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Coffea arabica lines with resistance to nematode *Meloidogyne paranaensis* derived from crossings with IPR 100

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Abstract

The aim of this work was to evaluate the reaction of IPR 100 derived Arabica coffee lines to the nematode *Meloidogyne paranaensis* and to investigate the association between the parameters used to select resistant genotypes. The experiment was carried out in a greenhouse at IAPAR in Londrina - PR, Brazil. The resistance to *M. paranaensis* of nineteen *Coffea arabica* F_3 lines derived from the cross "PRFB E9705-9" × 'IPR 100' and three from the cross 'IPR 100' × "Sarchimor E9601 III-19-1" were assessed. Plants with three to four pairs of leaves were inoculated with 5000 *M. paranaensis* eggs and J_2 juveniles. After 90 days of inoculation, the variables reproduction factor (RF), fresh weight of roots (FWR) and number of eggs and J_2 juveniles per gram of roots (Nematodes.g^{-1}) were assessed. Reduction in the reproduction factor (RRF) and host susceptibility index (HSI) were used to classify the resistant levels. Resistant lines were identified and the use of RRF, HSI and RF indices together helped to identify genotypes with resistance to nematodes. In the present study, statistical difference between the FWR of the genotypes was observed. Thus, if the indices are used alone, it is likely that HSI is better, since it can minimize possible interference in the classifications due to differences between the root volumes of the assessed genotypes. The percentage of plants with different resistance levels based on the classification of RRF and HSI and percentage of resistant plants based on RF are useful to identify homozygous and heterozygous genotypes.

Keyword: coffee, breeding, homozygous resistant, root-knot nematodes.
Abbreviations: IP_initial population; FP_final population; RF_reproduction factor; J2_second stage juveniles; Nematodes_number of eggs and J2; FWR_fresh weight of roots; Nematodes.g^{-1}_number of eggs and second stage juveniles per gram of roots; RL_resistance levels; RRF_reduction in the reproduction factor; HSI_host susceptibility index; HS_highly susceptible; S_susceptible; MS_moderately susceptible; MR_moderately resistant; R_resistant; HR_highly resistant; % PRL_percentage of plants with different resistance levels; %RP_percentage of resistant plants.

Introduction

Coffee is one of the main commodities in international agricultural trade, and Brazil is the largest producer and the second largest consumer (Conab, 2016). The root-knot nematode stands out among the main limiting factors to Brazilian coffee. It belongs to the *Meloidogyne* genus (Chitwood, 1949), and is widely disseminated and distributed in coffee plantations, causing huge losses to producers and to the economy (Campos and Villain, 2005). *Meloidogyne paranaensis* Carneiro et al. (1996) is one of the nematodes that parasitize coffee plants in Brazil. This species induces leaf necrosis, reduces growth, causes leaf fall and general decline of vegetative vigor, and may even cause plant death (Campos and Villain, 2005). Generally, nematode control is difficult to achieve, since in infested areas its eradication is virtually impossible (Gonçalves and Silvarolla, 2007). The main management strategy is to prevent the spread of soil, water and crops contaminated with this pathogen. Other management strategies available are: genetic, chemical, biological and cultural control (Gonçalves and Silvarolla, 2001). Plant resistance has been considered as one of the main nematode management strategies, especially for sedentary endoparasites, such as those from the *Meloidogyne* genus, which have a specialized interaction with their hosts (Roberts, 2002). Resistance to *M. paranaensis* has been found in *Coffea canephora* Pierre ex Froehner (Sera et al., 2006; Gonçalves and Silvarolla, 2007; Andreazi et al., 2015a), *C. congensis* Froehner (Gonçalves et al., 1988) and wild *C. arabica* L. accessions from Ethiopia (Anthony et al., 2003; Boisseau et al., 2009). Arabica coffee carrying *C. canephora* genes, such as "Icatu" and its derivatives (Gonçalves and Silvarolla, 2007; Andreazi et al., 2015b; Shigueoka et al., 2016a) and Híbrido de Timor derivatives (Salgado et al., 2014; Shigueoka et al., 2016b) also showed resistance to this nematode.

Although there are resistance sources to nematodes, few ungrafted Arabica coffee cultivars present resistance. Actually, *C. arabica* cv. IPR 100 is the only one cultivar that has been widely grown in infested areas in Brazil and presents resistance level to *M. paranaensis* similar to root-stock *C. canephora* cv. Apoatã IAC 2258 (Andreazi et al., 2015a; Sera et al., 2017). In the coffee breeding program of IAPAR, Arabica coffee cultivars resistant to nematodes have been developed using 'IPR 100' as resistance source, which is resistant to *M. paranaensis*. In contrary, it is susceptible to leaf rust (*Hemileia vastatrix* Berk. et Br.) (Sera et al., 2010; Del Grossi et al., 2013). For this reason, 'IPR 100' was crossed with rust resistant genotypes to develop cultivars with simultaneous resistance.

To assess the resistance to nematodes in *Coffea* spp., several parameters are used such as gall index (Gonçalves and Pereira, 1998; Noir et al., 2003; Muniz et al., 2009), egg mass index (Gonçalves and Ferraz, 1987, Muniz et al., 2009), reproduction factor (Salgado et al., 2005; Boisseau et al., 2009; Muniz et al., 2009), and number of nematodes per gram of roots (Gonçalves and Ferraz, 1987; Salgado et al., 2005; Boisseau et al., 2009). Some studies used the reduction in reproduction factor (Gonçalves and Pereira, 1998; Salgado et al., 2005; Andreazi et al., 2015) and the host susceptibility index (Gonçalves and Ferraz, 1987; Andreazi et al., 2015) to classify the resistance level of coffee plants. However, there are no studies on the association between these different parameters.

Therefore, the objective of this study was to evaluate the response of Arabica coffee lines derived from IPR 100 to *M. paranaensis* nematode and to investigate the association between the parameters used to select resistant genotypes.

Results and Discussion

Resistance to Meloidogyne paranaensis

Four lines presented less Nematodes.g^{-1} and statistically differed from the resistant check 'Apoatã IAC 2258'. Using these variables, nineteen F_3 lines did not statistically differ from the resistant check (Table 1).

Based on the mean values of RRF, 12 lines were classified as HR; 3 as MR; 6 as MS; and 1 as S, while the resistant check was MR. These 12 genotypes showed 100% RP (HR, R and MR) (Table 2), and showed RF lower than 1.00, whereas in resistant and susceptible checks RF values were 1.95 and 12.28, respectively (Table 1). Ten genotypes exhibited 100% classified as HR or R by RRF (Table 2).

Based on the mean values of HSI, 12 lines were classified as HR; 5 as MR; four as MS; and 1 as S, while the resistant check was MR (Table 3). Eleven genotypes showed 100% HR or R plants.

The susceptible check presented 100% of susceptible plants by both RRF and HSI indices. The 12 genotypes classified as HR by RRF had the same classification by HSI. Furthermore, they presented 100% RP. Therefore, in these 12 lines, the resistance is in homozygous condition. Eight of these 12 genotypes also showed 100% RP by RF (Table 4). The high %RP in the lines of this study can be explained by the fact that the F_3 generation was installed in areas infested by *M. paranaensis,* and thus coffee plants with high yield selected in these areas, likely with resistant status. In contrast, low yield coffee plants were susceptible and were not selected.

The resistant check Apoatã IAC 2258 was classified as MR both by RRF and HSI indices. This is because in 'Apoatã IAC 2258', 25.00% and 33.34% susceptible plants were observed by RRF and HSI indices, respectively. There are some disadvantages of using rootstock compared to ungrafted cultivars, such as segregation rate for susceptibility to nematodes of 10 to 15% due to the reproductive system of *C. canephora*, which has cross-pollination (Gonçalves and Silvarolla, 2007). The resistance level of 'Apoatã IAC 2258' would be similar to the F_3 resistant lines if there was no segregation of susceptible plants.

In this study, the assessed lines originated from the cross "PRFB E9705-9" × 'IPR 100' and 'IPR 100' × "Sarchimor E9601 III-19-1". In 10 genotypes, segregating susceptible plants were observed in nine lines derived from the parental "PRFB E9705-9", and one from the parental "Sarchimor E9601 III-19-1", indicating that these parents are susceptible, since 'IPR 100' is resistant (Ito et al., 2008; Salgado et al., 2014; Andreazi et al., 2015). The 'IPR 100' originated from the cross "Catuaí" × ("Catuaí" x "BA-10"), was similar to "PRFB E9705-9". It is likely that resistance comes from "BA-10", which is an Arabica coffee carrying *C. liberica* genes.

To date, 'IPR 100' (Sera et al., 2007; Ito et al., 2008; Salgado et al., 2014; Andreazi et al., 2015a) and 'IPR 106' (Ito et al., 2008) are Arabica coffee cultivars identified as resistant to this nematode. 'IPR 100' was released in 2012 and has already been planted by farmers in areas infested with *M. paranaensis*. The 12 F_3 lines which have been classified as HR by RRF and HSI will be advanced to the next self-pollinating generation and they have great potential to become new Arabica coffee cultivars resistant to *M. paranaensis.*

Correlation between RRF and HSI, and between %RP by RF, RRF and HSI indices

The Spearman's correlation coefficient between RRF and HSI was -0.8615 (p <0.0001), i.e., RRF and HSI indices correlated negatively, indicating that the increase in RRF value was associated with the decrease of HSI value. Thus, this negative correlation is consistent with the results obtained in this study. In general, it was observed that genotypes classified as resistant (HR, R or MR) by RRF had the same classification by HSI. The exceptions were IAPAR 12142, IAPAR 12149 e IAPAR 12151, which were classified as MS by RRF and as MR by HSI, besides IAPAR 12141, which was classified as MR by RRF and as MS by HSI.

As previously reported, the 12 lines classified as HR by RRF were also HR by HSI, and showed 100% RP in both indices. Using RRF in susceptible check, 75% plants were HS and 25% were S, while in HSI, 66.67% plants were HS, 25.00% were S, and 8.33% were MS. Correlations were statistically significant (p-value <0.0001) between %RP by RF and RRF, %RP by RF and HSI, and %RP by RRF and HSI. The values of the correlations were 0.9413, 0.8973 and 0.9331, respectively, indicating that the associations between these %RP using the three parameters were high. However, in general, lower %RP was observed when RF was used, compared to %RP by RRF and HSI (Table 4). Based on %RP by RF, 8 resistant homozygous genotypes were observed, whereas 12 were resistant homozygous genotypes by RRF and HSI. Therefore, using %RP by RF was more difficult to identify resistant homozygous, or even heterozygous genotypes. Through %RP by RF, higher disposal of resistant genotypes may occur, since heterozygous by RRF and HSI would not be selected by RF, as in the cases of IAPAR 12136 and IAPAR 12149. However, it is interesting to consider RF in the selection. It would be a mistake to select genotypes with 100% of plants classified as MR, R or HR by RRF and HSI, but with RF>1.0, since planting these genotypes in the field could lead to an increase in the nematode population.

Table 1. Mean of FWR, Nematodes.g^{-1}, and RF of *M. paranaensis* in Arabica coffee F_3 lines.

F_3 lines	FWR (g) [(1)]	Nematodes.g^{-1} [(1)]	RF [(1)]
IAPAR 12146	10.84 c	134.65 a	0.30 a
IAPAR 12145	9.31 c	154.61 a	0.32 a
IAPAR 12144	9.74 c	158.33 a	0.32 a
IAPAR 12148	9.94 c	166.43 a	0.27 a
IAPAR 12139	11.85 c	229.03 b	0.47 a
IAPAR 12150	9.43 c	258.17 b	0.38 a
IAPAR 12140	10.61 c	258.63 b	0.52 a
IAPAR 12147	3.78 a	269.12 b	0.20 a
IAPAR 12133	10.32 c	327.69 b	0.62 a
IAPAR 12153	4.79 a	415.63 b	0.36 a
IAPAR 12137	7.31 b	428.35 b	0.51 a
IAPAR 12152	4.18 a	446.43 b	0.35 a
IAPAR 12132	7.50 b	943.34 b	2.55 b
IAPAR 12149	6.26 b	960.90 b	3.11 b
IAPAR 12142	12.87 c	1250.32 b	3.49 b
IAPAR 12141	6.89 b	1435.59 b	2.44 b
IAPAR 12151	11.37 c	1505.94 b	4.05 b
'Apoatã IAC 2258' (resistant check)	8.41 b	1642.58 b	1.95 b
IAPAR 12135	8.30 b	1738.58 b	5.10 c
IAPAR 12143	4.58 a	1930.58 b	1.96 b
IAPAR 12134	7.69 b	2053.92 b	4.17 b
IAPAR 12138	13.23 c	2059.88 b	5.85 c
IAPAR 12136	6.81 b	4630.26 c	8.68 c
'Catuaí V. IAC 81' (susceptible check)	6.32 b	9639.71 d	12.28 d
Mean	8.43	1766.30	2.51
CV (%)	45.22	29.37	31.03

[(1)] Means followed by the same letter do not differ by the Scott-Knott test (p=0.05). Data of Nematodes.g^{-1} were transformed to log (x + 1).

Table 2. Means of reduction in reproduction factor (RRF), resistance level (RL), and percentage of highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) coffee plants to the nematode *Meloidogyne paranaensis* based on RRF.

F_3 lines	RRF[(1)]	RL[(2)]	HR%	R%	MR%	MS%	S%	HS%
IAPAR 12147	98.34 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12148	97.82 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12146	97.53 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12144	97.39 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12145	97.39 a	HR	83.34	8.33	8.33	0.00	0.00	0.00
IAPAR 12153	97.07 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12152	97.04 a	HR	75.00	25.00	0.00	0.00	0.00	0.00
IAPAR 12150	96.88 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12139	96.15 a	HR	66.67	33.33	0.00	0.00	0.00	0.00
IAPAR 12137	95.82 a	HR	83.34	8.33	8.33	0.00	0.00	0.00
IAPAR 12140	95.79 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12133	95.01 a	HR	50.00	50.00	0.00	0.00	0.00	0.00
'Apoatã IAC 2258'[(3)]	84.12 a	MR	50.00	0.00	25.00	8.33	16.67	0.00
IAPAR 12143	84.02 a	MR	41.67	25.00	8.33	16.67	8.33	0.00
IAPAR 12141	80.14 a	MR	50.00	25.00	0.00	8.34	8.33	8.33
IAPAR 12132	79.24 a	MR	50.00	33.34	0.00	8.33	0.00	8.33
IAPAR 12149	74.65 a	MS	33.33	16.67	0.00	41.67	0.00	8.33
IAPAR 12142	71.61 a	MS	50.00	16.67	8.33	0.00	0.00	25.00
IAPAR 12151	67.03 a	MS	50.00	25.00	0.00	8.33	0.00	16.67
IAPAR 12134	66.05 a	MS	33.34	33.34	8.33	0.00	8.33	16.66
IAPAR 12135	58.51 b	MS	41.67	16.67	8.33	0.00	0.00	33.33
IAPAR 12138	52.40 b	MS	16.67	33.33	16.67	0.00	0.00	33.33
IAPAR 12136	29.36 a	S	25.00	0.00	25.00	0.00	0.00	50.00
'Catuaí V. IAC 81'[(4)]	0.00 a	HS	0.00	0.00	0.00	0.00	25.00	75.00
Mean	79.56							
CV (%)	45.05							

[(1)] Means followed by the same letter do not differ by the Scott-Knott test (p=0.05). [(2)] RL were based on means of RRF. [(3)] Resistant check. [(4)] Susceptible check.

Table 3. Means of host susceptibility index (HSI), resistance level (RL), and percentage of highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and highly susceptible (HS) coffee plants to the nematode *Meloidogyne paranaensis* based on HSI.

F_3 lines	HSI [(1)]	RL[(2)]	HR %	R %	MR %	MS %	S %	HS %
IAPAR 12146	1.28 a	HR	100.00	0.00	0.00	0.00	0.00	0.00
IAPAR 12145	1.47 a	HR	100.00	0.00	0.00	0.00	0.00	0.00
IAPAR 12144	1.50 a	HR	100.00	0.00	0.00	0.00	0.00	0.00
IAPAR 12148	1.58 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12139	2.17 a	HR	100.00	0.00	0.00	0.00	0.00	0.00
IAPAR 12140	2.45 a	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12150	2.45 a	HR	83.33	16.67	0.00	0.00	0.00	0.00
IAPAR 12147	2.55 a	HR	83.33	16.67	0.00	0.00	0.00	0.00
IAPAR 12133	3.11 b	HR	91.67	8.33	0.00	0.00	0.00	0.00
IAPAR 12153	3.94 b	HR	66.67	33.33	0.00	0.00	0.00	0.00
IAPAR 12137	4.06 b	HR	66.67	33.33	0.00	0.00	0.00	0.00
IAPAR 12152	4.24 b	HR	66.67	33.33	0.00	0.00	0.00	0.00
IAPAR 12142	11.86 b	MR	66.67	8.33	0.00	8.33	8.33	0.00
IAPAR 12151	14.29 b	MR	66.67	0.00	8.33	0.00	25.00	0.00
'Apoatã IAC 2258'[(3)]	15.59 b	MR	50.00	8.33	8.33	33.33	0.00	0.00
IAPAR 12143	18.32 c	MR	33.33	16.67	16.67	16.67	16.67	0.00
IAPAR 12132	19.02 c	MR	66.67	16.67	0.00	0.00	8.33	8.33
IAPAR 12149	21.95 c	MR	41.67	8.33	41.67	0.00	0.00	8.33
IAPAR 12141	23.77 c	MS	58.33	16.67	0.00	8.33	0.00	16.67
IAPAR 12138	27.66 c	MS	58.33	8.33	0.00	8.33	8.33	16.67
IAPAR 12134	30.28 c	MS	58.33	16.67	0.00	0.00	0.00	25.00
IAPAR 12135	35.58 c	MS	50.00	16.67	0.00	0.00	16.67	16.67
IAPAR 12136	53.12 d	S	25.00	0.00	25.00	0.00	8.33	41.67
'Catuaí V. IAC 81'[(4)]	100.00 e	HS	0.00	0.00	0.00	8.33	25.00	66.67
Mean	16.76							
CV (%)	58.64							

[(1)] Means followed by the same letter do not differ by the Scott-Knott test (p=0.05). [(2)] RL were based on means of HSI. [(3)] Resistant check.[(4)] Susceptible check.

Table 4. Percentage of resistant plants (%RP) based on the reproduction factor (RF), reduced in reproduction factor (RRF) and host susceptibility (HSI) indices.

F_3 lines	%RP by RF[(1)]	%RP by RRF[(2)]	%RP by HSI[(3)]
IAPAR 12132	66.67	83.34	75.00
IAPAR 12133	100.00	100.00	100.00
IAPAR 12134	58.33	75.00	75.00
IAPAR 12135	50.00	66.67	66.67
IAPAR 12136	25.00	50.00	50.00
IAPAR 12137	91.67	100.00	100.00
IAPAR 12138	50.00	66.67	66.67
IAPAR 12139	100.00	100.00	100.00
IAPAR 12140	91.67	100.00	100.00
IAPAR 12141	66.67	75.00	75.00
IAPAR 12142	66.67	75.00	75.00
IAPAR 12143	66.67	75.00	66.67
IAPAR 12144	100.00	100.00	100.00
IAPAR 12145	91.67	100.00	100.00
IAPAR 12146	100.00	100.00	100.00
IAPAR 12147	100.00	100.00	100.00
IAPAR 12148	91.67	100.00	100.00
IAPAR 12149	41.67	50.00	91.67
IAPAR 12150	100.00	100.00	100.00
IAPAR 12151	66.67	75.00	75.00
IAPAR 12152	100.00	100.00	100.00
IAPAR 12153	100.00	100.00	100.00
'Apoatã IAC 2258'[(4)]	50.00	75.00	66.67
'Catuaí V. IAC 81'[(5)]	0.00	0.00	0.00
Mean	73.96	81.95	82.64

[(1)] Plants with RF $\leq$ 1.0 were considered resistant. [(2)] Plants classified as HR, R and MR by RRF were considered resistant. [(3)] Plants classified as HR, R and MR by HSI were considered resistant. [(4)] Resistant check. [(5)] Susceptible check.

Table 5. Arabica coffee F_3 lines derivative from the hybridizations "PRFB E9705-9" x 'IPR 100' and 'IPR 100' x "Sarchimor E9601 III-19-1" assessed for resistance to the nematode *Meloidogyne paranaensis.*

F_3 lines	Genealogy	Hybridization [(1)]
IAPAR 12132	H9932-01-87-34	"PRFB E9705-9" x 'IPR 100'
IAPAR 12133	H9932-05-12-38	"PRFB E9705-9" x 'IPR 100'
IAPAR 12134	H9932-05-12-49	"PRFB E9705-9" x 'IPR 100'
IAPAR 12135	H9932-05-14-22	"PRFB E9705-9" x 'IPR 100'
IAPAR 12136	H9932-05-14-29	"PRFB E9705-9" x 'IPR 100'
IAPAR 12137	H9932-07-81-02	"PRFB E9705-9" x 'IPR 100'
IAPAR 12138	H9932-07-81-60	"PRFB E9705-9" x 'IPR 100'
IAPAR 12139	H9932-08-94-15	"PRFB E9705-9" x 'IPR 100'
IAPAR 12140	H9932-08-94-54	"PRFB E9705-9" x 'IPR 100'
IAPAR 12141	H9932-08-97-17	"PRFB E9705-9" x 'IPR 100'
IAPAR 12142	H9932-08-97-32	"PRFB E9705-9" x 'IPR 100'
IAPAR 12143	H9932-08-110-19	"PRFB E9705-9" x 'IPR 100'
IAPAR 12144	H9932-08-98-38	"PRFB E9705-9" x 'IPR 100'
IAPAR 12145	H9933-03-35-43	"PRFB E9705-9" x 'IPR 100'
IAPAR 12146	H9933-03-35-44	"PRFB E9705-9" x 'IPR 100'
IAPAR 12147	H9933-03-51-24	"PRFB E9705-9" x 'IPR 100'
IAPAR 12148	H9933-17-43-12	"PRFB E9705-9" x 'IPR 100'
IAPAR 12149	H9933-17-43-29	"PRFB E9705-9" x 'IPR 100'
IAPAR 12150	H9934-07-51-10	"PRFB E9705-9" x 'IPR 100'
IAPAR 12151	H9921-02-71-13	'IPR 100' x "Sarchimor E9601 III-19-1"
IAPAR 12152	H9921-07-102-3	'IPR 100' x "Sarchimor E9601 III-19-1"
IAPAR 12153	H9921-07-102-5	'IPR 100' x "Sarchimor E9601 III-19-1"
IAPAR 12154	'Catuaí Vermelho IAC 81'	susceptible check
IAPAR 12155	'Apoatã IAC 2258'	resistant check

[(1)] "PRFB E9705-9" = ["Catuaí" x ("Catuaí" x "BA-10")].

The use of RRF and HSI indices together can help to identify genotypes resistant to nematodes. Few differences were observed between the two indices. By HSI, higher percentage of HR plants was observed. However, if the indices are used alone it is likely that HSI is better. The HSI takes the number of Nematodes.g^{-1} into account to classify the resistance levels, and may minimize the possible interference in the classification due to differences between the root volumes of the assessed genotypes. In the present study, statistical difference between the fresh weight of roots of genotypes it was observed (Table 1) and resistant genotypes tended to present a more developed root system. Boisseau et al. (2009) reported that it was important to use the Nematodes.g^{-1} variable in addition to the total number of nematodes extracted per plant, since the coffee plant tested for *M. paranaensis* presented statistical differences in the weight of roots.

Materials and methods

Plant materials

In year 1999, three artificial hybridizations (H9932, H9933, H9934) from three different "PRFB E9705-9" F_4 plants at Instituto Agronômico do Paraná (IAPAR), Londrina was carried out having 'IPR 100' as pollinator. "PRFB E9705-9" originated from the cross "Catuaí" × ("Catuaí" x "BA-10 coffee"). An artificial hybridization (H9921) using 'IPR 100' as mother plant and a "Sarchimor E9601 III-19-1" F_6 plant as pollinator were also carried out. F_4 seeds were collected from 22 individual plants of the F_3 lines, derived from the H9932, H9933, H9934 and H9921, which showed desirable agronomic traits in an area infested with *M. paranaensis* in Lupionópolis, PR, Brazil. Afterwards, plants were tested for resistance to *M. paranaensis* in this study (Table 5).

Experimental conduction and installation

The experiment was carried out in a greenhouse at the IAPAR, in Londrina-PR, Brazil (lat. 23°21'20,0"S; long. 51°09'58,2"W), between February and June 2012. *C. arabica* cv. Catuaí Vermelho IAC 81 and *C. canephora* cv. Apoatã IAC 2258 were used as susceptible and resistance checks, respectively. The experiment was installed in a randomized blocks design with 24 treatments, 12 replications and one plant per plot. The average maximum and minimum temperature during the period of the experiment was 35.3°C and 22.2°C, respectively. Seedlings were obtained by sowing in germinators containing sand. When plants reached the cotyledon stage, they were transplanted into 700ml plastic cups to complete their development, until they presented three to four pairs of leaves. After that, they were inoculated. The substrate was formulated containing a mixture of soil and sand (1:1), previously sterilized in an oven at 100°C for three hours with moisture in field capacity. For every 72 liters of soil, 230 g of super simple phosphate, 22 g KCl; 24 g urea and 72 g dolomitic limestone were added. Fertilization and pH correction were carried out as a result of the chemical analysis of the soil.

Quantification and inoculation of nematodes

M. paranaensis inoculum was obtained from the municipality of Apucarana (Paraná, Brazil) and recorded in the Nematology Laboratory of IAPAR under 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 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 J_2

were extracted from the roots of coffee plants and inoculated into cv. Santa Clara.

Eggs and J_2 were extracted from tomato roots using the Boneti and Ferraz (1981) method and the suspension was calibrated to 1000 eggs and J2/mL. Five thousand *M. paranaensis* (IP) eggs and J2 were inoculated in three holes of approximately 1 cm depth carried out with a glass rod around the plants.

Resistance assessment

The assessments were carried out 90 days after inoculation. The shoot was discarded and the root systems were collected, washed in running water and weighted. Then, the extraction of the eggs and J_2 was carried out, using the methodology proposed by Boneti and Ferraz (1981). After extraction, the FP of *M. paranaensis* of the plants was measured by counting the number of eggs and J_2 (Nematodes) per root system, using the Peters chamber under an optical microscope. With the data of FWR and of the quantification of nematodes the Nematodes.g^{-1} was determined. The RF was calculated using the formula: RF = FP/IP (Oostenbrink, 1966).

Classification of resistance levels

To classify the RL of the lines, RRF and HSI were used. RRF was calculated based on the formula: RRF = [(RF of the susceptible check - RF of the treatment) / RF of the susceptible check] x 100 (Moura & Regis, 1987). Based on RRF, genotypes were classified according to the modified scale of Moura and Regis (1987), where: < 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.

HSI was obtained using the formula HSI = (Nematodes.g^{-1} of the treatment/ Nematodes.g^{-1} of the susceptible check) x 100. HSI values were used to classify the resistance levels, as follows: 0.00 to 5.00% = HR; 5.01 to 10.00% = R; 10.01 to 25.00% = MR; 25.01 to 50.00% = MS; 50.01 to 75.00% = S; > 75.00% = HS.

For each genotype, the mean RF, RRF and HSI was calculated based on the data of the mean plots. Since the mean values of RRF and HSI are based on the values of RF and Nematodes.g^{-1} of the susceptible check, respectively, the RRF and HSI values of this check were 0.00.

Homozygous and heterozygous genotypes for resistance

To identify homozygous and heterozygous genotypes, the % PRL was calculated. %PRL was based on the classification of RRF and HSI, and was calculated using RF and Nematodes.g^{-1} data of individual plots of each treatment, including individual plots of the susceptible check, in addition to the mean data of the plots of RF and Nematodes.g^{-1} of the susceptible check. Therefore, RRF formula was RRF = [(mean of the plots of RF of the susceptible check - RF of the individual plot)/ mean of the plots of RF of the susceptible check] x 100; and HSI formula was HSI = (Nematodes.g^{-1} of the individual plot/mean of the plots of Nematodes.g^{-1} of the susceptible check) x 100. %PRL was calculated based on the mean of the plots of the susceptible check to calculate %PRL of this check and also to facilitate the identification of homozygous resistant genotypes. This is because if the data used was those of the individual plots of the susceptible check, it would increase the percentage of susceptible plants of the genotypes, in cases in which some plots of this check presented lower values of RF and Nematodes.g^{-1}.

The %RP based on RF was calculated classifying genotypes with RF ≤ 1 as resistant, and RF> 1 as susceptible (Sasser et al., 1984 with modifications). The %RP was also calculated based on RRF and HSI, considering HR, R, and MR plants as resistant, and MS, S and HS plants as susceptible. Using %RP of RRF and HIS, the resistant homozygous genotypes were those with 100% resistant plants (HR, R, MR), and heterozygous were those with up to 50% susceptible plants.

Statistical analysis

FWR, Nematodes.g^{-1}, RF, RRF and HSI data were subjected to the Shapiro-Wilk normality test and to the Hartley's *Fmax* test. Data were transformed to log (x + 1) for variable Nematodes.g^{-1}. After data transformations, analysis of variance and the Scott-Knott mean clustering test (p=0.05) were conducted. The association between RRF and HSI indices was assessed using the Spearman's correlation coefficient (p<0.0001).

Conclusion

Ten Arabica coffee F_3 lines derived from the cross "PRFB E9705-9" x 'IPR 100' and two lines from the cross 'IPR 100' x "Sarchimor E9601 III-19-1" are homozygous resistant to the nematode *Meloidogyne paranaensis.* The use of RRF, HSI and RF indices together helped identify genotypes resistant to nematodes. The %PRL based on the classification of RRF and HSI and %RP based on RF were useful to identify homozygous and heterozygous genotypes.

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References

Andreazi E, Sera GH, Faria RT de, Sera T, Fonseca IC de B, Machado ACZ, Shigueoka LH, Carvalho FG, Carducci FC (2015a) Behavior of 'IPR 100' and 'Apoatã IAC 2258' coffee cultivars at different levels of *Meloidogyne paranaensis* inoculum. Aust J Crop Sci. 9: 1069-1074.

Andreazi E, Sera GH, Faria RT de, Sera T, Shigueoka LH, Carvalho FG, Carducci FC (2015b) Resistance to *Meloidogyne paranaensis* in *Coffea arabica* L. progenies. Austr J Crop Sci. 9: 1190-1196.

Anthony F, Topart P, Astorga C, Anzueto F, Bertrand B (2003) La resistencia genética de *Coffea* spp. a *Meloidogyne paranaensis*: identificación y utilización para la caficultura latinoamericana. Manejo Integr Plagas Agroec. 67: 5-12.

Boisseau M, Aribi J, Sousa FR de, Carneiro RMDG, Anthony F (2009) Resistance to *Meloidogyne paranaensis* in wild *Coffea arabica*. Trop Plant Pathol. 34: 38-41.

Bonetti JI, Ferraz S (1981) Modificações no método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. Fitopat Bras. 6: 533.

Campos VP, Villain L (2005) Nematode parasites of coffee, cocoa and tea. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB Int, Wallingford UK. 529-579.

Carneiro RMDG, Almeida MRA, Quénéhervé P (2000) Enzyme phenotypes of *Meloidogyne* spp. populations. Nemat. 2: 645-654.

Carneiro RMDG, Carneiro RG, Abrantes IMO, Santos MSNA, Almeida MRA (1996) *Meloidogyne paranaensis* n. sp. (Nemata: Meloidogynidade), a root-knot nematode parasiting coffee in Brazil. J Nemat. 28: 177-189.

Conab (Companhia Nacional de Abastecimento) (2016). Acompanhamento da Safra Brasileira de Café, Primeiro Levantamento, jan. 2016. Conab, Brasília.

Del Grossi L, Sera T, Sera GH, Fonseca IC de B, Ito DS, Shigueoka LH, Andreazi E, Carvalho FG (2013) Rust Resistance in Arabic Coffee Cultivars in Northern Paraná. Braz Arch Biol Tech. 56: 27-33.

Gonçalves W, Ferraz LCCB (1987) Resistência do cafeeiro a nematoides. II. Teste de progênies e híbridos para *Meloidogyne incognita* raça 3,1. Nemat Bras. 11: 125-142.

Gonçalves W, Lima MMA de, Fazuoli LC (1988) Resistência do cafeeiro a nematoides: III. Avaliação da resistência de espécies de *Coffea* e de híbridos interespecíficos a *Meloidogyne incognita* raça 3. Nemat Bras. 12: 47-54.

Gonçalves W, Pereira AA (1998) Resistência do cafeeiro a nematoides IV – Reação de cafeeiros derivados do Híbrido de Timor a *Meloidogyne exigua*. Nemat Bras. 22: 39-50.

Gonçalves W, Silvarolla MB (2001) Nematoides parasitos do cafeeiro. In: Zambolim L (ed) Tecnologias de produção de café com qualidade. UFV, Viçosa. p. 199-268.

Gonçalves W, Silvarolla MB (2007) A luta contra a doença causada pelos nematoides parasitos do cafeeiro. O Agronôm. 59: 54-56.

Hartman R, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (eds) An advanced treatise on *Meloidogyne*, v 2, Methodology. NCSU Graphics, Raleigh. 115-123.

Ito DS, Sera GH, Sera T, Santiago DC, Kanayama FS, Del Grossi L (2008) Progênies de café com resistência aos nematoides *Meloidogyne paranaensis* e raça 2 de *Meloidogyne incognita.* Coff Sci. 3: 156-163.

Moura R, Regis EMO (1987) Reações de cultivares de feijoeiro comum (*Phaseolus vulgaris*) em relação ao parasitismo de *Meloidogyne javanica* e *M. incognita*. Nemat Bras. 11: 215-225.

Muniz M de FS, Campos VP, Moita AW, Gonçalves W, Almeida MRA,Sousa FR de, RMDG Carneiro. (2009) Reaction of coffee genotypes to different populations of *Meloidogyne* spp.: detection of a naturally virulent *M. exigua* population*.* Trop Plant Pathol. 34: 370-378.

Noir S, Anthony F, Bertrand B, Combres MC, Lashermes P (2003) Identification of major gene (*Mex*-1) from *Coffea canephora* confering resistance to *M. exigua* in *Coffea arabica.* Plant Pathol. 52: 97-103.

Oostenbrink M (1966) Major characteristic of the relation between nematodes and plants. Meded. Landbouwhogesch., Wageningen. 66. 46 p.

Salgado SML, Resende MLV, Campos VP (2005) Reprodução de *Meloidogyne exigua* em cultivares de cafeeiros resistentes e suscetíveis. Fitopat Bras. 30: 413-415.

Salgado SML, Rezende JC de, Nunes JAR (2014) Selection of coffee progenies for resistance to nematode *Meloidogyne paranaensis* in infested area. Crop Breed Appl Biotech. 14: 94-101.

Sasser JN, Carter CC, Hartman KM (1984) Standardization of host suitability studies and reporting of resistance to root-knot nematodes. North Carolina State University.

Shigueoka LH, Sera GH, Sera T, Fonseca IC de B, Andreazi E, Carvalho FG, Carducci FC, Ito DS (2016a) Reaction of Arabica coffee progenies derivative from Icatu to *Meloidogyne paranaensis*. Bragantia. 75: 193-198.

Shigueoka LH, Sera GH, Sera T, Silva SA, Fonseca IC de B, Machado ACZ (2016b) Host reaction of arabica coffee genotypes derived from Sarchimor to *Meloidogyne paranaensis*. Nematoda. 3: 10-16.

Sera GH, Sera T, Ito DS, Mata JS da, Doi DS, Azevedo JA de, Ribeiro-Filho C (2007) Progênies de *Coffea arabica* cv IPR 100 resistentes ao nematoide *Meloidogyne paranaensis*. Bragantia. 66: 43-49.

Sera GH, Sera T, Fonseca IC de B, Ito DS (2010) Resistência à ferrugem alaranjada em cultivares de café. Coff Sci. 5: 59-66.

Sera T, Sera GH, Fazuoli LC, Machado ACZ, Ito DS, Shigueoka LH, Silva SA da (2017) IPR 100 – Rustic dwarf Arabica coffee cultivar with resistance to nematodes *Meloidogyne paranaensis* and *M. incognita*. Crop Breed Appl Biotech. 17: 175-179