

Research Note

Resistance to nematode *Meloidogyne paranaensis* in Arabica coffee genotypes introgressed with *Coffea liberica*

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Abstract

The aim of this study was to prove that Arabica coffee introgressed with *C. liberica*, have resistance to *Meloidogyne paranaensis* (Mp). Open pollinated fruits were harvested from mother plants of 29 Arabica coffee genotypes from the IAPAR germplasm bank. Seeds were collected from the fruits and were sown to obtain seedlings to test the resistance to Mp. The experiment was set up in a completely randomized design with 29 coffee genotypes, 8 replications, and one plant per plot. Cultivars Catuaí Vermelho IAC 99 and IPR 100 were used as susceptible and resistant checks, respectively. Seedlings with three to four pairs of leaves were inoculated with 1,400 eggs and juveniles J2 of Mp (IP). At 120 days after inoculation, seedlings were evaluated by counting the nematodes per gram of roots, and the final nematodes population was obtained (FP). The reproduction factor (RF) was calculated using the formula:  $RF = FP/IP$ . The reproduction factor reduction was used to classify the resistance levels of genotypes, which were classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS). All genotypes differed from Catuaí in resistance factor (RF), five of which did not differ from IPR 100 for RF, and only the line IAPAR 15242 had  $RF < 1.0$ . Out of 28 Arabica genotypes introgressed with *C. liberica*, five HR, 11 R, 11 MR and one MS were identified. However, only IAPAR 15242 and IPR 100 were classified as HR and presented 100% of HR plants, but only the first showed an  $RF < 1.0$ . Results revealed that these Arabica coffee genotypes with introgression of *C. liberica* genes have great potential to be used in breeding programs and they are a new alternative as a source of resistance.

**Keywords:** BA coffees, breeding, root-knot nematodes, *S<sub>H3</sub>* gene.

**Abbreviations:** Mp\_ *Meloidogyne paranaensis*; RF\_reproduction factor; RFR\_ reproduction factor reduction; HR\_highly resistant; R\_resistant; % PRL\_percentage of plants with different resistance levels; MR\_moderately resistant; HS\_highly susceptible; S\_susceptible; MS\_moderately susceptible; IP\_initial population; FP\_final population; J2\_second stage juveniles; NGR\_nematodes per gram of roots.

Introduction

Brazilian coffee crops are subject to great economic losses due to phytonematode *Meloidogyne paranaensis* Carneiro et al. (1996). This nematode affects coffee trees by inducing leaf necrosis, reducing growth, causing leaf fall and the general decline of the plant. It can even lead to plant death (Campos and Villain, 2005).

In Brazil, *Meloidogyne paranaensis* (Mp) is widely disseminated and has been found in many coffee regions such as states of Paraná (Carneiro et al., 1996), São Paulo (Carneiro et al., 2005), Minas Gerais (Castro et al., 2008; Salgado et al., 2015) and Espírito Santo (Barros et al., 2011). This nematode also affects coffee plantations in Guatemala (Carneiro et al., 1996; Carneiro et al., 2004) and México (Lopez-Lima et al., 2015).

Phytonematodes are difficult to control, and their eradication is practically impossible in contaminated areas. Genetic, chemical, biological, and cultural managements can be used in these areas to reduce the nematode population (Gonçalves and Silvarolla, 2007). For perennial crops such as coffee, the use of resistant cultivars is the more efficient measure to control Mp.

In the past, hypocotyl grafting was the only procedure recommended for nematode-infested areas, using the *Coffea canephora* cv. Apoatã IAC-2258 as rootstock [resistant to *M. exigua* (Salgado et al., 2005), *M. incognita* (Sera et al., 2006), and Mp (Andreazi et al., 2015a)]. Nowadays, besides the rootstock Apoatã, the cultivar IPR 100 [*Coffea arabica*, resistant to Mp and *M. incognita*,

released in 2012 (Sera et al., 2017)], has also been recommended for infested areas without the requirement of grafting. In 2017, IAPAR released another Arabica cultivar, named IPR 106, which is resistant to Mp and *M. incognita* (Ito et al., 2008).

IPR 100 is highly resistant to Mp and it is derived from the coffee plant BA 10, which has *C. liberica* genes (Sera et al., 2017) and was probably the source of resistance of this cultivar. This fact arises the hypothesis that BA coffees are resistant to Mp. However, no studies have proved this assumption. The germplasm bank of the IAPAR (Londrina, PR, Brazil) holds coffee plants derived from BA-10 and BA-21 and from other coffee plants that carry *C. liberica* genes.

The aim of this study was to prove that Arabica coffees introgressed with *C. liberica*, have resistance to *Meloidogyne paranaensis*.

## Results

### Reproduction factor

For the three variables, four different groups were obtained. The control 'Catuaí Vermelho' presented reproduction factor (RF) of 92.24, indicating high infestation in the experiment, while IPR 100 showed RF of 1.71. All genotypes differed from Catuaí, five of which did not differ from IPR 100 for RF (Table 1).

### Resistance level

Out of 28 Arabica genotypes having *C. liberica* introgression, five were highly resistant (HR), 11 resistant (R), 11 moderately resistant (MR) and one moderately susceptible (MS).

Genotypes IAPAR 15261, H151/1, IAPAR 15247, IPR 100, and IAPAR 15242 were classified as HR, with RF ranging from 0.68 to 3.04. Genotypes classified as R presented RF between 5.14 and 9.15. Genotypes classified as R or HR and with RF > 1.0 should be carefully analyzed before stating their high resistance level. This is because the Mp population may occur in infested areas, which can lead to losses in production in a perennial crop, such as coffee. An explanation for the high RF values, including that of the resistant check (RF = 1.71), is because Mp population density was very high in the experiment. This is evidenced by the RF of 92.24 reported for the check 'Catuaí Vermelho' (Table 1).

Based on % PRL, it is possible to observe high frequency of MR plants in genotypes IAPAR 15250, 15246, 15252, 15257, 15255 and 15249, respectively, with 62.5, 62.5, 75.0, 62.5, 62.5 and 62.5% of MR plants, while Catuaí present only 14.2% (Table 2).

None of these genotypes presented highly susceptible (HS) and susceptible (S) plants, except for IAPAR 15243, which had 25% of S plants.

### Homozygous and heterozygous resistance

Based on the percentage of plants with different resistance levels (% PRL), the genotypes IAPAR 15242, IPR 100, IAPAR 15247, H151/1, IAPAR 15261, IAPAR 15268, IAPAR 15267, and H147/1 presented 100% of HR or R. IAPAR 15242 and IPR 100 stood out with 100% of HR plants, which are likely to present homozygous resistance (Table 2).

The genotypes IAPAR 15253, 15239, 15251 and 15256 seem to have resistance in heterozygous condition, since they presented high percentage of plants with high resistance

levels (50% of HR plants), but also had 12.5 to 25.0% of MS plants.

## Discussion

### RFR, RF and %PRL used in combination for genotype selection

Shigueoka et al. (2017) concluded that selection of MR, R, or HR coffee plants should consider the RFR classification, preferably selecting those genotypes with RF < 1.0. Considering this selection criterion, only genotype F<sub>3</sub> of Catuaí x IAC 1110-8 (IAPAR 15242) presented 100% of HR plants by RFR and it was classified as resistant by Sasser et al. (1984) criterion with modifications, because it had RF < 1.0. All genotypes of RF groups c and d will be advanced to the next self-pollination generation to identify individual plants with homozygous resistance. For the next generation, the lines IAPAR 15242, IAPAR 15247, and IAPAR 15261 and the hybrid H151/1 are more likely to present individual plants with homozygous resistance.

In our study, the need to use RFR in combination with RF to identify nematode resistant genotypes was evidenced, as reported by Shigueoka et al. (2017). When the RF of the susceptible check is very high, the RFR values of the other genotypes are also high, classifying as HR and R, genotypes classified as susceptibles by RF. Nevertheless, the classifications of RFR resistance levels should not be ruled out, since they may assist for the identification of genotypes with partial or intermediate resistance, which may be a quantitative trait and important for coffee breeding.

### Resistance of the genotypes

Several lines derived from the coffees BA-10 and BA-21 presented different levels of resistance to Mp. These lines were derived from hybrids H3437, H6963, H7314, H8187, H8518, and H8598, which were generated from the cross of BA 10 and BA 21 with different genitors, such as Catuaí, Mundo Novo, Acaíá, and Geisha (Table 1). These 25 genotypes were chosen to be tested for this nematode, because their mother plants showed high yield and high resistance to rust on the field at IAPAR. Resistance to rust is probably conferred by the gene *S<sub>H</sub>3* from the genotypes BA-10 and BA-21. This result is fundamental since, currently, only the ungrafted seedling of cultivars IPR 100 and IPR 106 are recommended for the areas infested with Mp, both susceptible to leaf rust and with late fruit ripening cycle (Pereira and Baião, 2015; Sera et al., 2017).

Since Catuaí, Mundo Novo, and Acaíá are susceptible to *M. paranaensis*, the source of resistance of these genotypes are the coffee plants BA-10 and BA-21, which are also important sources for drought tolerance (Mazzafera and Carvalho, 1987; Carvalho et al., 2017) and resistance to leaf rust (*Hemileia vastatrix* Berk. et Br) (Fazuoli et al., 2005).

Resistance to Mp was also identified in the hybrids H151/1 and H147/1, especially in the first. These two genotypes are important sources of resistance to rust as they have different *S<sub>H</sub>* genes, including the *S<sub>H</sub>3* (Bettencourt, 1981), which still provides high resistance to races found in Brazil (Sera et al., 2007; Sera et al., 2010).

A very low resistance level was observed in the genotypes classified in the groups b and c, based on the variables RF (Table 1) and %PRL (Table 2). Several genotypes showed more than 50% of MR plants, and many moderately

**Table 1.** Number of eggs and J2 of *Meloidogyne paranaensis* per gram of roots (NGR), reproduction factor (RF), reproduction factor reduction (RFR) and resistance levels (RL) of Arabica coffee genotypes with introgression of *Coffea liberica* genes.

Genotype	Description <sup>1</sup>	NGR <sup>2</sup>	RF <sup>2</sup>	RFR <sup>2</sup>	RL <sup>3</sup>
Catuai V.	Caturra x MN (susceptible check)	8553.39 a	92.24 a	0.00 d	HS
IAPAR 15243	F <sub>3</sub> of Catuai x IAC 1110-8 (H7314)	2852.68 b	30.99 b	66.41 c	MS
IAPAR 15250	F <sub>3</sub> of MN x IAC 1107-5-6 (H8518)	1965.35 b	21.20 b	77.02 c	MR
IAPAR 15254	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	2067.93 b	19.00 b	79.40 c	MR
IAPAR 15246	F <sub>3</sub> of IAC 1110-8-5 x Acaiá IAC 474-7 (H8187)	2458.96 b	17.95 b	80.54 c	MR
IAPAR 15252	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	1319.53 b	16.88 b	81.70 c	MR
IAPAR 15257	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	2084.22 b	16.47 b	82.14 c	MR
IAPAR 15266	F <sub>4</sub> of (IAC 1110-1 x MN) x MN (H5978)	1830.61 b	15.87 b	82.79 c	MR
IAPAR 15256	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	1205.13 c	12.39 c	86.56 b	MR
IAPAR 15255	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	1409.26 b	11.06 c	88.01 b	MR
IAPAR 15249	F <sub>3</sub> of MN x IAC 1107-5-6 (H8518)	1013.41 c	10.87 c	88.21 b	MR
IAPAR 15251	F <sub>3</sub> of MN x IAC 1107-5-6 (H8518)	831.13 c	10.61 c	88.49 b	MR
IAPAR 15239	F <sub>3</sub> of Catuai x (Catuai x IAC 1110-8) (H8598)	778.99 c	9.98 c	89.18 b	MR
IAPAR 15248	F <sub>3</sub> of MN x IAC 1107-5-6 (H8518)	714.60 c	9.15 c	90.08 b	R
H147/1	F <sub>1</sub> of 34/13 S353 4/5 x 110/5 S4 Agaro	815.78 c	9.15 c	90.08 b	R
IAPAR 15253	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	1002.32 c	9.01 c	90.23 b	R
IAPAR 15258	F <sub>4</sub> of IAC 1110-1 x MN (H3437)	751.51 c	8.56 c	90.72 b	R
IAPAR 15259	F <sub>4</sub> of IAC 1110-1 x MN (H3437)	680.24 c	8.48 c	90.81 b	R
IAPAR 15267	F <sub>5</sub> of IAC 1110-1 x MN (H3437)	805.92 c	7.65 c	91.70 b	R
IAPAR 15260	F <sub>4</sub> of IAC 1110-1 x MN (H3437)	660.31 c	7.21 c	92.19 b	R
IAPAR 15262	F <sub>4</sub> of IAC 1110-1 x MN (H3437)	551.07 c	6.55 c	92.90 b	R
IAPAR 15245	F <sub>3</sub> of Catuai x IAC 1110-8 (H7314)	381.46 d	5.39 c	94.16 b	R
IAPAR 15244	F <sub>3</sub> of Catuai x IAC 1110-8 (H7314)	842.66 c	5.18 c	94.38 b	R
IAPAR 15268	F <sub>3</sub> of Geisha IAC 1137-1 x IAC 1110-8 (H6963)	495.01 d	5.14 d	94.43 a	R
IAPAR 15261	F <sub>4</sub> of IAC 1110-1 x MN (H3437)	237.07 d	3.04 d	96.70 a	HR
H151/1	F <sub>1</sub> of 33/1 S288-23 x 110/5 S4 Agaro	205.67 d	2.53 d	97.26 a	HR
IAPAR 15247	F <sub>3</sub> of IAC 1110-8-5 x Acaiá IAC 474-7 (H8187)	290.95 d	2.50 d	97.29 a	HR
IPR 100	Catuai x (Catuai x IAC 1110-8) (resistant check)	194.47 d	1.71 d	98.15 a	HR
IAPAR 15242	F <sub>3</sub> of Catuai x IAC 1110-8 (H7314)	41.98 d	0.68 d	99.26 a	HR
General mean		1245.80	12.67	86.26	
CV		48.81%	48.03%	35.81%	

<sup>1</sup>IAC 1107 was originated from BA-21 coffee and IAC 1110 from BA-10. MN= Mundo Novo. The self-pollination generations described are from the mother plants, which were harvested the seeds for the resistance test. <sup>2</sup>Means followed by the same letter did not differ by the Scott-Knott mean clustering test ( $\alpha = 0.05$ ). Data was transformed by  $\sqrt{x}$ .

<sup>3</sup>Based on RFR, 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.

**Table 2.** Percentage of plants with different resistance levels (%PRL) to the nematode *Meloidogyne paranaensis* based on reproduction factor reduction (RFR).

Genotype	% PRL <sup>(1)</sup>					
	HR	R	MR	MS	S	HS
Catuai V. <sup>(2)</sup>	0	0	14.2	42.9	0	42.9
IAPAR 15243	12.5	0	25.0	37.5	25.0	0
IAPAR 15250	0	0	62.5	37.5	0	0
IAPAR 15254	0	25.0	37.5	37.5	0	0
IAPAR 15246	0	12.5	62.5	25.0	0	0
IAPAR 15252	0	0	75.0	25.0	0	0
IAPAR 15257	0	25.0	62.5	12.5	0	0
IAPAR 15266	12.5	0	50.0	37.5	0	0
IAPAR 15256	50.0	12.5	25.0	12.5	0	0
IAPAR 15255	12.5	25.0	62.5	0	0	0
IAPAR 15249	25.0	12.5	62.5	0	0	0
IAPAR 15251	50.0	0	25.0	25.0	0	0
IAPAR 15239	50.0	12.5	12.5	25.0	0	0
IAPAR 15248	12.5	62.5	25.0	0	0	0
H147/1	62.5	37.5	0	0	0	0
IAPAR 15253	50.0	12.5	25.0	12.5	0	0
IAPAR 15258	12.5	37.5	50.0	0	0	0
IAPAR 15259	12.5	62.5	25.0	0	0	0
IAPAR 15267	50.0	50.0	0	0	0	0
IAPAR 15260	50.0	25.0	25.0	0	0	0
IAPAR 15262	25.0	62.5	12.5	0	0	0
IAPAR 15245	50.0	25.0	25.0	0	0	0
IAPAR 15244	50.0	37.5	12.5	0	0	0
IAPAR 15268	75.0	25.0	0	0	0	0
IAPAR 15261	50.0	50.0	0	0	0	0
H151/1	75.0	25.0	0	0	0	0
IAPAR 15247	75.0	25.0	0	0	0	0
IPR 100 <sup>(3)</sup>	100.0	0	0	0	0	0
IAPAR 15242	100.0	0	0	0	0	0

<sup>(1)</sup> HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; HS = highly susceptible.

<sup>(2)</sup> Catuai Vermelho IAC 99 = susceptible check. <sup>(3)</sup> Resistant check.

susceptible (MS) plants, demonstrating they may have quantitative resistance (Table 2). It is possible to observe that the genotypes with heterozygous resistance such as IAPAR 15253 and IAPAR 15239 have qualitative resistance, since they showed 50% of HR plants, but also have quantitative, since they had 12.5 to 25% of MS plants and 0% of S and HS plants. This fact could be used in breeding programs or integrated management of nematodes using these partially resistant genotypes together with chemical, biological, and cultural control.

Only IAPAR 15242 and IPR 100 were classified as HR and presented 100% of HR plants, but only the first showed an RF < 1.0. High levels of resistance to Mp and RF < 1.0 were also identified in *C. canephora* genotypes (Sera et al., 2006; Andreazi et al., 2015a), Arabica coffees with introgression of *C. canephora* genes, such as Icatu (Andreazi et al., 2015b; Shigueoka et al., 2016a) and wild Arabica coffees from Ethiopia (Boisseau et al., 2009; Fatobene et al., 2017). Many of the genotypes showed high frequency of MR plants such as genotypes IAPAR 15252 and 15255, respectively, with 75.0 and 62.5%. This was also found in Timor Hybrid (Salgado et al., 2014) and Sarchimor (Shigueoka et al., 2016b), both Arabica coffees with *C. canephora* introgression. Therefore, Arabica genotypes carrying *C. liberica* genes with high and intermediate resistance levels could be new options to be used by breeding programs for development of new cultivars or as sources of resistance.

IAPAR 15242 presented 100% of HR plants and showed the same resistance level of IPR 100, which is also derived from BA-10 (Sera et al., 2017). Therefore, this genotype has great potential to become a new cultivar resistant to Mp and simultaneously resistant to leaf rust, as coffee plants derived from BA-10 may have the *S<sub>H</sub>3* gene, which promotes high resistance according to Fazuoli et al. (2005).

## Materials and methods

### Plant materials

Open pollinated fruits were harvested from mother plants of 29 Arabica coffee genotypes from the IAPAR's germplasm bank. Seeds were produced using these fruits, which were sown to obtain seedlings to test the resistance to *M. paranaensis* (Mp).

Twenty-five genotypes, in different self-pollinated generations, derived from the Indian coffee plants named BA-10 (IAC 1110-1, IAC 1110-8) and BA 21 (IAC 1107-5-6) were harvested. These genotypes were selected to test the resistance because they have high yield, rusticity, and high resistance to coffee leaf rust on field conditions at IAPAR.

Two F<sub>1</sub> hybrids from the CIFC were also harvested, H147/1 and H151/1, which carry the resistance genes to coffee leaf rust, respectively, *S<sub>H</sub>2*, *S<sub>H</sub>3*, *S<sub>H</sub>4*, *S<sub>H</sub>5* and *S<sub>H</sub>3*, *S<sub>H</sub>4*, *S<sub>H</sub>5* (Bettencourt, 1981).

Catuaí Vermelho IAC 99 and IPR 100 were used as susceptibility and resistance checks, respectively. The origin and self-pollination generations of genotypes are presented in Table 1. In our study, we are analyzing if the harvested mother plants have resistance and if it is homozygous or heterozygous. Therefore, Table 1 describes the self-pollination generations of harvested mother plants.

F<sub>1</sub> hybrids that originated the genotypes derived from BA-10 and BA-21 were generated at the IAC (Campinas, SP, Brazil) and were denominated H3437, H5978, H6963, H7314, H8187, H8518, and H8598 (Table 1).

### Experiment conduction and installation

The experiment was carried out in a greenhouse at the IAPAR, in Londrina-PR, Brazil (lat 23° 21' 20" S lat, 51° 09' 58" W long, alt 578 m asl), between May and October 2016. The experiment was installed in a completely randomized design with 29 coffee genotypes, 8 replications, and one plant per plot. The average temperature was 26.0°C, and the average maximum and minimum temperatures during the period of the experiment were 30.5°C and 19.5°C, respectively. Seedlings with four pairs of leaves were transplanted into 700 mL plastic cups and after one month they were inoculated with Mp. 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.

### *Meloidogyne paranaensis*

Mp inoculum was obtained in the municipality of Apucarana (Paraná, Brazil) and recorded in the Nematology Laboratory of IAPAR under the number 98.1. The population was identified as Mp through  $\alpha$ -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. This multiplied and purified inoculum was kept in the susceptible coffee cultivar Mundo Novo IAC 376-4. About 60 days before inoculation of the experiment, eggs and J2 were extracted from the roots of Mundo Novo plants and inoculated into Santa Clara tomato cultivar.

Eggs and J2 were extracted from tomato roots (Boneti and Ferraz, 1981) and the suspension was calibrated to 1,000 eggs and J2 per mL. 1,400 Mp eggs and J2 (IP) were inoculated in three holes of approximately 1 cm depth.

### Resistance assessment

The assessments were carried out 120 days after inoculation. The shoot was discarded and the root systems were collected, washed in running water and weighted. The extraction of the eggs and J2 was carried out on the washed root systems (Boneti and Ferraz, 1981). After extraction, the final population (FP) was measured by counting the number of eggs and J2 per root system, using the Peters chamber under an optical microscope. With the data of fresh weight of roots and of the quantification of nematodes, it was determined the nematodes per gram of roots (NGR).

The reproduction factor (RF) was calculated using the formula:  $RF = \frac{FP}{IP}$  (Oostenbrink, 1966). Based on this formula, the RF of each plot (RF<sub>p</sub>) was calculated using the formula  $RF_p = \frac{FP_{pTreat}}{IP}$ , where FP<sub>pTreat</sub> is the FP of each plot of the treatment.

### Classification of resistance levels

To classify the resistance level of the genotypes, we used the reproduction factor reduction (RFR), that was calculated based on the following formula (Shigueoka et al., 2017). The RFR of each plot (RFR<sub>p</sub>) was calculated by the following formula:

$$RFR_p = \frac{(RF_{mpSusc} - RF_{pTreat})}{RF_{mpSusc}} \times 100, \text{ where: } RF_{mpSusc} = \text{RF mean}$$

of the plots of the susceptible check;  $RF_{pTreat}$  = RF of each plot of the treatment, including of the susceptible check.

Based on RFR values, genotypes were classified according to the scale: < 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 (Shigueoka et al., 2017).

$RF_{mpSusc}$  was used to calculate  $RFR_p$  because if RF of individual plants of susceptible check ( $RF_{pSusc}$ ) is used. It can increase the percentage of susceptible plants of the treatments. This may occur in cases where some plots of the susceptible check have low RF values. Therefore, by using  $RF_{mpSusc}$  it is easier to identify resistant homozygous genotypes than using  $RF_{pSusc}$  (Shigueoka et al., 2017).

Genotypes were also classified based on RF values, where those with  $RF > 1.0$  were classified as susceptible and those with  $RF \leq 1.0$  as resistant (Sasser et al., 1984 with modifications).

### Homozygous and heterozygous resistance

Homozygous and heterozygous resistance were identified by the calculation of the percentage of plants with different resistance levels (% PRL). The % PRL is based on the resistance levels (HR, R, MR, MS, S, HS) of each plant, classified by the RFRp values (Shigueoka et al., 2017).

### Statistical analysis

ANOVA, Bartlett test of homogeneity of variances, Shapiro-Wilk normality test, and Scott-Knott mean clustering test at 5% significance were performed using the R software version 3.3.0 (R Core Team, 2016), package ScottKnott (Jelihovschi et al., 2014). Data were transformed to  $\sqrt{x}$  for all variables.

### Conclusions

The genotype IAPAR 15242 ( $F_3$  of Catuaí x IAC 1110-8) have high potential to present individual plants with homozygous resistance in the next self-pollination generation. Several Arabica coffee genotypes with *C. liberica* introgression (e.g. hybrids H151/1 and H147/1) derived from BA-10 and BA-21). They presented high and intermediate resistance to *M. paranaensis* and they could be used in breeding programs as resistance sources or to release new cultivars. Many genotypes showed high levels of resistance (e.g. highly resistant and resistant) when RFR was used, but were susceptible by RF. This happened because the RF of the susceptible control was very high in the experiment. Therefore, only the use of the RFR is not suitable for the identification of resistant genotypes, and RF must be used in combination with RFR.

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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. Aust J Crop Sci. 9:1190-1196.

Barros AF, Oliveira RDL, Zambolim L, Ferreira AO, Coutinho RR (2011) *Meloidogyne paranaensis* attacking coffee trees in Espírito Santo State, Brazil. Aust Plant Dis Notes. 6:43-45.

Bettencourt AJ (1981) Melhoramento genético do cafeeiro: transferência de fatores de resistência à *Hemileia vastatrix* Berk. & Br. para as principais cultivares de *Coffea arabica* L. Lisboa: ULTRAMAR/CIFC.

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. Fitopatologia Brasileira. 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. 529-579.

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

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

Carneiro RMDG, Randig O, Almeida MRA, Gonçalves W (2005) Identificação e caracterização de espécies de *Meloidogyne* em cafeeiro nos Estados de São Paulo e Minas Gerais através dos fenótipos de esterase e SCAR-multiplex-PCR. Nematologia Brasileira. 29: 233-241.

Carneiro RMDG, Tigano MS, Randig O, Almeida MRA, Sarah JL (2004) Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. Nematol. 6:287-298.

Carvalho FG, Sera GH, Andreazi E, Sera T, Fonseca IC de B, Carducci FC, Shigueoka LH, Holderbaum MM, Costa KC (2017) Tolerância ao déficit hídrico em mudas de genótipos de café portadores de genes de diferentes espécies. Coffee Sci. 12:156-163.

Castro JMC, Campos VP, Pozza EA, Naves RL, Andrade Júnior WC, Dutra MR, Coimbra JL, Maximiniano C, Silva JRC (2008) Levantamento de fitonematoides em cafezais do Sul de Minas Gerais. Nematologia Brasileira. 32:56-64.

Fatobene BJ dos R, Andrade VT, Aloise GS, Silvarolla MB, Gonçalves W, Guerreiro Filho, O (2017) Wild *Coffea arabica* resistant to *Meloidogyne paranaensis* and genetic parameters for resistance. Euphytica. 213:196.

Fazuoli LC, Oliveira ACB de, Toma-Braghini M., Silvarolla MB (2005) Identification and use of sources of durable resistance to coffee leaf rust at the IAC. In: Zambolim L, Zambolim EM, Várzea VMP (eds.) Durable resistance to coffee leaf rust. Editora UFV, Viçosa. 137-185.

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

Hartman RM, 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*. 2<sup>nd</sup> vol: methodology. NC State University Graphics, Raleigh. 69-77.
- Ito DS, Sera GH, Sera T, Santiago DC, Kanayama FS, Del Grossi L (2008) Progenies de café com resistência aos nematóides *Meloidogyne paranaensis* e raça 2 de *Meloidogyne incognita*. *Coffee Sci.* 3:156-163.
- Jelihovschi EG, Faria JC, Allaman IB (2014) ScottKnott: a package for performing the Scott-Knott clustering algorithm in R. *Trends Appl Computacional Math.* 15:3-17.
- Lopez-Lima D, Sánchez-Nava P, Carrion G, Monteros AE de los, Villain L (2015) Corky-root symptoms for coffee in central Veracruz are linked to the root-knot nematode *Meloidogyne paranaensis*, a new report for México. *Europ J Plant Pathol.* 141:623-629.
- Mazzafera PI, Carvalho A (1987) Produção e tolerância à seca de cafeeiros. *Bragantia.* 46:403-415.
- Oostenbrink M (1966) Major characteristics of the relation between nematodes and plants. *Mededelingen Landbouwhogeschool Wageningen.* 66:1-46.
- Pereira A, Baião AC (2015) Cultivares. In: Sakiyama NS, Martinez HEP, Tomaz MA, Borém A (eds.) *Café arábica: do plantio à colheita*. Editora UFV, Viçosa. 24-45.
- R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Salgado SML, Guimarães NMRB, Botelho CE, Tassone GAT, Marcelo AL, Souza SR de, Oliveira RDL, Ferreira DF (2015) *Meloidogyne paranaensis* e *Meloidogyne exigua* em lavouras cafeeiras na região Sul de Minas Gerais. *Coffee Sci.* 10:475-481.
- Salgado SML, Resende MLV, Campos VP (2005) Reprodução de *Meloidogyne exigua* em cultivares de cafeeiros resistentes e suscetíveis. *Fitopatologia Brasileira.* 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 Biotechnol.* 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.
- Sera GH, Sera T, Azevedo JA de, Mata JS da, Ribeiro Filho C, Doi DS, Ito DS, Fonseca IC de B (2006) Porta-enxertos de café robusta resistentes aos nematoides *Meloidogyne paranaensis* e *M. incognita* raças 1 e 2. *Semina: Ciências Agrárias.* 27:171-184.
- Sera GH, Sera T, Fonseca IC de B, Ito DS (2010) Resistência à ferrugem alaranjada em cultivares de café. *Coffee Sci.* 5:59-66.
- Sera GH, Sera T, Ito DS, Azevedo JA de, Mata JS da, Doi DS, Ribeiro Filho C (2007) Resistance to leaf rust in coffee carrying  $S_H3$  gene and others  $S_H$  genes. *Brazil Arch Biol Tech.* 50:753-757.
- 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 Biotechnol.* 17:175-179.
- 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 da, Fonseca IC de B, Machado ACZ (2016b) Host reaction of arabica coffee genotypes derived from Sarchimor to *Meloidogyne paranaensis*. *Nematoda.* 3:10-16.
- Shigueoka LH, Sera T, Fonseca IC de B, Andreazi E, Carducci FC, Sera GH (2017) *Coffea arabica* lines with resistance to nematode *Meloidogyne paranaensis* derived from crossings with IPR 100. *Aust J Crop Sci.* 11:1203-1209.