Bragantia

ISSN: 0006-8705

editor@iac.sp.gov.br

Secretaria de Agricultura e Abastecimento do Estado de São Paulo

Brasil

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Bragantia, vol. 75, núm. 2, abril-junio, 2016, pp. 193-198

Secretaria de Agricultura e Abastecimento do Estado de São Paulo

Campinas, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=90845585008

DOI: http://dx.doi.org/10.1590/1678-4499.229

Reaction of Arabica coffee progenies derivative from Icatu to *Meloidogyne paranaensis*

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ABSTRACT: The aim of this study was to evaluate the reaction of Arabica coffee progenies derived from Icatu to *Meloidogyne paranaensis*. The experiment was conducted under screenhouse conditions at Instituto Agronômico do Paraná (IAPAR) in Londrina, Paraná State, Brazil. Seedlings with three to four pairs of leaves were inoculated with 5,000 *M. paranaensis* eggs and second-stage juveniles (J2). Four F_4 progenies of HN 87609 derived from Icatu H4782-7-925 were evaluated. *C. arabica* cv. Catuaí Vermelho IAC 81 and *C. arabica* cv. IPR 100 were susceptible and resistant checks, respectively. The experiment was conducted in a randomized blocks design with 14 replications of one plant per plot. Assessments were performed 120 days after inoculation. The number of eggs and second-stage juveniles (J2) per gram of roots (Nematodes·g^{-1}) and reproduction factor (RF) were evaluated. Host susceptibility index (HSI) was used to classify the resistance levels of coffees. In relation to Nematodes·g^{-1}, IAPAR 12232 and IAPAR 12231 progenies were not significantly different from the resistant check IPR 100. All F_4 progenies of HN 87609 were classified as highly resistant by HSI and presented 100% of plants classified as highly resistant or resistant. Therefore, these progenies are homozygously resistant to *Meloidogyne paranaensis*.

Key words: *Coffea*, breeding, root-knot nematode, resistance.

INTRODUCTION

In coffee crops, nematodes are one of the main factors contributing to lower production as they parasitize the roots throughout the crop cycle (Salgado and Rezende 2010). The most important nematodes for the culture belong to the genus *Meloidogyne* Goeldi 1887, being found in the major coffee producing regions in Brazil, and cause losses in productivity, which vary with the species, population density and susceptibility of the cultivar (Salgado and Rezende 2010).

Meloidogyne paranaensis Carneiro, Carneiro, Abrantes, Santos and Almeida 1996, was identified in many samples from coffee crops grown in the State of São Paulo (Carneiro et al. 2005), in addition to coffee cultivations in the states of Goiás (Silva et al. 2009), Espírito Santo (Barros et al. 2011) and in some municipalities of Alto Parnaíba and Southern Minas Gerais (Castro et al. 2003, 2008; Castro and Campos 2004).

M. paranaensis is highly aggressive to coffee plants and is a species that limits the implementation of coffee crops in infested areas and the maintenance of those already planted (Gonçalves and Silvarolla 2007).

The control of plant-parasitic nematode is difficult to perform and the eradication is practically impossible in infested areas (Gonçalves and Silvarolla 2007). The use of resistant coffee varieties is an effective measure of control of *M. paranaensis*.

Sources of resistance to *M. paranaensis* have been found in *C. canephora* Pierre ex Froehner (Sera et al. 2006;

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Received: Jun. 1, 2015 – Accepted: Oct. 8, 2015

Gonçalves and Silvarolla 2007) and *C. arabica* L. from Ethiopia (Anthony et al. 2003; Boisseau et al. 2009).

The cultivar Apoatã IAC 2258 of *C. canephora* is resistant to *M. paranaensis* and is used as rootstock (Sera et al. 2006; Fonseca et al. 2008). IPR 100 and IPR 106 are *C. arabica* cultivars, carriers of genes of *C. liberica* Hiern. and *C. canephora*, respectively, and are also resistant to this nematode (Ito et al. 2008; Sera et al. 2007, 2009).

Arabica coffees carrying genes of *C. canephora*, such as Icatu populations, have plants resistant to *M. paranaensis*, whose progeny segregates for the trait (Mata et al. 2002; Sera et al. 2004; Gonçalves and Silvarolla 2007; Matiello et al. 2010), and have potential for use in breeding programs to transfer this resistance.

In most coffee genotypes, there is still segregation for resistance, such as the Icatu H4782-7-925 (Carneiro et al. 2013). It is possible to select progenies derived from Icatu H4782-7-925 with resistance in homozygous to *M. paranaensis*.

Therefore, this study aimed to identify Arabica coffee progenies derived from Icatu H4782-7-925 with resistance to nematode *M. paranaensis*.

MATERIAL AND METHODS

Genetic material

An open pollinated progeny (S1) was obtained from a mother plant Icatu H4782-7-925 in Astorga, State of Paraná, in 1987, which was planted in 1988 in area infested by *Meloidogyne paranaensis* in the municipality of Centenário do Sul, State of Paraná. A plant with higher yield than other plants, smaller size than the mother plant and resistant to rust (*Hemileia vastatrix* Berk. et Br.), named HN 87609, was selected in 1991, and its progeny (generation S2) was also planted in 1992 in an infested area in the same municipality. Three S2 plants (HN 87609-10; HN 87609-81; HN 87609-15) were selected and advanced to the S3 generation in an area infested by *M. paranaensis* in the municipality of Munhoz de Melo, State of Paraná, in 1995. Four S3 plants (HN 87609-10-23; HN 87609-81-50; HN 87609-81-87; HN 87609-15-6) were selected and advanced to the S4 generation in 2001, also in an area infested by the same nematode in São Jorge do Patrocínio, State of Paraná. Resistance to *M. paranaensis* was assessed using seeds of four individual S4 plants (HN 87609-10-23-3; HN 87609-81-50-7; HN 87609-81-87-8; HN 87609-15-6-12). The treatments consisted of four S4 HN 87609 progenies. *C. arabica* cv. Catuaí Vermelho IAC 81 and *C. arabica* cv. IPR 100 were susceptible and resistant checks, respectively (Table 1).

Table 1. Description of F_4 progenies of HN 87609 assessed for resistance to nematode *Meloidogyne paranaensis*.

Progeny	Description
IAPAR 12229	F_4 of HN 87609-10-23-3
IAPAR 12230	F_4 of HN 87609-81-50-7
IAPAR 12231	F_4 of HN 87609-81-87-8
IAPAR 12232	F_4 of HN 87609-15-6-12
'Catuaí Vermelho IAC 81'	Susceptible check
'IPR 100'	Resistant check

Experimental setup

The experiment was conducted in screenhouse at Instituto Agronômico do Paraná (IAPAR) in Londrina, Paraná State, Brazil (23°21'20,0"S 51°09'58,2"W), between January and April 2012. The average maximum and minimum temperatures during the experimental period were 33.3 and 19.3 °C, respectively. Seedlings were obtained by sowing in sand. At the cotyledon stage, seedlings were transplanted into 700-mL plastic cups to complete their development until they reach three to four pairs of leaves when they were inoculated. The substrate was formulated containing a 1:1 mixture of soil and sand, previously sterilized in an oven at 100 °C for 3 h with moisture at field capacity. For every 72 L of soil, 230 g of simple superphosphate, 22 g KCl, 24 g urea and 72 g of dolomitic limestone were added. Fertilization and pH correction were performed according to soil chemical analysis.

The experiment was arranged in randomized blocks with 14 replications and one plant per plot.

Inoculum

M. paranaensis inoculum was obtained from the municipality of Apucarana (State of Paraná, Brazil) and registered at the Nematology Laboratory of IAPAR with the number 98.1. The population was identified as *M. paranaensis* through α-esterase phenotypes (Carneiro et al. 2000), morphological characteristics (Hartman and

Sasser 1985) and examination of the perineal pattern of females. To obtain purified population, one egg mass was multiplied in tomato plants of the cultivar Santa Clara. Afterwards, the inoculum was kept in coffee cv. Mundo Novo IAC 376-4. For multiplication of inoculum used in the experiment, about 60 days before inoculation, eggs and second-stage juveniles (J2) were extracted from the roots of coffee and inoculated into tomato plants cv. Santa Clara.

Eggs and J2 were extracted from tomato roots using the method of Bonetti and Ferraz (1981) and the suspension was calibrated for 1,000 eggs and J2 per mL; 5,000 eggs and J2 of *M. paranaensis* (initial population = Pi) were inoculated into three holes approximately 1 cm deep, made with a glass rod around the plants.

Resistance evaluation

Evaluations were made 120 days after inoculation, by discarding shoots and collecting roots, which were washed in running water and weighed. Subsequently, eggs and J2 were extracted using the method of Bonetti and Ferraz (1981). After extraction, the final population (Pf) of *M. paranaensis* in plants was determined by counting the number of eggs and J2 per root system using Peters chamber under an optical microscope. With data of weight of the root and number of nematodes, we determined the number of eggs and J2 per gram of roots (Nematodes·g^{-1}).

The reproduction factor (RF) was calculated using the formula: RF = Pf / Pi. Genotypes with RF ≤ 1 were considered as resistant and with RF > 1 as susceptible (Oostenbrink 1966).

Classification of resistance levels

The resistance levels (RL) of the progenies were classified by host susceptibility index (HSI).

The HSI was estimated by the formula HSI = (treatment Nematodes·g^{-1} / susceptible check Nematodes·g^{-1}) × 100 according to Gonçalves and Ferraz (1987) with modifications. HSI values were used to classify the RL of coffee according to modified criteria of Fassuliotis (1985) which correspond to: 0 to 1% = highly resistant (HR); 1.01 to 10% = resistant (R); 10.01 to 25% = moderately resistant (MR); 25.01 to 50% = moderately susceptible (MS); 50.01 to 75% = susceptible (S); 75.01 to 100% = highly susceptible (HS).

RF and HSI were calculated on the basis of data of the plots mean. The percentage of plants with different resistance levels, obtained from HSI, was calculated using data of individual plots of the susceptible check with data of the respective plots of the treatments.

Statistical analysis

Data on RF and Nematodes·g^{-1} were tested for normality by Shapiro-Wilk test and homogeneity of variances by Hartley test, at 5% probability. Data were transformed to log (x + 1) for analysis of variance and Scott-Knott test for means comparisons, at 5% probability.

RESULTS AND DISCUSSION

IAPAR 12232 and IAPAR 12231 had lower values of Nematode·g^{-1}, compared to other treatments, and were not statistically different from the resistant check IPR 100. All F_4 progenies, as well as the resistant check, were resistant, according to RF, and significantly differed from the susceptible check, which showed a large increase in the population of nematodes with RF of 21.81 (Table 2).

All progenies from HN 87609 were classified as HR by HSI, similarly to the resistant check. In the cultivar IPR 100, 79% of the plants were classified as HR and 21% as R. In all progenies, we verified 100% of the plants between HR and R; the progeny IAPAR 12232 stood out since it showed 100% of plants as HR and % HSI of 0.24 (Table 3).

The four F_4 progenies showed 100% of plants classified as HR or R; therefore, the resistance to *M. paranaensis* can be in homozygotic condition. This high frequency of resistant plants in HN 87609 progenies can be explained by the fact that F_1, F_2, F_3 and F_4 generations have been planted in areas infested by *M. paranaensis* and therefore the coffee plants with high yield, selected with these areas, were probably resistant, in contrast with the low productive plants that have not been selected and were susceptible.

Probably, Icatu H4782-7-925 was the source of resistance for the four F_4 progenies and it is not known the genotype pollinating HN 87609. Several studies report →

resistance to *M. paranaensis* in Icatu genotypes, such as line 925 (Matiello et al. 2010; Carneiro et al. 2013), 'IPR 106' (Ito et al. 2008) and 'Icatu Vermelho IAC 3888' (Gonçalves and Silvarolla 2007). The line 925 of Icatu reported by other authors (Matiello et al. 2010; Carneiro et al. 2013) as resistant to *M. paranaensis* is the same used herein as a mother plant.

The rootstock 'Apoatã IAC 2258' presents some drawbacks for its use in relation to ungrafted Arabica cultivars, such as segregation rate for susceptibility to nematodes (10 – 15%) and the greater need for replanting (about 10 to 15%) due to this segregation (Gonçalves and Silvarolla 2007) in addition to the higher cost of seedlings. Furthermore, some authors observed that, in an area free of nematodes, coffees grafted on 'Apoatã IAC 2258' were less productive (Dias et al. 2009; Paiva et al. 2012) and showed lower vegetative development (Oliveira et al. 2004; Dias et al. 2011) than the same coffee without grafting. Despite some disadvantages, rootstock cultivars Apoatã IAC 2258, in Brazil, and Nemaya, in Central America, have allowed the maintenance of crops in areas infested by root-knot nematodes.

Currently, the only ungrafted Arabica cultivar released resistant to *M. paranaensis* is IPR 100. The F_4 HN 87609 progenies identified as resistant herein are of great importance because they showed no susceptible segregating plants. These progenies will be self-pollinated and advanced to next generation and have great potential to become new cultivars of Arabica coffee resistant to *M. paranaensis*.

Table 2. Mean number of eggs and second-stage juveniles of *Meloidogyne paranaensis* per gram of roots (Nematodes·g^{-1}) and reproduction factor in Arabica coffee progenies.

Progeny	Nematodes g^{-1}(1)	RF(1)
IAPAR 12232	15,93 a	0,03 a
'IPR 100'	21,65 a	0,05 a
IAPAR 12231	36,26 a	0,11 a
IAPAR 12229	42,47 b	0,10 a
IAPAR 12230	56,50 b	0,23 a
'Catuaí Vermelho IAC 81'	6.632,19 c	21,81 b
Overall mean	1.134,17	3,72
CV%	**32,32**	**33,02**

(1)Mean values followed by different letters are significantly different by Scott-Knott test at 5% probability. Data transformed to log (x + 1). RF = reproduction factor; CV = Coefficient of variation.

CONCLUSION

The four F_4 progenies of HN 87609, derived from Icatu H4782-7-925, were highly resistant, in the same manner as the IPR 100 check. Furthermore, they showed no susceptible segregating plants and are homozygously resistant to *M. paranaensis*.

Table 3. Host susceptibility index, resistance levels and percentage of coffee plants highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible to the nematode *Meloidogyne paranaensis*.

Progeny(1)	%HSI	RL	%HR	%R	%MR	%MS	%S	%HS
IAPAR 12232	0,24	HR	100	0	0	0	0	0
'IPR 100'	0,33	HR	79	21	0	0	0	0
IAPAR 12231	0,55	HR	71	29	0	0	0	0
IAPAR 12229	0,64	HR	79	21	0	0	0	0
IAPAR 12230	0,85	HR	79	21	0	0	0	0
'Catuaí Vermelho IAC 81'	–	–	–	–	–	–	–	–

(1)The progenies were ordered decreasingly based on the number of eggs and juveniles per gram of roots (Nematodes·g–1). HSI = Host susceptibility index; RL = Resistance level; HR = Highly resistant; R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible; HS = Highly susceptible.

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