academicJournals

Vol. 12(11), pp. 916-922, 16 March, 2017
DOI: 10.5897/AJAR2016.11999
Article Number: 7D9E97C63186
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Reaction of *Coffea canephora* clones to the root knot nematode, *Meloidogyne incognita*

Anderson Vieira Santos[1], Rodrigo Barros Rocha[2], Cléberson de Freitas Fernandes[2], Silvaldo Felipe da Silveira[3], André Rostand Ramalho[2] and José Roberto Vieira Júnior[2]*

[1]Centro Universitário Luterano de Ji-Paraná- CEULJI/ULBRA - Av. Engenheiro Manfredo Barata Almeida da Fonseca, nº 762, Caixa Postal 61, CEP 76.907-438, Ji-Paraná/RO, Brazil.
[2]Empresa Brasileira de Pesquisa Agropecuária - Embrapa Rondônia, BR 364, Km 5,5 Zona Rural CEP:76815-800, Porto Velho/RO, Brazil.
[3]Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Av. Alberto Lamego, nº 2000 - Parque Califórnia, CEP: 28013-602, Campos dos Goytacazes/RJ, Brazil.

Received 25 November, 2016; Accepted 14 February, 2017

In the Western Amazon, Brazil, *Coffea canephora* Pierre is the main species cultivated because it has good adaptation to the climate and soils of the region. Among the factors that limit the yield of this crop, *Meloidogyne* or root-knot nematode, caused by the root knot nematode, is an aggressive disease present in the state of Rondonia. The aim of this study was to evaluate the reaction of fifteen clones of *C. canephora*, belonging to the cultivar BRS Ouro Preto, to *Meloidogyne incognita* (Est I2). Therefore, six seedlings with six months of growth in a nursery from each one of the clones of the cultivar were transferred to pots containing a sterilized substrate in a greenhouse. Following the same design, three clonal genotypes selected from the cultivar IAC Apoatã (*C. canephora* botanical variety Robusta) served as resistant controls, and seedlings of *C. arabica* of the Obatã line and tomato (*Solanum lycopersicum*) cv. Santa Clara (20 days after germination) as susceptible controls, for a total of 120 pots. A 10 ml suspension containing 5000 eggs of *M. incognita* was inoculated on each plant, and after 150 days, they were evaluated with regards to root fresh weight (FWR), number of galls (NG), number of eggs (NE), and the nematode reproduction factor (RF = final population/initial population). Except for the clone K98M-0061, which exhibited galls (29.17), all the clones of the variety BRS Ouro Preto showed resistance at levels equivalent to those of the controls of the var. Apoatã (RF <1) and good root development. Thus, it can be concluded that the total composition of clones of the variety BRS Ouro Preto is resistant to *M. incognita* in Rondonia.

Key words: Coffee, *Meloidogyne*, plant selection.

INTRODUCTION

Brazil is the world's largest producer and exporter of coffee (*Coffea* spp.), with a total production area of 1,942.1 thousand hectares (Conab, 2016). The state of Rondonia is the largest coffee-growing area in the North region of Brazil, and is the second largest producing state of conilon coffee (*Coffea canephora*) in Brazil, exceeded

only by Espírito Santo (Conab, 2016). In addition to soil and climate conditions, other factors can limit the yield of conilon coffee, such as the occurrence of pests and diseases (Faganello, 2006). Among the diseases that damage the coffee crop, those caused by plant parasite nematodes can cause a decline of approximately 15% in world production and 20% in Brazilian production (Ito et al., 2008). Among the plant nematodes that have important economic consequences in diverse crops throughout the world, those of the genus *Meloidogyne*, also known by the generic name “root-knot nematodes”, are responsible for approximately 75% of these losses (Lordello, 1984). In recent years, surveys have shown an extensive array of *Meloidogyne* spp. ability to parasitize coffee roots, and distribution of this species that differs widely from one country to another. Such species are widely distributed in coffee fields in Brazil, where they cause considerable losses and damage according to the species, the population density and the susceptibility of the cultivar (Barros et al., 2014; Contarato et al., 2014; Salgado; Rezende, 2010). Currently, 18 species have already been described as parasites in coffee fields throughout the world (Humphreys-Pereira et al., 2014). Five of them occur in Brazilian coffee fields: *Meloidogyne coffeicola* Lordello and Zamith, *Meloidogyne exigua* Goeldi, *Meloidogyne hapla* Chitwood, *Meloidogyne incognita* (Kofoid & White) Chitwood, and *Meloidogyne paranaensis* Carneiro (Salgado et al., 2015; Silva et al., 2013), and all have been classified as regulated non-quarantined pests (Brasil, 2009), that is, their spread between crop fields and regions should be impeded through prohibition of sale of contaminated seedlings.

In Brazil, *M. incognita* is considered as one of the most harmful to coffee fields because the damage it causes might not only lead to death of the plant but might expose the root system of the plants to the attack of other diseases and reduce the capacity of the plant to absorb water, leaving it more susceptible to drought, which occurs from May to September, as has been observed with regard to the occurrence of *Fusarium* wilt and *Rhizoctonia* disease in crop fields of up to two years of age in Rondonia (Vieira Júnior et al., 2015).

Although, *C. canephora* is considered more resistant to nematodes than *C. arabica* (Barbosa et al., 2014; Ito et al., 2008). Vieira Junior et al. (2008) report the occurrence and the symptoms of root-knot nematode in different municipalities of the state of Rondonia. A recent study confirmed two main species as parasites of *C. canephora* in production areas in Rondonia: *M. exigua* and *M. incognita*. Of these two, *M. incognita* has greater occurrence in all the municipalities evaluated (Vieira Júnior et al., 2015). In another study, the species *M. incognita* was also identified in a sample of coffee roots from five coffee fields in the municipalities of Cacoal (two areas), Nova Londrina, Ji-Paraná and Ouro Preto do Oeste, showing that it is one of the species associated with coffee growing in the state (Pissinati et al., 2015). Therefore, *M. incognita* is believed to be the most widespread and most important species in the state of Rondonia.

With the intention of service to regional coffee growers, Embrapa Rondonia released a synthetic cultivar in 2012 composed of 15 clones of *C. canephora*, designated as ‘BRS Ouro Preto’ (RNC/MAPA No. 29486). Some of the main characteristics of this cultivar are uniform maturation of fruit, lower biennial yield differences, mean yield of 70 bags/hectare of hulled coffee under medium technology conditions, large coffee beans, good beverage, and tolerance to diseases and to the main abiotic stresses- low altitude, high mean temperature and relative humidity, and medium to high water deficit (annual water deficit = 150-200 mm) (Ramalho et al., 2015). Although, the variety released offers adequate levels of resistance to rust and *Cercospora* leaf spot (Sera et al., 2007; Vieira Junior et al., 2015), a study has not yet been carried out to evaluate the reaction of the clones that make up this cultivar to the species of *Meloidogyne* present in the state.

Due to the potential of this cultivar for growing in the state of Rondonia, and the impact of the root-knot nematode on coffee growing in Rondonia, the aim of this study was to characterize the clones of the cultivar ‘BRS Ouro Preto’ with regard to resistance to the root-knot nematode, *M. incognita* (Est I2).

MATERIALS AND METHODS

To obtain inocula of *M. incognita*, samples of the nematode were collected from under the canopy of naturally infested plants from a coffee field in the municipality of Ji-Paraná, RO. The inoculum was then multiplied in a greenhouse by inoculating tomato plants cv. Santa Clara with egg masses derived from a single female.

With establishment of the pure sample, enzymatic characterization of the esterase profile was carried out using the electrophoresis technique (Carneiro and Almeida, 2001) in the Plant Pathology Laboratory of Embrapa Clima Temperado – Pelotas, RS. Recognition of a single pattern of esterase allowed identification of *M. incognita* (Est I2) in a pure sample. In the following step, seedlings of the fifteen clones that make up the cultivar of *C. canephora* ‘BRS Ouro Preto’ (K98M-0016; 0057; 0061; 0069; 0073; 0088; 0089; 0125; 0130; 0160; 0167; 0187;

*Corresponding author. E-mail: jose-roberto.vieira@embrapa.br.

Table 1. Analysis of variance (ANOVA) on fresh weight of roots (FWR), number of galls (NG), number of eggs (NE), and reproduction factor (RF) of the nematode *M. incognita* evaluated in 15 clones of *C. canephora* (conilon) 'BRS Ouro Preto' in comparison with four controls.

SV	DF	FWR	NG[1]	NE[1]	RF
Treatment	18	4.54**	23.49**	9.89**	11.68**
Clones	14	3.44**	15.19**	2.26*	4.17**
Controls	3	9.67**	69.75**	37.57**	48.79**
Clones vs Controls	1	4.50*	4.01*	33.69**	5.60*
Residue	95				
Total	113				
$Mean_{General}$		36.76	5.39	1.78	0.36
$Mean_{Clones}$		35.78	4.64	1.86	0.37
$Mean_{Controls}$		40.46	8.17	1.48	0.30

[1]Data transformed to square root of the value. **: Significant at 1% probability.

00189; 00197; 0199) with six months of age, were transplanted to polyethylene pots (8 L capacity) containing a sterilized substrate composed of sand, vermiculite, natural soil and organic compost (1:1:1:1). Two weeks after transplanting the seedlings, extraction of eggs from the tomato plants was carried out using the Hussey and Barker (1973) technique. Each coffee plant (or pot) was inoculated separately with a 10 ml suspension containing 5,000 eggs of *M. incognita* (Est I2). Three clonal genotypes ('A1322', 'A1326', 'A1327') selected for resistance to *M. incognita* in the cultivar IAC Apoatã (*C. canephora*, botanical variety Robusta) were evaluated as resistant controls. The *C. arabica* variety Obatã IAC 1669-20 (RNC/MAPA Nº 2956) was used as a susceptible control, arising from a cross (Sarchimor x Catuaí), as well as tomato cv. Santa Clara seedlings. The coffee plants were inoculated after transplanting at six months of age and those of the tomato plant at twenty days of age.

The trials were carried out in a post-and-rafter type greenhouse, a structure with treated wood, covered with anti-UVB plastic film of 120 µ, with front ventilation and free sides at the Universidade Luterana do Brasil in the municipality of Ji-Paraná (10°51'44.36"S, 61°57'29.33"O).

At 90 days after inoculation (DAI), the tomato plants were evaluated with regards to reaction to *M. incognita* (Est I2). Evaluation of the coffee plants occurred at 150 days after the date of inoculation. For that purpose, the roots of each plant were separated from the shoots, washed, weighed, evaluated with regards to the number of galls, and processed (Hussey and Barker, 1973) for evaluation of the number of eggs and determination of the reproduction factor (RF = final population / initial population) in the different genotypes. Cultivars with RF< 1.00 were considered resistant; with RF = 0.00, immune; and with RF > 1.00, susceptible (Oostenbrink, 1966).

To assist in data interpretation, the RF values were used to classify the reaction of the coffee plants to *M. incognita* by the criteria of Seinhorst (1967), in which plants with RF<1 are considered poor hosts (PH); with RF≥1, good hosts (GH); and RF = 0, non-hosts (NH). The susceptibility of the plants was also classified following the criteria recommended by Sasser et al. (1984), in which plants whose number of galls throughout the root system is less than ten are considered resistant (R) and greater than or equal to ten as susceptible (S). To quantify the resistance response, a completely randomized design was used with six replications for each treatment (clone), considering the following model:

$$Y_{ij} = u + G_i + e_{ij}$$

Where, $Y_{ij} =$ observation of the i-th clone in the j-th replication, $u =$ overall mean, $G_i =$ effect of the i-th clone and $e_{ij} =$ random error involving the i-th clone and in the j-th replication. To test the equality hypothesis between the means of groups, the Scott-Knott test was used at 5% probability. For that purpose, a single plant (or pot) was considered an experimental unit, and each genotype was evaluated through 6 plants (or pots).

The estimates of genotypic, environmental and phenotypic variance were obtained from the least squares estimation method so as to quantify the proportion of total variance due to the effects of genotypes (clones) and environment (error) (Cruz et al., 2012). Broad-sense heritability, the coefficients of genotypic and environmental variation, and the intraclass correlation were estimated from the components of variance (Rocha et al., 2015). Broad-sense heritability measures the relative proportion between the genotypic and environmental effects in expression of the characteristics. It is considered the most important component of the estimates of genetic progress obtained from asexual propagation, which, according to Vencovsky and Barriga (1992), can be estimated by the expression:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}$$

Where, h^2 is broad-sense heritability, σ_g^2 is genotypic variance and σ_e^2 is environmental variance.

The coefficient of environmental variation, estimated by the ratio between the root of environmental variance and the mean of the experiment, was used to provide a measure of experimental precision. For its part, the coefficient of genetic variation, estimated by the ratio between the root of genotypic variance and the mean of the experiment was interpreted to verify the predominance of the genetic component in expression of this characteristic.

RESULTS AND DISCUSSSION

According to the F test of analysis of variance, the effects of clones, controls and of the clone × control contrast were significant at 1% probability for fresh weight of roots (FWR), number of galls (NG), number of eggs (NE) and the reproduction factor of the nematode *M. incognita* (RF) (Table 1). The significance of the effect of clones × control contrast indicates that the three clones of the *C. canephora* variety Apoatã and *C. arabica* of the Obatã line showed significant differences from the genotypes evaluated.

The coefficient of variation that weights the mean value of the experiment and the variance of the residue was interpreted to quantify the precision of the experiment (Table 2). When there is no previous knowledge of the

Table 2. Estimates of the genetic parameters of fresh weight of roots (FWR), number of galls (NG), number of eggs (NE), and the reproduction factor (RF) of the nematode *M. incognita* evaluated in 15 clones of *C. Canephora* (conilon) 'BRS Ouro Preto'.

Genetic parameters	FWR	NG	NE	RF
σ_g^2	52.76	1.23	0.04	0.01
σ_e^2	15.35	0.08	0.02	0.00
σ_p^2	37.41	1.15	0.02	0.01
h^2	70.91	93.41	55.72	75.99
$\hat{\rho}$	28.89	70.28	17.34	34.54
CV_g	17.10	61.40	11.03	26.94
CV_e	26.11	39.17	25.84	32.74
CV_g/CV_e	0.65	1.57	0.43	0.82

σ_g^2: Genotypic variance, σ_e^2: environmental variance, σ_p^2: phenotypic variance, h^2: heritability for selection based on the mean of the genotypes, $\hat{\rho}$: intraclass correlation, CV_g: coefficient of genotypic variation, CV_e: coefficient of environmental variation, CV_g/CV_e: ratio between the coefficients of genotypic and environmental variation.

difficulty of measuring a characteristic, the coefficient of variation can be classified in accordance with the criteria proposed by Pimentel-Gomes (2009), which classifies values of the coefficient of variation from 20 to 30 as associated with high data dispersion. It was observed that the estimates of the coefficient of variation were equivalent to those obtained by Ito et al. (2008) and Gonçalves et al. (1996), and due to the heterogeneity of variance, the evaluations coming from counting were transformed using the square root of the original value (Tables 1 and 2).

Cloning of plants, whether by planting of orthotropic stem cuttings or by tissue culture, allows the complete genotypic value of the individual to be exploited. The magnitudes of genotypic variance and of environmental variance indicate a predominance of the effect of genotypes on the expression of this characteristic, which is the result of the differentiated genetic expression among clones of the variety. The estimates of heritability of 0.7 for FWR, 0.93 for NG, 0.55 for NE, and 0.75 for RF indicated the predominance of the genotypic component in expression of these characteristics (Table 2).

The susceptible controls represented by the tomato cv. Santa Clara and Arabica coffee acted as good hosts (GH) for *M. incognita* at 90 DAI (tomato) and 150 DAI (Arabica coffee), leading to a high reproduction factor RF>1 (11.71 and 1.3, respectively) and high number of galls (791 and 34, respectively), confirming the pathogenicity of the inoculum of the nematode used in this experiment. The fifteen clonal genotypes of the cultivar 'BRS Ouro Preto' reacted in a resistant manner to *M. incognita* at 150 DAI, exhibiting a reproduction factor less than one (RF<1), and were classified as poor hosts (Seinhorst, 1967) (Table 3).

Nevertheless, the clone K98M-0061 had a susceptibility (S) response by the classification of Sasser et al. (1984) in relation to the number of galls produced in the roots of the coffee plant (Table 3). Moura et al. (1997) reported that the presence of galls is a symptomatological aspect of the plant resistance response, and that galls should not be considered in an isolated manner in evaluation of resistance because resistant plants can also form galls in the presence of the nematode in resistance trials. In a study carried out by Silva et al. (2006), it was also found that part of the clones considered resistant (RF<1) responded as susceptible in relation to number of galls. This fact suggests that although the nematode induced formation of galls, the parasite reproduced very little in all the clones evaluated in this study (Table 3).

In relation to the controls of Apoatã evaluated in this experiment, all were classified as non-hosts (RF=0); they did not form galls or formed only a small number, and all the genotypes responded as resistant (Table 3). Genotypes of *C. canephora* of the botanical variety Robusta Apoatã have been used as an alternative in control of root-knot nematode in the form of rootstock or otherwise. Among them, the cultivar IAC 2258 is recommended for planting in areas infested with the nematodes *M. exigua*, *M. incognita* (Kofoid & White) Chitwood and *M. paranaensis* (Andreazi et al., 2013). In areas infested by the pathogen, results were found in which the mean yield over six crop seasons of non-grafted susceptible genotypes was up to 55% less than the yield of those genotypes grafted onto IAC Apoatã 2258 (Barbosa et al., 2014). In a study performed in an area naturally infested by *M. incognita* in Paraná, Dias-Arieira et al. (2012) found that 34 months after planting, the cultivar Iapar 59 grafted onto Apoatã 2258 had 448% greater coffee bean production than the treatment with the non-grafted Iapar 59 cultivar. This study shows the effectiveness of the rootstock Apoatã in maintaining production of the scion, even in areas infested by the nematode.

In the present study, it was also observed that the clones K98M-0057and K98M-184 obtained root development (FWR= 44.48 and 52.89) similar to the Apoatã controls according to the Scott-Knott mean grouping test at 5% probability. The other clones exhibited less developed root systems, with the two lowest values of FWR being identified in clone K98M-073 and K98M-167 (Table 3). According to Sera et al. (2006), there is a possibility of success in selecting clones that have a

Table 3. Fresh weight of roots (FWR), number of galls (NG), number of eggs (NE), reproduction factor (RF), and response of clones of *C. canephora* 'BRS Ouro Preto', in comparison with three standard clones for resistance from cv. IAC Apoatã, and for susceptibility from cv. Obatã (*C. arabica*), in relation to the standard for susceptibility (tomato, *S. lycopersicum* cv. Santa Clara) to root-knot nematode *M. incognita* (Est I2) at 150 days after inoculation with 5000 eggs of the nematode/plant.

Clone	FWR[a]	NG[b]	C[1]	NE[c]	RF[d]	C[2]
K98M-0016	38.45[b]	1.50[c]	R	670[c]	0.27[c]	PH
K98M-0057	44.49[a]	2.00[c]	R	670[c]	0.19[c]	PH
K98M-0061	36.51[c]	29.17[a]	S	1000[c]	0.23[c]	PH
K98M-0069	37.87[c]	2.00[c]	R	650[c]	0.18[c]	PH
K98M-0073	22.81[d]	5.67[b]	R	1000[c]	0.33[c]	PH
K98M-0088	38.08[c]	3.50[c]	R	500[c]	0.15[c]	PH
K98M-0089	32.2[c]	5.00[b]	R	1170[c]	0.23[c]	PH
K98M-0125	37.79[b]	4.00[b]	R	1000[c]	0.20[c]	PH
K98M-0130	31.29[c]	2.50[c]	R	2000[b]	0.45[b]	PH
K98M-0160	35.28[c]	2.33[c]	R	670[c]	0.16[c]	PH
K98M-0167	24.53[d]	2.50[c]	R	500[c]	0.16[c]	PH
K98M-0184	52.9[a]	2.50[c]	R	1000[c]	0.28[c]	PH
K98M-0189	31.42[b]	1.50[c]	R	170[c]	0.00[d]	PH
K98M-0194	36.03[b]	1.33[c]	R	330[c]	0.21[c]	PH
K98M-0199	37.02[c]	2.83[c]	R	330[c]	0.11[c]	PH
C. Arabica cv. Obatã	23.13[d]	34.00[a]	S	6500[a]	1.30[a]	GH
Apoatã 1322	51.51[a]	0.83[c]	R	0[d]	0.00[d]	NH
Apoatã 1326	45.08[a]	1.17[c]	R	0[d]	0.00[d]	NH
Apoatã 1327	42.09[a]	0.67[c]	R	0[d]	0.00[d]	NH
Tomato Sta, Clara	15.66	791	S	508000	11.71	GH

[1] Response according to Sasser et al. (1984), in which R= resistant and S= susceptible; [2] Response according to Seinhorst (1967), in which NH= non-host; PH= poor host, and GH= good host; [a] fresh weight of roots; [b] number of galls; [c] number of eggs; [d] reproduction factor; Significant at 1 and 5% probability by the Scott-Knott test.

more voluminous root system, because this is a favorable characteristic for a good rootstock cultivar.

According to Pasqualotto et al. (2015), the management strategies for reducing the plant nematode population are crop-based, biological, chemical and genetic, the last of which is the most effective and economically viable. Therefore, selection of resistant clones is one of the most promising alternatives for minimizing losses caused by the nematodes in the coffee crop because it allows nematode populations to be maintained below the level of economic damage (Wangai et al., 2014). However, it is important to emphasize that generally, the levels of resistance of coffee genotypes are related to the species and/or strains of associated Meloidogyne. Sera et al. (2006) found that 24 clones of *C. canephora* exhibited a resistance response to *M. incognita* strain 1, but when exposed to *M. incognita* strain 2, only 12 clones showed resistance.

Therefore, due to the occurrence of other species of *Meloidogyne* associated with the coffee plant in the state of Rondonia, it becomes necessary to carry out new trials so as to evaluate the reaction of the cultivar BRS Ouro Preto to other nematodes. Among them, *M. exigua* is one of the most important because in surveys undertaken in the state, this species was considered as the most frequent in coffee-producing areas (Vieira Junior et al., 2015).

In addition, one of the obstacles of nematology research in agricultural crops is the absence of methodological standardization in relation to the best population density of the nematode that is able to express adequate levels of resistance/susceptibility of the genotypes, and in relation to the best date for evaluation, especially in the case of perennial crops, like coffee. Since species like *M. incognita* and *M. paranaenses* are more aggressive to the coffee plant (Ito et al., 2008), Sera et al. (2006) suggested that these nematodes should be tested with low levels of inoculum because inoculum pressure could induce a resistant plant to act as susceptible, generating misleading results. According to Machado et al. (2015),

using very high initial populations of the nematode, the roots of the host plant can be severely damaged by the attack of the pathogen and there is substantial competition between the individuals for feeding sites of the host, making the reproduction factor at the end of the experiment remain below 1.0, which characterizes a resistance reaction, even in plants susceptible to the nematode (Greco and Di Vito, 2009).

In this study, a concentration of 5000 eggs/eight-liter pot was used; however, new experiments with different nematode concentrations, as well as trials in a greenhouse and in the field should be carried out to better understand the resistance response of this cultivar to the different species and strains of the root-knot nematodes that act as parasites of the coffee plant.

Conclusion

All the clones of the cultivar, BRS Ouro Preto can be considered resistant to *M. incognita* (Est I2) (RF<1) in spite of the larger number of galls observed in clone 61, indicating that this cultivar is resistant to the pathogen. The clones of Apoatã evaluated are not hosts of *M. incognita* (Est I2) and they can serve as an important tool in the control of the pathogen and in plant breeding programs aiming at resistance to this nematode. New studies with other species and strains of root-knot nematode under different environmental conditions should be carried out to quantify the resistance response of this variety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a scholarship, and the Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CPC) for financial support.

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