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# Resistance to Meloidogyne paranaensis in Coffea arabica L. progenies

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# Abstract

The aggressiveness and rapid spread of *Meloidogyne paranaensis* in several coffee-producing regions of Brazil has drawn considerable attention. Some coffee cultivars are resistant to root-knot nematode. Especially a limited number of ungrafted Arabica cultivars have shown resistance. Therefore, this study aimed to identify resistance to *M. paranaensis* in *C. arabica* progenies. Seedlings were inoculated with 5,000 *M. paranaensis* eggs at second-stage juveniles, and the treatments consisted of 19 F<sub>5</sub> progenies of *C. arabica* derived from a probable natural "Icatu H4782-7-925" × "Sarchimor 1669-33" cross. Resistance was identified by the reduced reproduction factor (RRF) and host susceptibility index (HSI). In all of the progenies, fewer eggs and second-stage juveniles were observed per gram of root (nematodes.g<sup>-1</sup>) relative to the susceptible control 'Catuaí Vermelho IAC 99.' In almost all of the progeny, 100% of the plants showed a resistance response (HR, R and MR) according to either index (RRF and HSI). This resistance is probably originated from the parental "Icatu H4782-7-925", which is the source of resistance. All of the F<sub>5</sub> progenies were resistant to *M. paranaensis*, and 17 of the 19 progenies studied did not segregate for this trait. Individual plants of these progenies with good agronomic traits will be advanced to next generation to obtain new cultivars. All the resistant progenies might be advanced individually, especially those that present the best agronomic characteristics are selected and has the potential to become new cultivar.

**Keywords**: breeding, coffee, cultivars, Icatu, root-knot nematodes.

Abbreviations: 'Apoatã' 'Apoatã IAC 2258'; 'Catuaí' 'Catuaí Vermelho IAC 99'; HR highly resistant; HS highly susceptible; HSI\_host susceptibility index; IAPAR\_Instituto Agronômico do Paraná; FP\_final populations; IP\_initial populations; MR\_moderately resistant; MS\_moderately susceptible; nematodes.g<sup>-1</sup>\_eggs and second-stage juveniles per gram of root; R\_resistant; RF\_reproduction factor; RRF\_reduction in reproduction factor; S\_susceptible.

### Introduction

Coffee is a major commodity worldwide, and Brazil is its largest producer and exporter (Nishijima et al., 2012). *Coffea arabica* L. is the most commercially important coffee species and accounts for more than 70% of the cultivated coffee area worldwide. Plant-parasitic nematodes, especially from the genus *Meloidogyne* Goeldi, are a major problem because they reduce coffee crop yield and expansion (Campos and Villain, 2005).

The aggressiveness and rapid spread of *M. paranaensis* (Carneiro et al., 1996) in several coffee-producing regions of Brazil have drawn attention to its negative effects (Castro et al., 2008; Barros et al., 2011). The species is difficult to control, and there are few resistant cultivars, and once established in an area, *M. paranaensis* eradication is practically impossible (Gonçalves and Silvarolla, 2007). Additionally, a number of naturally occurring weeds throughout the majority of agricultural regions in Brazil are hosts for *M. paranaensis* (Roese and Oliveira, 2004).

The main strategy for managing plant-parasitic nematodes is preventing their spread. For areas already infested, the most recommended control method is genetic through the use resistant cultivars. However, sources of resistance are considered rare in *C. arabica* (Gonçalves and Silvarolla, 2007). The most widely planted coffee cultivars in the world, including 'Caturra', 'Catuai' and 'Mundo Novo', have low genetic variability and are susceptible to the major pests and diseases that attack coffee plants, including nematodes (Bertrand et al., 1999). Therefore, the resistance to *M. paranaensis* reported in other *Coffea* sp., including *C. canephora* Pierre ex Froehner (Sera et al., 2006), is essential for developing novel cultivars.

The "Icatu" is an Arabica coffee genotype carrying *C. canephora* genes and have shown resistance to *M. paranaensis* (Gonçalves and Silvarolla, 2007; Ito et al., 2008; Matiello et al., 2010). The cultivar 'IPR 100,' which is derived from a cross between "Catuai" and coffee plants from the BA-10 series and carries *C. liberica* Hiern genes, is resistant to *M. paranaensis* as well as to races 1 and 2 of *M. incognita*, with the resistance most likely arising from *C. liberica* (Sera et al., 2007, 2009; Ito et al., 2008; Kanayama et al., 2009). Wild accessions of *C. arabica* from Ethiopia that are resistant to *M. paranaensis* have also been identified (Anthony et al., 2003; Boisseau et al., 2009).

One technique that has been widely recommended is hypocotyledonary grafting using rootstock composed of cultivar 'Apoata IAC 2258' of *C. canephora*, which is resistant to *Meloidogyne exigua* Goeldi (Salgado et al., 2005), *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. paranaensis* (Sera et al., 2006; Fonseca et al., 2008). This method has allowed for the short-term cultivation of coffee in infested areas (Fonseca et al., 2008). However, using it in place of ungrafted cultivars causes certain disadvantages, including its segregation rate for nematode susceptibility (10 to 15%) and increased replanting requirements, approximately 10 to 15%, due to this segregation (Gonçalves and Silvarolla, 2007).

Although certain coffee cultivars are resistant to nematodes only a limited number of ungrafted Arabica cultivars have shown resistance. Thus, the aim of this study was to identify resistance to *M. paranaensis* in *C. arabica* progenies.

#### Results

# Nematodes.g<sup>-1</sup>, RF, RRF and HSI

By the Scott-Knott test, the susceptible control, cultivar 'Catuai', was significantly different from the resistant control 'Apoatã' and the studied progenies in all parameters (nematodes.g<sup>-1</sup>, RF, RRF and HSI) (Tables 2, 3 and 4). It showed a higher mean number of nematodes.g<sup>-1</sup> (16,322.6) as expected (Table 2). Basing on the same test, all the progenies did not differ from the resistant control 'Apoatã', for all parameters.

The means for the progenies were approximately 19 to 77 times lower than for the susceptible control 'Catuaí'. In 'Apoatã', the number of nematodes.g<sup>-1</sup> was 23 times lower than in 'Catuaí'. The data showed a small increase in the number of nematodes relative to the initial population for 'Apoatã' (RF = 1.48). This increase did not occur in the progenies that presented RF < 1.0, classified as resistant, except for progeny IAPAR 12306 with RF of 1.33. In turn, 'Catuaí' had an RF of 26.19, which is consistent with a good susceptibility pattern.

#### Resistance level of the progenies

Based on RRF (Table 3), all of the progenies were classified as HR, surpassing the 'Apoatā' resistant control, which was classified as R. Except for progenies IAPAR 12315 and IAPAR 12321, in which 91% of the plants were between HR and MR and 9% were MS plants, all of the remaining progenies were classified between HR and MR. In seven progenies, 100% of the plants were classified between HR and R, and in progenies IAPAR 12320 and IAPAR 12322, 100% of the plants were classified as HR. All of the 'Apoatā' plants were between HR and MR. The low rate of susceptible plants in progenies IAPAR 12315 and IAPAR 12321 may have been caused by cross-fertilization (5 to 10%) that can occur in *C. arabica* in the field, where the seeds used for this experiment were collected.

Using the HSI, both the progenies and resistant control were classified as R (Table 4). For all progenies, 100% of the plants were classified as HR, R and MR. For 'Apoatã' 91% of the plants were classified as resistant (HR, R and MR) and 9% were classified as MS. In eight progenies, 100% of the plants were classified between HR and R, and none of the plants of these progenies were classified as 100% HR.

# Discussion

*Meloidogyne paranaensis* has a strong ability to destroy the root system of coffee plants (Gonçalves and Silvarolla, 2007; Silva et al., 2009). This behavior may have negatively impact

the resistance classification of the plants when only the reproduction rate of the nematodes is considered, which also occurs when only the RF and RRF are considered. Thus, the HSI (Gonçalves and Ferraz, 1987 modified) was used as an alternative analysis parameter because it considers the nematodes.g<sup>-1</sup> root. In this study, the mean response of the progenies differed between the RRF and HSI. According to the RRF, the mean response was HR for all progenies but only R for 'Apoatã'. However, using the HSI, the mean response of the progenies and 'Apoatã' were both R.

Resistance to M. paranaensis has been reported in genotypes of coffee plants carrying genes from C. canephora and C. liberica (Goncalves and Silvarolla, 2007; Ito et al., 2008; Sera et al., 2009; Matiello et al., 2010). The "Icatu" was originated from a cross between C. canephora and C. arabica cv. Bourbon Vermelho, and then was backcrossed with "Mundo Novo". The presence of resistance has been detected in "Icatu" selections, such as line 925 (Matiello et al., 2010; Carneiro et al., 2013), IPR 106 ("Icatu") (Ito et al., 2008) and 'Icatu Vermelho IAC 3888' (Goncalves and Silvarolla, 2007). The Line 925 of "Icatu", which has been identified by other authors as resistant to M. paranaensis, is the same line that was used in this study as the parent plant. Therefore, it is likely that resistance of the  $F_5$  progenies, observed in this study, originated from the parent plant "Icatu H4782-7-925". However, it cannot be ruled out that the pollinator "Sarchimor 1669-33" conferred resistance because another study has shown the resistance to M. paranaensis in a selection (IAPAR 88480-8) made inside of "Sarchimor 1669-33" (Sera et al., 2009).

In this study, the  $F_5$  progenies identified as resistant are important for coffee farming because the rootstock 'Apoatã IAC 2258' has several disadvantages and cultivar IPR 100 is currently the only cultivar recommended for use without grafting. Furthermore, several arabica coffee lines have been segregated for resistance. The resistance of "Icatu H4782-7-925" was shown in a field study, although susceptible segregants have also been found for this genotype (Carneiro et al., 2013).

For all of the progenies studied here, 100% of the plants showed resistance (HR, R and MR) according to both indices (RRF and HSI) except for those in treatments IAPAR 12315 and IAPAR 12321, where 9% of the plants were MS as classified by the RRF. When 100% of the plants showed some level of resistance (HR, R or MR), these progenies are likely homozygous for resistance to *M. paranaensis*. The high frequency of resistant plants in these progenies may have been caused by the establishment of the  $F_1$ ,  $F_2$  and  $F_3$ generations in areas infested with *M. paranaensis*; therefore, the coffee plants with high yield selected in these areas were most likely resistant, and coffee plants with low yield were likely susceptible and not selected.

The rootstock 'Apoatã IAC 2258' has some disadvantages, including a low rate of segregation for susceptibility (Gonçalves and Silvarolla, 2007). In this study, we observed 9% of the 'Apoatã' plants classified as MS by the HSI. In nematode-free areas, coffee plants grafted onto 'Apoatã IAC 2258' are less productive (Dias et al., 2009; Paiva et al., 2012) and have less vegetative growth (Oliveira et al., 2004; Dias et al., 2011) than the same coffee plants grown without grafting. Therefore, it is likely that this increased yield and vegetative growth in ungrafted plants would also occur in nematode-infested areas.

As reported previously, sources of resistance to *M. paranaensis* already exist, but there are few ungrafted coffee cultivars available that show such a response. Resistant

| F <sub>5</sub> Progenies | Pedigree            |
|--------------------------|---------------------|
| IAPAR 12306              | HN 87609-15-6-6-2   |
| IAPAR 12307              | HN 87609-15-6-6-3   |
| IAPAR 12308              | HN 87609-15-6-6-4   |
| IAPAR 12309              | HN 87609-15-6-6-6   |
| IAPAR 12310              | HN 87609-15-6-6-7   |
| IAPAR 12311              | HN 87609-15-6-6-10  |
| IAPAR 12312              | HN 87609-15-6-6-11  |
| IAPAR 12313              | HN 87609-15-6-6-12  |
| IAPAR 12314              | HN 87609-15-6-6-13  |
| IAPAR 12315              | HN 87609-15-6-6-14  |
| IAPAR 12316              | HN 87609-15-6-6-15  |
| IAPAR 12317              | HN 87609-15-6-8-5   |
| IAPAR 12318              | HN 87609-15-6-8-6   |
| IAPAR 12319              | HN 87609-15-6-9-1   |
| IAPAR 12320              | HN 87609-15-6-9-2   |
| IAPAR 12321              | HN 87609-15-6-9-11  |
| IAPAR 12322              | HN 87609-15-6-14-4  |
| IAPAR 12323              | HN 87609-15-6-14-5  |
| IAPAR 12324              | HN 87609-15-6-14-15 |
| 'Catuaí Vermelho IAC 99' | Susceptible control |
| 'Apoatã IAC 2258'        | Resistant control   |

**Table 1**. Pedigree of 19  $F_5$  progenies of *Coffea arabica* from a probable natural "Icatu H4782-7-925" x "Sarchimor 1669-33" cross and controls tested for resistance to *Meloidogyne paranaensis*.

The F5 progenies 12306 to 12316 were originated from the same F4 plant (HN 87609-15-6-6) (Table 1). The progenies 12317 and 12318 were originated from F4 plant HN 87609-15-6-8. The progenies 12317 and 12318 were originated from F4 plant HN 87609-15-6-9. The progenies 12322 to 12324 were originated from F4 plant HN 87609-15-6-14. The selection by the genealogical method was initiated in the F2 generation, in plant number HN 87609-15.

| F <sub>5</sub> Progenies             | Nematodes.g <sup>-1(1)</sup> | $RF^{(2)}$ | Reaction <sup>(3)</sup> |  |
|--------------------------------------|------------------------------|------------|-------------------------|--|
| IAPAR 12307                          | 210 a                        | 0.62 a     | R                       |  |
| IAPAR 12310                          | 238 a                        | 0.55 a     | R                       |  |
| IAPAR 12314                          | 239 a                        | 0.76 a     | R                       |  |
| IAPAR 12311                          | 264 a                        | 0.78 a     | R                       |  |
| IAPAR 12324                          | 272 а                        | 0.43 a     | R                       |  |
| IAPAR 12313                          | 289 a                        | 0.64 a     | R                       |  |
| IAPAR 12317                          | 325 a                        | 0.61 a     | R                       |  |
| IAPAR 12321                          | 360 a                        | 0.99 a     | R                       |  |
| IAPAR 12319                          | 367 a                        | 0.82 a     | R                       |  |
| IAPAR 12309                          | 389 a                        | 0.82 a     | R                       |  |
| IAPAR 12312                          | 391 a                        | 0.58 a     | R                       |  |
| IAPAR 12315                          | 417 a                        | 0.85 a     | R                       |  |
| IAPAR 12308                          | 436 a                        | 0.75 a     | R                       |  |
| IAPAR 12318                          | 457 a                        | 0.61 a     | R                       |  |
| IAPAR 12320                          | 476 a                        | 0.42 a     | R                       |  |
| IAPAR 12322                          | 520 a                        | 0.40 a     | R                       |  |
| IAPAR 12316                          | 584 a                        | 0.76 a     | R                       |  |
| 'Apoatã IAC 2258'(resistant control) | 719 a                        | 1.48 a     | S                       |  |
| IAPAR 12306                          | 772 a                        | 1.33 a     | S                       |  |
| IAPAR 12323                          | 856 a                        | 0.83 a     | R                       |  |
| 'Catuaí IAC 99'(susceptible control) | 16,324 b                     | 26.19 b    | S                       |  |
| CV%                                  | 13.86                        | 23.12      |                         |  |

**Table 2.** Mean number of *Meloidogyne paranaensis* eggs and second-stage juveniles per gram of roots (nematodes.g<sup>-1</sup>) and reproduction factor (RF) in *Coffea arabica* progenies.

<sup>(1)</sup>Means followed by the same letter were not different by the Scott-Knott test (p<0,01). Data were log(x) transformed. <sup>(2)</sup>Means followed by the same letter were not different by the Scott-Knott test (p<0,01). Data were  $\sqrt{x+1}$  transformed. <sup>(3)</sup>R = resistant; S = susceptible. Reaction by RF.

**Table 3.** Mean response and percentage of coffee plants classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) to the nematode *Meloidogyne paranaensis* based on the reduced reproduction factor (RRF).

| Progenies $F_5$                         | RRF <sup>(1)</sup> | RL <sup>(2)</sup> | HR  | R  | MR | MS | S | HS |
|---|--------------------|-------------------|-----|----|----|----|---|----|
| IAPAR 12306                             | 95 a               | HR                | 73  | 9  | 18 | 0  | 0 | 0  |
| IAPAR 12307                             | 97 a               | HR                | 73  | 27 | 0  | 0  | 0 | 0  |
| IAPAR 12308                             | 97 a               | HR                | 91  | 9  | 0  | 0  | 0 | 0  |
| IAPAR 12309                             | 97 a               | HR                | 82  | 9  | 9  | 0  | 0 | 0  |
| IAPAR 12310                             | 98 a               | HR                | 91  | 0  | 9  | 0  | 0 | 0  |
| IAPAR 12311                             | 97 a               | HR                | 82  | 18 | 0  | 0  | 0 | 0  |
| IAPAR 12312                             | 98 a               | HR                | 82  | 9  | 9  | 0  | 0 | 0  |
| IAPAR 12313                             | 98 a               | HR                | 91  | 0  | 9  | 0  | 0 | 0  |
| IAPAR 12314                             | 97 a               | HR                | 73  | 9  | 18 | 0  | 0 | 0  |
| IAPAR 12315                             | 97 a               | HR                | 73  | 18 | 0  | 9  | 0 | 0  |
| IAPAR 12316                             | 97 a               | HR                | 91  | 9  | 0  | 0  | 0 | 0  |
| IAPAR 12317                             | 98 a               | HR                | 82  | 0  | 18 | 0  | 0 | 0  |
| IAPAR 12318                             | 98 a               | HR                | 82  | 9  | 9  | 0  | 0 | 0  |
| IAPAR 12319                             | 97 a               | HR                | 82  | 9  | 9  | 0  | 0 | 0  |
| IAPAR 12320                             | 98 a               | HR                | 100 | 0  | 0  | 0  | 0 | 0  |
| IAPAR 12321                             | 96 a               | HR                | 73  | 18 | 0  | 9  | 0 | 0  |
| IAPAR 12322                             | 98 a               | HR                | 100 | 0  | 0  | 0  | 0 | 0  |
| IAPAR 12323                             | 97 a               | HR                | 73  | 18 | 9  | 0  | 0 | 0  |
| IAPAR 12324                             | 98 a               | HR                | 91  | 9  | 0  | 0  | 0 | 0  |
| 'Apoatã IAC 2258'                       | 95 a               | R                 | 55  | 27 | 18 | 0  | 0 | 0  |
| 'Catuaí Vermelho IAC 99' <sup>(3)</sup> | 0 b                | HS                | -   | -  | -  | -  | - | -  |
| CV%                                     | 10.89              |                   |     |    |    |    |   |    |

<sup>(1)</sup>Means followed by the same letter were not different by the Scott-Knott test (p<0,01). <sup>(2)</sup>Resistance level (RL) by RRF. <sup>(3)</sup>Cultivar used as reference to calculate the RRF.

**Table 4**. Mean response and percentage of coffee plants classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) to the nematode *Meloidogyne paranaensis* based on the host susceptibility index (HSI).

| F <sub>5</sub> Progeny         | HSI <sup>(1)</sup> | <b>R</b> L <sup>(2)</sup> | HR% | R% | MR% | MS% | <b>S%</b> | HS% |
|--------------------------------|--------------------|---------------------------|-----|----|-----|-----|-----------|-----|
| IAPAR 12306                    | 4.8 a              | R                         | 9   | 73 | 18  | 0   | 0         | 0   |
| IAPAR 12307                    | 1.3 a              | R                         | 45  | 55 | 0   | 0   | 0         | 0   |
| IAPAR 12308                    | 2.7 a              | R                         | 18  | 82 | 0   | 0   | 0         | 0   |
| IAPAR 12309                    | 2.3 a              | R                         | 18  | 73 | 9   | 0   | 0         | 0   |
| IAPAR 12310                    | 1.4 a              | R                         | 27  | 73 | 0   | 0   | 0         | 0   |
| IAPAR 12311                    | 1.4 a              | R                         | 27  | 73 | 0   | 0   | 0         | 0   |
| IAPAR 12312                    | 2.5 a              | R                         | 36  | 64 | 0   | 0   | 0         | 0   |
| IAPAR 12313                    | 1.7 a              | R                         | 27  | 73 | 0   | 0   | 0         | 0   |
| IAPAR 12314                    | 1.4 a              | R                         | 45  | 45 | 9   | 0   | 0         | 0   |
| IAPAR 12315                    | 2.5 a              | R                         | 27  | 64 | 9   | 0   | 0         | 0   |
| IAPAR 12316                    | 3.6 a              | R                         | 9   | 82 | 9   | 0   | 0         | 0   |
| IAPAR 12317                    | 1.7 a              | R                         | 18  | 73 | 9   | 0   | 0         | 0   |
| IAPAR 12318                    | 2.8 a              | R                         | 27  | 55 | 18  | 0   | 0         | 0   |
| IAPAR 12319                    | 2.3 a              | R                         | 9   | 82 | 9   | 0   | 0         | 0   |
| IAPAR 12320                    | 2.8 a              | R                         | 18  | 73 | 9   | 0   | 0         | 0   |
| IAPAR 12321                    | 2.1 a              | R                         | 9   | 82 | 9   | 0   | 0         | 0   |
| IAPAR 12322                    | 3.2 a              | R                         | 9   | 91 | 0   | 0   | 0         | 0   |
| IAPAR 12323                    | 5.4 a              | R                         | 9   | 64 | 27  | 0   | 0         | 0   |
| IAPAR 12324                    | 1.7 a              | R                         | 55  | 45 | 0   | 0   | 0         | 0   |
| 'Apoatã IAC 2258'              | 4.4 a              | R                         | 45  | 36 | 9   | 9   | 0         | 0   |
| 'Catuaí IAC 99' <sup>(3)</sup> | 100.0 b            | HS                        | -   | -  | -   | -   | -         | -   |
| CV%                            | 31.82              |                           |     |    |     |     |           |     |

CV% 31.82 <sup>(1)</sup>Means followed by the same letter were not different by the Scott-Knott test (p<0,01). Data were  $\sqrt{x+1}$  transformed. <sup>(2)</sup>Resistance level (RL) by HSI. <sup>(3)</sup> Cultivar used as a reference to calculate HSI. ungrafted cultivars, such as IPR 100 (Sera et al., 2009) have become more popular in Paraná State, Brazil and shown good results in the field. Another cultivar with good potential that has yet to be released is 'IPR 106' ("Icatu") (Ito et al., 2008). The progenies studied here are not significantly different from 'Apoatã' because they have a lower nematode multiplication rate, do not segregate for susceptibility, and were classified as resistant at nearly 100%. Therefore, the  $F_5$ progenies with 100% resistant plants will be used to create the next generation by self-fertilization because they show great potential for development as *M. paranaensis*-resistant coffee cultivars. By the Scott-Knott test, all the progenies were classified in only one group which also included the tough standard Apoatã.

# **Materials and Methods**

# Plant materials

In 1987, a natural hybridization between an "Icatu H4782-7-925" plant (female) and "Sarchimor 1669-33" occurred in the municipality of Astorga, state of Paraná, Brazil. Seeds of one plant of "Icatu H4782-7-925" were collected in Astorga and planted in a Meloidogyne paranaensis-infested area located in Centenário do Sul, state of Paraná, Brazil, in 1988. From this population in Centenário do Sul, one plant, presumably the natural F<sub>1</sub> hybrid (HN 87609), was identified that had a higher yield inside the population and was smaller than "Icatu H4782-7-925" and completely resistant to rust. The dwarf stature trait is controlled by one pair of dominant factors named Ct (Carvalho et al., 1984) and complete resistance to rust is also controlled by dominant major genes (Bettencourt et al., 1980, 1992). "Icatu H4782-7-925" is a tall plant with susceptibility to coffee leaf rust (Hemileia vastatrix Berk. et Br.) and "Sarchimor 1669-33" is a dwarf plant with resistance to rust. In Astorga, "Sarchimor 1669-33" plants were located in proximity to the harvested "Icatu H4782-7-925" plants with the unique dwarf genotype with resistance to rust, indicating that the "Sarchimor 1669-33" plants were the pollinators of the natural hybrid.

Seeds from this single rust-resistant plant (HN 87609), were harvested in 1991, and F2 plants were planted in 1992 in another area of Centenário do Sul, which was infested with the same nematode. The F<sub>2</sub> population segregated according to plant size and rust resistance, confirming that a natural hybridization had occurred. Seeds from an F<sub>2</sub> plant (HN 87609-15) were collected and used to produce an F<sub>3</sub> generation in another M. paranaensis-infested area located in the municipality of Munhoz de Melo, Paraná, Brazil, in 1995. The seeds from an F<sub>3</sub> plant (HN 87609-15-6) were collected, and the F<sub>4</sub> plants were used in a study in 2002 conducted at the IAPAR, Londrina, Paraná, Brazil, using an experimental design without nematodes. In an experiment at IAPAR in 2006, individual F<sub>4</sub> plants (HN 87609-15-6-6; HN 87609-15-6-8; HN 87609-15-6-9; HN 87609-15-6-14) were selected to produce the F<sub>5</sub> generation in a nematode-free area using an experimental design. In this study, resistance to M. paranaensis was analyzed using seeds from 19 distinct F<sub>5</sub> individuals from four F<sub>4</sub> plants (Table 1). The C. arabica cv. 'Catuaí Vermelho IAC 99' and C. canephora cv. 'Apoatã IAC 2258' were used as susceptible and resistant controls, respectively (Table 1).

The experiment was conducted in a randomized block design with 21 treatments, 11 replicates and plots containing one plant.

The experiment was conducted in a greenhouse at the IAPAR headquarters in Londrina, Paraná, Brazil (23°21'20.0"S 51°09'58.2"W) between March and July 2013. The maximum and minimum air temperature during the experiment was 26.5 °C and 15.6 °C, respectively. Seedlings were obtained by planting in germinators that contained sand and were located in the IAPAR seedling nursery. When the seedlings reached the cotyledon stage, they were transplanted into 700 mL plastic pots to grow until they had developed three to four pairs of leaves, and they were subsequently inoculated.

The substrate was formulated to contain a 1:1 mixture of soil and sand and previously sterilized in an oven dryer at 100°C for three hours with moisture at field capacity. In every 72 L soil, 230 g single super phosphate, 22 g KCl, 24 g urea and 72 g dolomite limestone were added. Soil fertilization and soil pH correction were performed based on a chemical analysis of the soil.

# Collection, quantification and inoculation of Meloidogyne paranaensis

The *M. paranaensis* inoculum was extracted from pure populations confirmed by electrophoresis and multiplied in tomato plants (cultivar 'Santa Clara') and coffee plants (cultivar 'Catuai') for approximately nine months in a greenhouse. Eggs were collected using the method described by Hussey and Barker (1973) with modifications. Egg concentration was measured in a Peters' counting chamber, and the suspension was adjusted to 1,000 eggs. ml<sup>-1</sup>. A total of 5,000 *M. paranaensis* eggs (Initial population = IP) were inoculated in three holes (approximately 1 cm deep) around each plant.

# Extraction of nematodes

Extraction was performed 110 days after inoculation. The root system of the plants was carefully washed in running water and weighed. The eggs were then collected from the roots using the method in Hussey and Barker (1973) with modifications, and after extraction, the number of eggs and juveniles was counted in a Peters' counting chamber to obtain the final population (FP). The number of nematodes.g<sup>-1</sup> was calculated from the root system weight and nematode count data.

### **Resistance** evaluation

The RF was calculated using the formula FP/IP, where RF  $\leq 1$  = resistant and RF > 1 = susceptible (Oostenbrink, 1966). The RRF and HSI were used to classify the levels of resistance of the progenies. The RRF was calculated using the following formula: RRF = [(RF of the susceptible control] – RF of the treatment) / RF of the susceptible control] × 100 (Moura and Regis, 1987). Based on the RRF, the genotypes were classified using a modified version of the scale of Moura and Regis (1987), where 0 to 25% = HS; 25.1 to 50% = S; 50.1 to 75% = MS; 75.1 to 90% = MR; 90.1 to 95% = R; and 95.1 to 100% = HR. The HSI was obtained using the following formula:  $HSI = (nematodes.g^{-1} \text{ for the treatment/nematodes.g}^{-1} \text{ for the susceptible control}) \times 100 (Gonçalves and Ferraz, 1987, modified). The HSI values were used to classify the level of resistance of the coffee plants using a modified version of the criteria in Fassuliotis (1985) where 0 to 1% = HR; 1.1 to 10% = R; 10.1 to 25% = MR; 25.1 to 50% = MS; 50.1 to 75% = S; and 75.1 to 100% = HS.$ 

The RF, RRF and HSI were calculated from the means of the plots. The percentage of plants with different levels of resistance, which was determined by the RRF and HSI, was calculated using the data from individual plots of the susceptible control and data from the respective treatment plots. The percentages of resistant (HR, R and MR) and susceptible (MS, S and HS) plants were used to determine whether the allele(s) for nematode resistance in the progeny were homozygous or heterozygous.

#### Statistical analysis

The nematodes.g<sup>-1</sup>, RF, RRF and HSI data were tested for normality using the Shapiro-Wilk test, and the homogeneity of the variances was evaluated by Hartley's test at 5% probability. The data were transformed to log(x) for nematodes.g<sup>-1</sup> and to  $\sqrt{x + 1}$  for RF and HSI. For RRF was not used transformation. A subsequent analysis of variance and the means were compared by the Scott-Knott test (p<0.01).

### Conclusions

All of the  $F_5$  progenies of the coffee plants derived from a probable natural cross between "Icatu H4782-7-925" and "Sarchimor 1669-33" showed resistance to *M. paranaensis*. Seventeen of the 19 progenies studied did not segregate for this trait.

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