplanted into plastic cups with a capacity of 700 mL and 1200 eggs and/or J₂ of *M. paranaensis* (IP) were inoculated after one month. The evaluations were performed 130 days after inoculation, when the data of the number of eggs and J₂ per gram of roots, the final population of nematodes (FP) were obtained and the reproduction factor (RF) was calculated using the formula: $RF = IP / FP$. To classify the resistance levels of the genotypes, the reduction of the reproduction factor (RRF) was used. The Ethiopian accessions were classified from highly resistant to highly susceptible. Most of the Ethiopian coffees were highly resistant to *M. paranaensis*. The genotype Geisha also showed resistance, but is still in heterozygous condition.

Keywords: breeding, *Coffea arabica*, Geisha, germplasm bank, root-knot nematodes.

Abbreviations: CATIE_Centro Agronómico Tropical de Investigación y Enseñanza, IAPAR_Instituto Agronômico do Paraná, IP_initial population, FP_final population, RF_reproduction factor, J2_second stage juveniles, NEL_number of eggs and J2, RL_resistance levels, RRF_reduction of reproduction factor, HS_highly susceptible, S_susceptible, MS_moderately susceptible, MR_moderately resistant, R_resistant, HR_highly resistant.

Introduction

The nematode *Meloidogyne paranaensis* is highly widespread in Brazil. It was found in Brazilian States such as Paraná, São Paulo (Campos and Villain, 2005), Espírito Santo (Barros et al., 2011) and Minas Gerais, which is the main coffee producer State of Brazil. This pathogen is a serious problem for the culture. It is also responsible for considerable losses in Guatemala coffee crop. This species has a large number of host plants like soybean, vegetables, weeds and others, being highly aggressive for coffee trees. It causes drastic root reduction, root thickness and disruption, low growth, defoliation and may induce plant death (Salgado and Rezende, 2010).

The nematode control is a big challenge, for which some specific strategies must be adopted to contain the dissemination, such as reduction in farm equipment transit, avoid soil and water movement and principally using healthful seedlings without contamination. Other manner strategies are genetics, chemical, biological and cultural (Gonçalves and Silvarolla, 2001). The most efficient and economically feasible way to control nematodes is by using resistant coffee cultivars. Since 1987, the resistant rootstock

'Apoatã IAC-2258' from *C. canephora*, brought the viability to coffee grow in infested areas in short term. This rootstock is resistant to *M. exigua* (Salgado et al., 2005), *M. incognita* (Sera et al., 2006) and *M. paranaensis* (Sera et al., 2006). In 2012, the IAPAR locate in Londrina, Paraná State, Brazil, released an Arabica coffee cultivar named IPR 100 resistant to *M. paranaensis* and *M. incognita* (Sera et al., 2017). In 2017, another Arabica coffee cultivar resistant to the same nematode species called IPR 106 was released (Ito et al., 2008). A few Arabica coffee cultivars is recommended to *M. paranaensis* infested areas, because of low quantity of genetic resistance sources. The resistance to *M. paranaensis* was identified in *C. canephora* (Sera et al., 2006) and in Arabica coffees with introgression of *C. canephora* genes, like the Icatu derivative genotypes (Shigueoka et al., 2016a), Híbrido de Timor (Salgado et al., 2014) and Sarchimor (Sera et al., 2009, Shigueoka et al., 2016b). *C. arabica* accessions from Ethiopia also have shown resistance to the nematode (Boisseau et al., 2009; Fatobene et al., 2017).

Table 1. Reproduction factor (RF) and number of eggs and J2 per grams of root (NEJ.g⁻¹) of *Meloidogyne paranaensis* in Arabica coffee plants from Ethiopia evaluated in two experiments (Exp1 and Exp2) in greenhouse conditions.

Genotypes	RF/ Exp1 ⁽¹⁾	RF/ Exp2 ⁽²⁾	NEJ.g ⁻¹ / Exp1 ⁽¹⁾	NEJ.g ⁻¹ / Exp2 ⁽¹⁾
E302/ CAF 83	159.74 a	86.24 a	18638 a	6155 a
Mundo Novo IAC 376-4	105.63 a	65.06 a	10694 a	5026 a
E007/ CAF 521	29.52 b	12.70 bc	3189 b	1398 bc
M7846/CAF257	3.05 bc	6.72 bc	557 bc	653 bc
E298/ CAF 382	2.01 bc	1.16 bc	165 bc	99 bc
E428/ CAF 368	1.91 bc	0.48 bc	181 bc	49 bc
E279/ CAF 618	1.76 bc	14.00 bc	152 bc	1409 bc
E546/ CAF 379	0.98 c	0.65 bc	123 bc	53 bc
E333/ CAF 201	0.96 c	0.32 c	86 c	21 c
E123/ CAF 231	0.88 c	0.16 c	73 bc	13 c
Geisha/ CAF 346	0.57 c	16.75 b	47 c	1874 ab
E464/ CAF 113	0.46 c	0.19 c	43 c	21 c
E209/ CAF 182	0.26 c	0.36 c	31 c	31 c
E131/ CAF 378	0.24 c	6.85 bc	34 c	727 bc
E228/ CAF 617	0.19 c	0.09 c	29 c	12 c
Means	20.54	14.12	2270	1169
CV	56.50%	57.92%	25.70%	36.83%

⁽¹⁾ Means within a column followed by the same letter are not significantly different according to the Tukey test at 5%. Data transformed by log (x+1).

⁽²⁾ Means within a column followed by the same letter are not significantly different according to the Tukey test at 5%. Data transformed by log $\sqrt{x+1}$.
CV, coefficient of variation.

Table 2. Resistance levels (RL) and reduction of reproduction factor (RRF) of *Meloidogyne paranaensis* in Arabica coffee plants from Ethiopia evaluated in two experiments (Exp1 and Exp2) in greenhouse conditions.

Genotypes	%RRF/ Exp1 ⁽¹⁾	RL/ Exp1	%RRF/ Exp2 ⁽¹⁾	RL/ Exp2
E302/ CAF 83	-51.22 c	HS	-32.54 b	HS
Mundo Novo IAC 376-4	0.00 b	HS	0.00 b	HS
E007/ CAF 521	72.04 a	MS	80.48 a	MR
M7846/CAF257	97.11 a	HR	89.66 a	MR
E298/ CAF 382	98.09 a	HR	98.20 a	HR
E428/ CAF 368	98.19 a	HR	99.25 a	HR
E279/ CAF 618	98.33 a	HR	78.48 a	MR
E546/ CAF 379	99.07 a	HR	98.99 a	HR
E333/ CAF 201	99.09 a	HR	99.50 a	HR
E123/ CAF 231	99.17 a	HR	99.74 a	HR
Geisha/ CAF 346	99.46 a	HR	74.25 a	MS
E464/ CAF 113	99.57 a	HR	99.70 a	HR
E209/ CAF 182	99.75 a	HR	99.85 a	HR
E131/ CAF 378	99.77 a	HR	89.46 a	MR
E228/ CAF 617	99.82 a	HR	99.85 a	HR
Means	80.55		78.30	
CV	6.79		6.06	

⁽¹⁾ Means within a column followed by the same letter are not significantly different according to the Tukey test at 5%. Data transformed by log (x+3).
CV, coefficient of variation.

C. arabica has very narrow genetic basis and most of the cultivars of this species was derived from varieties Typica and Bourbon (Anthony et al., 2001). A greater genetic diversity than this genotype is found in *C. arabica* plants from highlands of southwest of Ethiopia. These genetic resources from Ethiopia contain a great allele source to be used in genetic breeding (Silvestrini et al., 2007). Nowadays, worldwide breeding programs have used *C. arabica* accessions from Ethiopia aiming to explore the genetic variability, which presents in the coffee plants. The coffee breeding program of IAPAR has 132 *C. arabica* accessions from Ethiopia, which were not been evaluated for resistance to *M. paranaensis*.

The objective of this study was evaluate the resistance in *C. arabica* accessions from Ethiopia belonging to IAPAR's germoplasma bank to *M. paranaensis*.

Results

Reproduction factor and nematodes per gram of roots

Both two experiments showed high nematodes multiplication in the susceptible control, showing high means of RF and NEJ.g⁻¹. All the genotypes also presented lower RF and NEJ.g⁻¹, compared to Mundo Novo IAC 376-4, with the exception of E302, which did not differ. The resistant accessions were E228, E209, E464, E123, E333 and E546 with RF lower than 1.0 in both experiments. The accessions E428 and E298 showed low means of RF, but slightly higher than 1.0, at least in one experiment (Table 1).

Reduction of reproduction factor and resistance levels

Considering RRF, almost all the genotypes had statistically significant difference with higher means than Mundo Novo IAC 376-4, in exception of E302, which showed lower RRF only in Exp2, with no difference among the susceptible control. For this variable, the best accessions were E228, E209, E464, E123, E333, E546, E428 and E298 being classified as highly resistant in Exp1 and Exp2 (Table 2).

Discussion

Other studies have also shown resistance to *M. paranaensis* in *C. arabica* accessions from Ethiopia. Anthony et al. (2003) observed the accessions from Ethiopia T16733 and T16739 with respectively 100% and 87.1% of resistant plants. Boisseau et al. (2009) confirmed that the accessions Et 15, Et 25, Et 25B, Et 32B, Et 52, Et 57, Et 59, Ar 57 and Ar 59 presented RF < 1.0, indicating high resistance level to *M. paranaensis*. In another study with 71 plants from 20 Ethiopian accessions originated from the same FAO expedition (FAO, 1968), 16 accessions with resistance to *M. paranaensis* were identified (Fatobene et al., 2017). The genotypes E428 and E546 evaluated in our study also showed resistance, similar to those reported by Fatobene et al. (2017). Therefore, based on our studies, it is possible to verify high frequency of *C. arabica* from Ethiopia genotypes with resistance to *M. paranaensis*.

These accessions from Ethiopia have great importance for breeding programs because of their genetic diversity (Silvestrini et al., 2007) and variability such as resistance to nematodes *M. incognita* (Anzueto et al., 2001; Fatobene et al., 2017) and *M. exigua* (Fatobene et al., 2017), resistance to bacterial halo blight (Mohan et al., 1978), resistance to Coffee Berry Disease (Van der Vossen and Walyaro, 2009), drought tolerance (Queiroz-Voltan et al., 2014;), coffee quality (Bertrand et al., 2006), low caffeine (Silvarolla et al., 2004) and other chemical compounds (Scholz et al., 2016). Arabica coffee accessions from Ethiopia are important genetic source to *M. paranaensis*, as they have shown high resistance level, which has only been identified in *C. canephora* (Sera et al., 2006) and in *C. arabica* with introgression of genes of *C. canephora* such as Icatu derivate genotypes (Shigueoka et al., 2016a).

The genotypes E311, Geisha and E279 were HR only in Exp1, showing a heterozygous resistance. It is possible that susceptible segregating plants in those three cases are increased by the RF means. It is likely to identify homozygous HR plants, if we advance to next self-pollination generation with selection of HR individual plants. Geisha is an interesting genotype for breeding programs and it worth doing selections to generate homozygous resistant to *M. paranaensis* progenies, because it has genetic variability for resistance to bacterial halo blight (Mohan et al., 1978), drought tolerance (Queiroz-Voltan et al., 2014) and mainly exceptional coffee cup quality (Boot, 2013).

E007 presented an intermediary resistance level, being classified as MS in Exp1 and MR in Exp2 (Table 2) with respectively RF means of 29.52 and 12.70 (Table 1). That resistance can be quantitative due to minor gene effects and can be useful in breeding programs, if combined with other minor genes from coffee genotypes from different origins, aiming to increase the resistance level.

Materials and methods

Plant materials

The IAPAR's collection of *C. arabica* accessions from Ethiopia was originated from seeds coming from open pollination plants of CATIE's Germplasm Bank of Costa Rica. These plants of CATIE were originated from a FAO expedition in 1968.

The evaluation for resistance to *M. paranaensis* was made in 14 *C. arabica* accessions from Ethiopia, using open pollination seeds. As a susceptible control, the cultivar Mundo Novo IAC 376-4 were used. These accessions of IAPAR have a "CAF" initial followed by a specific numeration (Table 1).

Installation and conduction of experiments

Two experiments were carried out in a greenhouse in IAPAR, Londrina, Paraná State, Brazil (lat. 23°21'20.0"S; long. 51°09'58.2"W). The first experiment (Exp1) was done between August 03, 2017 and January 16, 2018. The second experiment (Exp2) was a replication of Exp1 aiming to confirm the resistance of the treatments. Exp2 was done between August 25, 2017 and February 15, 2018. A completely randomized design was used with 15 treatments, eight replications and one plant per plot. Temperature range in Exp1 was 11 °C and 44 °C and in Exp2 was 11 °C and 48 °C. The irrigation was done manually in two shifts, morning and afternoon.

The seedlings were obtained by sowing in sand germinators and transplanting after cotyledonary stage. Seedlings with three or four pairs of leaves were transplanted to 700 mL vessels, containing soil and sand substrate in proportion 1:1, previously sterilized in a stove with 100 °C for three hours. For each 72 liters of substrate we added 230 g of simple superphosphate, 22 g of KCl, 24 g of urea and 72 g of dolomitic limestone.

Quantification and inoculation of nematodes

M. paranaensis inoculum was obtained from the municipality of Apucarana (Paraná, Brazil) and recorded/stored in the Nematology Laboratory of IAPAR under the number 98.1. The population was identified as *M. paranaensis* through α -esterase phenotypes (Carneiro and Almeida, 2000), morphological characteristics (Hartman and Sasser, 1985), and examination of the females perineal pattern.

To obtain purified populations, one egg mass was multiplied in Santa Clara tomato cultivar. After this multiplication, the inoculum was kept in the coffee cultivar Mundo Novo IAC 376-4. For the multiplication of the inoculum that was used in the experiment, about 60 days before inoculation, eggs and J2 were extracted from the roots of coffee plants and inoculated into cv. Santa Clara.

Eggs and J2 were extracted from tomato roots using the Boneti and Ferraz (1981) method, and the suspension was calibrated to 1,000 eggs and J2/mL.

The inoculation was done 30 days after seedlings transplanting applying approximately 1,200 eggs and J2/mL close to the plant stalk. This number of nematodes was considered as the initial population (IP).

Resistance evaluation

The evaluation of Exp1 and Exp2 was done 132 and 134 days after inoculation, respectively, removing plant aerial part and washing and weigh up the root system. Then the nematodes were extracted by Boneti and Ferraz (1981) methodology. After extraction, the final population (FP) of *M. paranaensis* was quantified by counting the number of eggs and J2 (NEJ) per root system using Peters counting slide with 1 mL in optical microscope. With data of fresh root weight and NEJ, the NEJ per root grams were calculated (NEJ.g^{-1}). The nematodes reproduction factor (RF) was calculated using the formula $\text{RF} = \text{FP} / \text{IP}$ (Oostenbrink, 1966).

Resistance classification levels

To classify genotypes resistance levels (RL) the reduction of reproduction factor was used (RRF), calculated by the formula: $\text{RRF} = [(\text{RF of susceptible control} - \text{RF of treatment}) / \text{RF of susceptible control}] \times 100$ (Moura and Regis, 1987). Based on RRF the genotypes were classified according a scale: $< 25.00\%$ = 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) (Shigueoka et al., 2017). For each genotype, we calculated the mean RF and RRF based on the data of the mean plots. Since the mean values of RRF is based on the values of RF of the susceptible control, RRF values of this control were 0.00 (Shigueoka et al., 2017).

Statistical analyses

The RF data of Exp1 and NEJ.g^{-1} from Exp1 and Exp2 were transformed with $\log(x+1)$, while the RF data of Exp2 was transformed by $\sqrt{x+1}$. All the RRF data were transformed by $\log(x+3)$. All variables were submitted to the Shapiro-Wilk normality test and to the Hartley's *F*max test (R Core Team 2016). The analyses of variance and Tukey's test at 5% probability were estimated using Agricolae package (Mendiburu, 2015).

Conclusion

The Arabica coffee plants from Ethiopia E123, E209, E228, E298, E333, E428, E464 and E546 presented high level of resistance to *M. paranaensis*. They are important resistance sources to breeding programs. Geisha coffee also presented resistance; however, it is in heterozygous condition.

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