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Resistance of new *Coffea canephora* clones to root-knot nematode (*Meloidogyne incognita*) in the western amazon

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ABSTRACT

Root-knot disease is among the main diseases affecting coffee crop. The plant selection to the development new resistant cultivars is among one the most efficient methods of control. The present work aimed to quantify the resistance responses of *Coffea canepho*ra clones to root-knot nematode *Meloidogyne incognita* in the Western Amazon. For this, 17 previously selected clones were evaluated in three experimental trials, carried out in the municipalities of Ji-Paraná and Porto Velho, Rondônia. The resistance to root-knot nematodes *M. incognita* were evaluated by the numbers of gall in the roots (NG) and by the reproductive factor (RF). The resistance response was also interpreted according the genetic diversity of the clones based in their morphological traits. The clones BRS3210, C12, BRS2314, BRS3137 and BRS1216 are resistant to *M. incognita* with RF of 0.34, 0.62, 0.79, 0.86 and 0.98, respectively. BRS3213, C125, C15, BRS2336, BRS3220 and C09 clones were classified as susceptible, with RF of 1.93, 1.95, 2.00, 2.31, 2.32 and 2.35. The BRS3193, C160 and BRS2357 clones were classified as very susceptible, with RF values of 3.03, 4.41 and 5.82, respectively. The clustering based on the genetic diversity of morphological traits indicated that genotypes more similar to the Robusta botanic variety had lower RF. The hybrid plants showed intermediate degrees of resistance indicating the segregation for the character of the *M. incognita* resistance. The clones BRS3210, C12, BRS2299, BRS2314, BRS3137 and BRS1216 expressed resistance responses to *M. incognita* with potential for growing resistant genotypes in the Western Amazon.

Key words: Coffee; root-knot disease; plant breeding; Amazonian.

1 INTRODUCTION

Brazil is the second largest coffee producer of the species *Coffea canephora* with production of 14.3 million bags (60 kg) of hulled coffee (Companhia Nacional de Abastecimento - CONAB, 2020). This culture is a relevant source of income for hundreds of municipalities being important to the generation of jobs in the field. The state of Rondônia is the third largest producer of *C. canephora* in Brazil, after the states of Espírito Santo and Bahia. Its coffee-growing area consists of approximately 73 thousand hectares, with mean annual production of 1.9 million bags (60 kg) of hulled coffee (CONAB, 2020; Ministry of Agriculture, Livestock and Supply - MAPA, 2020).

Among the factors that can limit coffee yield, pests and diseases is one of the most important (Van Der Vossem et al., 2015; Zambolim, 2016a; Avelino et al., 2018; Oliveira et al., 2018a; Myers et al., 2020). Plant-parasitic nematodes have an important economic impact on coffee in most coffee-producing countries. Economic losses may vary considerably, depending on the species, the population density and the susceptibility of the host cultivar (Fatobene et al., 2018). Among the most harmful species are the *Meloidogyne exigua* Göldi 1887, *M. incognita* (Kofoid & White) Chitwood 1949 and *M. paranaensis* Carneiro, Almeida and Carneiro (1996). *M. incognita* has been the species with the highest occurrence in *C. Canephora* crops in the state of Rondônia, and the one with the greatest economic impact (Vieira Junior et al., 2015). Causing the death of plants up to two years old, the damage caused to the root system reduces the absorption capacity of water and nutrients (Goulart et al., 2019; Ventura et al., 2019). It also exposes the root to others diseases such as Fusarium wilt and Rizoctonia damping-off (Vieira Junior et al., 2015; López-Lima et al., 2018).

The field control of plant parasite nematodes is limited and usually does not bring satisfactory results (Ferraz; Brown, 2016; Zambolim, 2016a; Ebone; Kovaleski; Deuner, 2019). Some practices such as crop rotation may not be viable, once the *M. incognita* has a large host range. Chemical control is also limited, as it causes adverse effects to the environment and loses its efficacy over time (Zambolim, 2016b).

Plant selection of *C. canephora* genotypes with resistance to root knot nematodes may be considered in the development of new resistant cultivars (Lima et al., 2015; Fatobene et al., 2018). Of the 32 genotypes of *C. canephora* evaluated by Santos et al. (2018a), it was observed that nine showed higher

susceptibility to *M. incognita*. In a study of 73 wild *Coffea* spp, plants were identified 18 genotypes of *C. canephora* resistant to *Meloidogyne* spp. (Aribi et al., 2018). *C. canephora* clones resistant to *Meloidogyne* spp. are an alternative to coffee production in infested areas, including those areas traditionally cultivated with *C. arabica* (Salgado et al., 2020).

With the objective of selecting resistant genotypes, clones with characteristics of the Conilon and Robusta botanical varieties were characterized to their reaction to the root-knot nematode *M. incognita* (Est I2).

2 MATERIAL AND METHODS

Seventeen previously selected genotypes (Oliveira et al., 2018b; Spinelli et al., 2018) were evaluated by the following morphological and productive traits: plant height, number of productive plagiotropic branches, distance between rosettes of the plagiotropic branch, number of coffee beans per rosette, number of rosettes per plagiotropic branch, length of plagiotropic branch, length and width of leaves, number of days for fruit ripening and coffee bean size. The genotypic value of production was estimated based on production of hulled coffee using the BLUP (Best Linear Unbiased Prediction) method (Table 1).

Table 1: Clones of *Coffea canephora* evaluated for *Meloidogyne incognita* resistance on three experiments (trials) performed in the municipalities of Ji-Paraná and Porto Velho - RO.

Treatments	Clones	Origin
1	Apoatã-2258	Robusta IAC 2258-1 S2
2	K98M-0125	Cultivar BRS Ouro Preto
3	K98M-0160	Cultivar BRS Ouro Preto
4	BRS 2357	Cultivar BRS Ouro Preto
5	BRS 2299	Cultivar BRS Ouro Preto
6	C09	Emcapa 03 x Robusta 640
7	C12	Emcapa 03 x Robusta IAC2258-1 S2
8	C15	Emcapa 03 x Robusta IAC2258-1 S2
9	C750	Open pollinated hybrid
10	BRS 2336	Open pollinated hybrid
11	BRS 3137	Open pollinated hybrid
12	BRS 3193	Open pollinated hybrid
13	BRS 3210	Emcapa 03 x Robusta IAC2258-1 S2
14	BRS 3213	Emcapa 03 x Robusta IAC2258-1 S2
15	BRS 2314	Emcapa 03 x Robusta 640
16	BRS 1216	Emcapa03 x Robusta 1675
17	BRS 3220	Emcapa 03 x Robusta 1675

The *M. incognita* inoculation agent was extracted from samples of roots taken from under the canopy of naturally infested crop fields in the municipality of Ji-Paraná, RO (10°52'53''S, 61°30'45''W, altitude 159m) (Santos et al., 2017). In order to identify the species of the rootknot nematode, enzymatic characterization of the esterase profile was performed in the Embrapa Temperate Climate Plant Pathology Laboratory - RS, according to the methodology of Carneiro and Almeida (2001). Using females of *Meloidogyne javanica* as control samples, the observed esterase profile was of a single typical pattern of *M. incognita* (Est I2) (Santos et al., 2017). The inoculum was kept in greenhouse, alternating its multiplication in tomato and coffee plants forming an inoculum bank. This inoculum was registered in the "Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado - Sisgen" under access code number AF69FBC.

To quantify the resistance response to *M. incognita* of the *C. canephora* clones, three tests were carried out: the first and the second trials were evaluated at Embrapa Rondônia – Porto Velho, RO (8° 47'38, 44"S, 63° 50'47.93 "O) from September 2017 to February 2018 and from July to December 2018. The third trial was evaluated at the Lutheran University of Brazil (10° 51'44.36" S, 61 57'29.33 "O) in Ji-Paraná- RO from July to December 2018. All tests were performed in a greenhouse "chapel model", covered with 120 micron anti-UBV plastic film with front and side ventilation.

For the tests, seedlings with six months of development and six pairs of leaves were transplanted to 8-liter pots containing sterilized substrate composed of natural soil and sand 1:1. Each coffee plant was inoculated separately by irrigation of the substrate in the pot with 10 ml of suspension containing 5000 eggs + second stage juveniles (J2) of *M. incognita* (Est I2). For this evaluation, the roots of each plant were separated from the shoots, washed, and weighed, and the number of galls were counted in 3g of root. After that, the roots were processed according to the methodology of Boneti and Ferraz (1981) to determine the number of eggs and the reproduction factor (RF) of *M. incognita* (RF = final population / initial population) (Oostenbrink, 1966). To calculate the RF, the number of nematode eggs extracted from each coffee plant were counted on a Peter's slide under a light microscope.

The classification of the resistance levels was based on the criteria proposed by Moura and Regis (1987). The immune genotypes were those with 100% reduction in the reproduction factor (RF). The resistant were those with 99 to 51% of reduction and the susceptible ones those below 50% of reduction. The most susceptible genotypes presented RF estimates higher than the susceptible controls.

Each genotype inoculated with *M. incognita* represented a treatment, using six replications for each clone arranged in a completely randomized design. The Apoatã-2258 and BRS2299 were used as resistant controls, due to their previously known resistance to *M. incognita* (Santos et al., 2017). The open pollination clones C750 and C125 were used as susceptible

controls due to their susceptibility to nematodes, as observed in the tests carried out by Santos et al. (2017; 2018a).

The significance of the effect of clones on the resistance response was individually tested in each experiment, according to the model described by Cruz, Regazzi and Carneiro (2012) (Equation 1):

$$Y_{ij} = m + G_i + E_{ij} \quad (1)$$

Where Y_{ij} refers to the observation of the ith genotype in the jth replication; m is the experimental average; G_i is the effect of the ith genotype (clone); and E_{ij} is the experimental error that affects all the observations.

After that, the homogeneity of residual variances was verified and a joint variance analysis was performed, to quantify the effect of the interaction between genotypes and experiments. Each experiment was interpreted as a different environment, according to the Equation 2 (Cruz, Regazzi and Carneiro, 2012):

$$Y_{ijk} = m + G_i + A_j + GA_{ij} + E_{ijk} \quad (2)$$

Where Y_{ijk} refers to the observation of the ith genotype in the jth environment; m is the experimental average; G_i is the effect of the ith genotype (clone); A_j it is the effect of the jth environment; GA_{ij} is the effect of the interaction between the ith genotype and the jth environment and E_{ijk} is the experimental error.

In order to quantify the proportion of total variance due to genotype and environmental effects, estimates of genotypic, environmental and phenotypic variance were obtained using the least squares estimation method (Cruz; Regazzi; Carneiro, 2012). From the variance components were estimated the heritability in the broad sense, the genotypic and environmental coefficients of variation and the intraclass correlation (Rocha et al., 2015).

3 RESULTS AND DISCUSSION

The effect of the genotype x environment interaction was significant for the resistance traits: number of galls, number of eggs and reproduction factor; indicating differences in the resistance response of the clones (Table 2). To achieve higher precision and accuracy of the RF estimates, three biological tests were interpreted.

The change in the resistance response evaluated in different tests can be of simple nature, when there is no alteration in the classification of the resistant genotypes. Or this change can be of complex nature, when there is modification in the RF classification (Cruz; Regazzi; Carneiro, 2012). For this reason the RF estimates were interpreted individually in each experiment (Table 2).

Table 2: Analysis of variance of gall number (NG), number of eggs (NO) and nematode reproduction factor (FR) evaluated at 17 clones of the botanical varieties Conilon and Robusta in experiments carried out in Porto Velho - RO and Ji-Paraná, RO, 150 days after inoculation with 5000 eggs of *Meloidogyne incognita*.

FV	GL	NG[1]	NO[1]	RF
Treatments	16	3.60**	15.66**	24.28**
Genotypes (GEN)	12	1.22^{NS}	16.56**	24.44**
Control (CTRL)	3	14.53**	16.35**	30.03**
Groups	1	8.21*	7.17^{NS}	16.74^{NS}
Environments (ENV)	2	9.09**	14.18**	8.42**
GEN x ENV	32	9.33**	4.28**	5.29**
Residue	255			
Total	305			
Average $_{Overall}$		8.92	246.84	1.94
Average $_{Susceptible\ Control}$		10.23	358.56	2.25
Average $_{Resistant\ Control}$		3.54	39.57	0.37

[1]Data transformed to the square root of the value. **: significant at 1% probability. GL: degrees of freedom, NG: number of galls, NO: number of eggs, RF: Reproduction factor.

The heritability measures the relative proportion between the genotypic and environmental variances in expression of the resistance (Almeida; Cruz; Resende, 2016). The number of eggs (NO) and the reproduction factor (RF) presented mean heritability estimates higher than 90%, indicating predominant genetic control, with potential for selection gains (Table 3). Lower estimate of heritability was observed for the gall number trait, indicating higher environmental influence.

Several factors can affect the results of a challenge between pathogen and host. Biological factors such as inoculant virulence, seedling development, substrate composition, inoculation conditions, greenhouse humidity, temperature and solar irradiation, may be considered (Hua, 2013; Mohawesh; Karajeh, 2014; Carvalho et al., 2015; Özalp; Devran, 2018). In addition to standardizing the conditions of the biological tests, the principles of agricultural experimentation were considered to interpret all these factors together, as effects of the environment.

The characteristics that have the higher coefficients of variation were RF>NO>NG (Table 3). Santos et al. (2017) observed similar magnitude estimates in the the evaluation of the cultivar 'BRS Ouro Preto' to *M. incognita* (Est I2). Estimates of the coefficient of genetic variation (CV_g) above the coefficient of environmental variation (CV_e) characterize a favorable condition to obtain gains with the selection of resistant plants. The CV_g/CV_e ratio showed an amplitude of 0,35 for NG and 2,76 for RF indicating that the second trait had higher genetic variability than the first one.

Table 3: Genetic parameters estimates of nematode number of galls (NG), number of eggs (NO) and reproduction factor (RF) evaluated in 17 clones of the botanical varieties Conilon and Robusta in experiments carried out in Porto Velho - RO and Ji-Paraná, RO, 150 days after inoculation with 5000 eggs of *Meloidogyne incognita*.

Genetic Parameters	NG	NO	RF
σ_g^2	0.03	36.47	2.40
σ_p^2	0.37	5.10	0.25
σ_e^2	0.25	11.60	0.31
h^2	18.16	93.96	95.91
$\hat{\rho}$	4.67	68.60	80.82
CV_g	5.88	42.00	72.50
CV_e	17.83	24.92	28.89
CV_g/CV_e	0.35	1.77	2.76

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

Although used as a criterion to classify plant resistance to nematodes, other authors have reported limitations in the use of the number of galls for the diagnosis of resistance (Saucet et al., 2016; Barcala, et al., 2016; Sato; Kadota; Shirasu, 2019); since resistant plants can form galls in the presence of few nematodes and susceptible plants might not produce galls (Santos et al. 2017). According to Araujo Filho and Dallagnol (2018), the resistance response of plants does not prevent the penetration of roots by juveniles (J2). Lima et al. (2015) show that the defense response of the roots of *C. canephora* was later activated by the formation of giant cells, with inhibition and degradation of the nematode feeding sites, instead of obstructing the root infection. Thus, the characteristic number of galls was not considered for discrimination of resistant genotypes.

As expected, the treatments used as resistant and susceptible controls showed significant differences in the resistance traits means (Table 2). The reproduction factor of 0.37 of the Apoatã-2258 and BRS2299 treatments indicates that these clones, also classified as resistant by Santos et al., (2018a), showed resistance to *M. incognita.* Such results confirm the resistance of the Apoatã cultivar which has been used as an alternative in control of root-knot nematodes. The Apoatã IAC 2258 is recommended as rootstock resistant to *Meloidogyne* spp. in São Paulo for planting grafted seedlings in areas infested with the nematodes *M. exigua* and *M. incognita* (Kofoid & White) Chitwood and *M. paranaensis* (Barbosa et al., 2014; Andreazi et al., 2015).

The reproduction factor of 2.25 estimated from the susceptible treatments C750 and C125 indicates the higher susceptibility of these clones. The use of susceptible clones is also important to check on the inoculum's virulence, understood as the pathogen's ability to multiply within the host (Lima et al., 2015; Peres et al., 2017; Santos et al., 2018b). These results are similar to those obtained by Santos et al. (2017; 2018a) who observed reproduction factor averages of 0.36 and 1.59 in different experiments evaluated in Western Amazonia.

For the classification of resistance of *C. canephora* genotypes to *M. incognita* (Est I2) the clones were ordered according to the reproduction factor reduction (RFR), using Linn and Binns classification criteria that considers performance and stability in all experiments (Table 4). The classification of resistance or susceptibility was based on the criteria presented by Moura and Regis (1987).

The clones BRS 3210, C12, BRS 2314, BRS 3137 and BRS 1216 expressed reduction of the reproduction factor (RF) higher than 51% being considered as resistant. The clone BRS 3210 showed higher reductions in the RF than the positive control BRS 2299. Clones BRS 3213, KM98-0125, C15, BRS 2336 and C09 expressed a reduction in reproduction factor of less than 50% and were classified as susceptible to knot-root nematode *M. incognita*. In turn, clones BRS 2357, KM98-0160 and BRS 3193 were considered very susceptible, being more susceptible than susceptible control C750.

The reproduction factor (RF) showed an amplitude ranging from 0.34 (BRS 3210) to 5.82 (BRS 2357), similar as observed in another studies. Aribi et al. (2018) observed amplitude from 0.0 to 3.1 in the evaluation of 13 genotypes. Amplitude of 0.34 to 8.4 was also observed by Lima et al. (2015), after 8 months of inoculation with a population of 10.000 eggs.

In order to compare the genetic diversity to the resistance response, the clones were grouped according to their morphological characteristics (Figure 1). The plants of the Robusta botanical variety were distinguished by their higher vegetative vigor, which reflect in their higher height, plagiotropic branch length, distance between rosettes, number of rosettes per branch, leaf length and width compared to the clones of the Conilon botanical variety. The dendrogram indicated the formation of four distinct groups according to the similarity of the clones, whether from the botanical variety Conilon, Robusta or intervarietal hybrids (Figure 1).

The group I formed by clones C750, BRS 2357 with traits of the Conilon botanical variety presented higher average reproduction factor (RF = 4.18). The group II formed by clones KM98-0160, KM98-0125, BRS 2336, BRS 2299 and BRS 3137 and the group III by clones BRS 3210, BRS 3213, C15, BRS 3220, BRS 1216, C09, BRS 2314 and BRS 3193 are characterized by clustering plants with hybrid traits among the Conilon botanical varieties and Robusta. These groups had lower mean reproductive factor estimates than the first group of 2.08 and 1.63 respectively. And at last, the group IV formed by the clones Apoatã and C12 that have

Table 4: Reproduction factor (RF) and reproduction factor reduction (RFR) of 17 *Coffea canephora* clones of the Conilon and Robusta botanical varieties in experiments carried out in the municipalities of Porto Velho - RO and Ji-Paraná, RO, 150 days after inoculation with 5000 eggs of *Meloidogyne incognita*.

Genotypes	Exp1	Exp2	Exp3	Average (RF)	Linn and Binns Ordering	RFR (%)	Classification[3]
Apoatã-2258	0.18	0.03	0.03	0.08	1	96.86	Resistant
BRS 3210	0.28	0.32	0.43	0.34	2	86.67	Resistant
C12	0.43	0.35	1.08	0.62	3	75.69	Resistant
BRS 2299[1]	0.78	0.5	0.7	0.66	4	74.12	Resistant
BRS 2314	0.98	0.37	1.03	0.79	5	69.02	Resistant
BRS 3137	0.63	0.92	1.03	0.86	6	66.27	Resistant
BRS 1216	1	0.92	1.02	0.98	7	61.57	Resistant
BRS 3213	1.6	1.8	2.38	1.93	8	21.57	Susceptible
K98M-0125[2]	1.5	1.7	2.65	1.95	9	24.31	Susceptible
C15	1.87	2.23	1.9	2.00	10	23.53	Susceptible
BRS2336	2.78	1.93	2.23	2.31	11	9.41	Susceptible
BRS3220	2.07	1.78	3.1	2.32	12	9.02	Susceptible
C09	2.47	1.87	2.7	2.35	13	7.84	Susceptible
C750[2]	2.22	2.82	2.62	2.55	14	0.00	Highly Susceptible
BRS 3193	2.73	2.25	4.1	3.03	15	-18.82	Highly Susceptible
K98M-0160	4.97	3.07	5.2	4.41	16	-72.94	Highly Susceptible
BRS 2357	5.85	4.2	7.42	5.82	17	-128.24	Highly Susceptible

[1]Resistant control, [2]Susceptible control. Exp1: Porto Velho (September 2017 to February 2018). Exp2: Porto Velho (July to December 2018.), Exp3: Ji-Paraná (July to December 2018). [3]Classification according to Moura and Regis (1987).

predominant Robusta traits, presented lower mean of the reproduction factor (RF = 0,39).

In addition to the lower plant reproduction factor of the Robusta botanical variety, resistance segregation is also observed in hybrid plants, with very resistant hybrid clones such as BRS 3210 and very susceptible ones, like as BRS 3193. The segregation of the resistance response indicates that the genotypes must be evaluated individually regardless of their genealogy or other morphological traits.

Many studies of nematode resistance have indicated the action of few genes with greater effect (Passianotto, et al., 2017; Mota, et al., 2020). There are also other results, that indicate the action of lesser effect genes that have evolved over time, according to the gene-for-gene theory (Bell et al., 2019; Przybylska; Obrępalska-Stęplowska, 2020). In *C. canephora*, the plant-pathogen response mechanism is related to a hypersensitivity response, which occurs shortly after root penetration and establishment of a feeding site, starting an attack and defense gene interaction between pathogen and host (Lima et al., 2015; Maghuly; Jankowicz-Cieslak; Bado, 2020).

This pattern was also observed by Albuquerque et al. (2017) in *C. arabica* response against *M. incognita*. These authors observed that plant defense genes were suppressed in susceptible plants, unlike the resistant ones that showed a rapid hypersensitivity response shortly after the establishment of the feeding site.

According to Ferraz (2018) and Ventura et al. (2019) the strategies to reduce the population of phytonematodes in field are biological, chemical and genetic, the latter being the most efficient and economically viable. Therefore, the selection of resistant clones is one of the most promising alternatives to minimize the damage caused by nematodes in the coffee culture, keeping the nematode populations below the level of economic damage (Fatobene et al., 2018; Rezende et al., 2019; Salgado et al., 2020). The clones BRS 3210, C12, BRS 2314, BRS 3137 and BRS 1216, showed resistance to *M. incognita*. Thus constituting an important factor for the management and control of gall nematodes in the region.

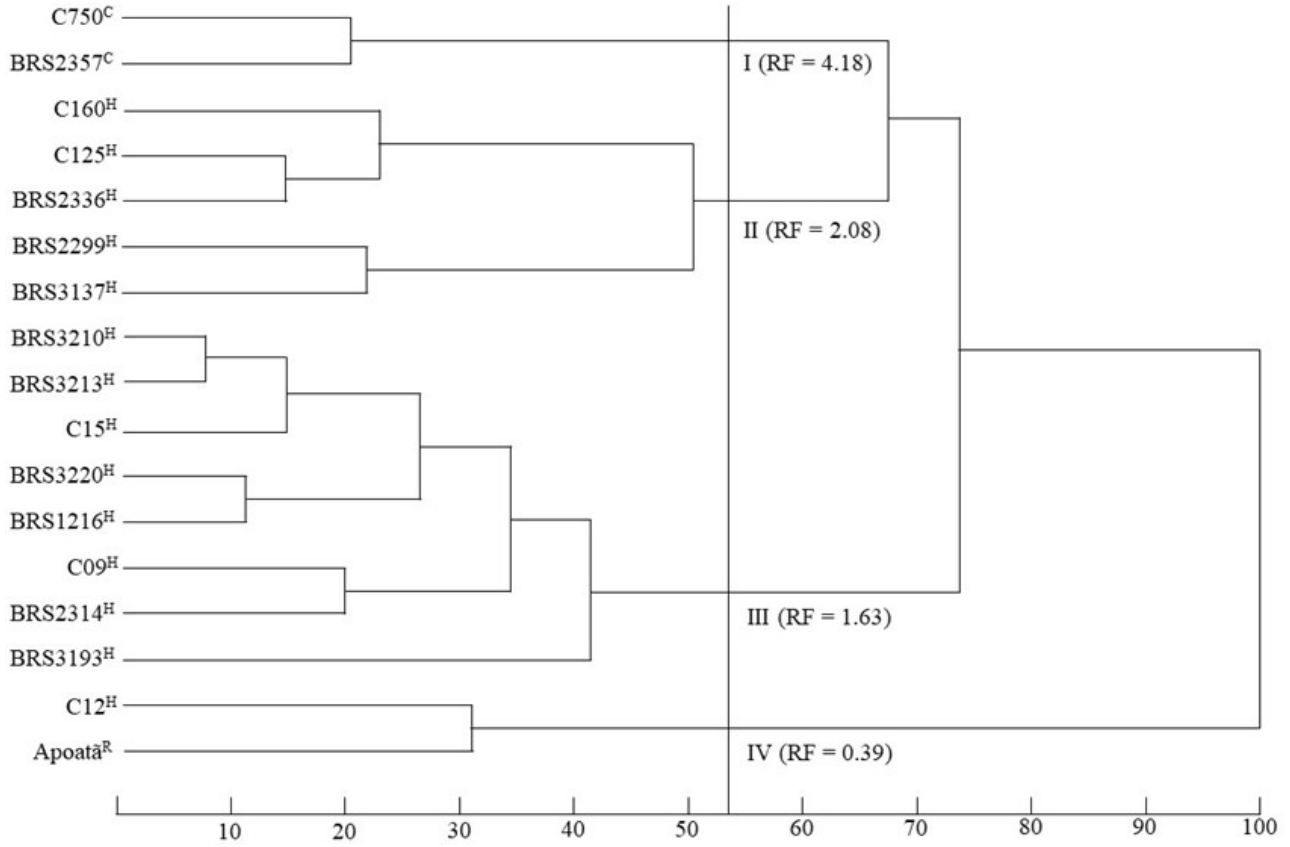

Figure 1: Dendrogram obtained by the UPGMA method, classifying the 17 *Coffea canephora* clones in relation to ten morphological traits evaluated in the first production. The letters C, R and H identify clones with predominant traits of the botanical varieties Conilon, Robusta or intervarietal hybrids. The cutoff point established at the greatest distance between the groups identifies the clusters I, II, III and IV that present different reproductive factors (RF).

4 CONCLUSIONS

At 150 DAI, the *C. canephora* clones BRS 3210, C12, BRS 2299, BRS 2314, BRS 3137 and BRS 1216 expressed resistance response to *M. incognita*, indicating potential for selection in coffee breeding programs of genotypes resistant to root nematodes. The number of eggs + J2 is a more reliable trait, for evaluating resistance to *M. incognita* in *C. canephora*, than to characteristic number of galls. The segregation of the resistance response indicates that the genotypes must be evaluated individually regardless of their genealogy or other morphological traits.

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