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IPR 106: new Arabica coffee cultivar, resistant to some *Meloidogyne paranaensis* and *M. incognita* nematode populations of Paraná

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Abstract: *Cultivar IPR 106 resulted from a spontaneous hybridization between "Icatu IAC 925" and an unknow dwarf plant. It is a dwarf cultivar with high rusticity, late ripening cycle, large grains, excellent cup quality and resistance to some populations of the nematodes Meloidogyne paranaensis and M. incognita found in the state of Paraná.*

Keywords*: breeding, Coffea arabica, Icatu 925, root-knot nematode.*

INTRODUCTION

Coffee growers in Brazil is limited mainly by nematodes, which reduce yields and hamper the planting of coffee in infested areas. Potentially promising regions in Brazil cannot be exploited for coffee cultivation due to these pathogens. *Meloidogyne exigua*, *M. incognita* and *M. paranaensis* are the main nematodes in Brazilian coffee plantations (Gonçalves and Silvarolla 2007).

Planting resistant coffee cultivars is the best alternative for infested areas, since this management method of nematode control is efficient and environmentally correct. However, few nematode-resistant coffee cultivars are currently available. Since 1987, the rootstock *Coffea canephora* cv. Apoatã IAC 2258, which is resistant to *M. exigua* (Fonseca et al. 2008), *M. incognita* (Fonseca et al. 2008) and *M. paranaensis* (Fonseca et al. 2008, Andreazi et al. 2015), has been the most widely used cultivar in infested coffee-growing areas.

To date, cv. IPR 100, released in 2012, has been the only available Arabica coffee that is resistant to *M. exigua* (Rezende et al. 2017), to *M. paranaensis* (Andreazi et al. 2015, Sera et al. 2017) and to some *M. incognita* populations (Sera et al. 2017). The release of cv. IPR 106 represents a new option for coffee farmers in areas infested with *M. paranaensis* and *M. incognita*.

PEDIGREE AND BREEDING METHOD

Cultivar IPR 106 was derived from a spontaneous hybridization between Icatu H4782-7-925 (Icatu IAC 925) as mother plant and an unknown dwarf Arabica coffee with yellow fruit. The hypothesis of spontaneous hybridization is explained by the fact that in 1982, a high-yielding dwarf plant with orange fruits was identified in the county of Astorga, Paraná (PR), within a population

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of tall Icatu H4782-7-925 plants. The mother plant Icatu IAC 925 is a resistance source to *Meloidogyne paranaensis*. In 1987, the seeds of this dwarf plant were collected (supposedly the spontaneous F_1 hybrid). In 1988, the F_2 generation was planted in an area infested with the nematodes *M. paranaensis* and *M. incognita* in the county of Centenário do Sul-PR. This F_2 population had normal and dwarf plants, as well as plants with red, orange and yellow fruits, confirming the hypothesis of a spontaneous hybridization between Icatu IAC 925 and a dwarf genotype with yellow fruits. In 1993, yellow fruits were harvested from an F_2 dwarf plant. The F_3 generation was planted in 1994, in another area infested with the same nematodes, in Cambira-PR. Seeds of an F_3 plant (IAPAR 93166-9) were harvested in 1999 and planted to obtain the F_4 generation in 2000, in Londrina-PR.

In 2005, seeds of 25 F_4 plants were harvested and F_5 plants were grown in Londrina. In 2014, seeds of four F_5 progenies were harvested (IAPAR 93166-9-9-16; IAPAR 93166-9-12-18; IAPAR 93166-9-14-19; IAPAR 93166-9-18-22), and a composite sample was established, representing the genetic seed stock (F_6) of cultivar IPR 106, which was released in 2017. From the F_1 to F_5 generations, plants were selected for plant yield and size, fruit size, maturation cycle and resistance to coffee leaf rust. From F_1 to F_3, field plants were selected for nematode resistance, based on the high yield of the selected plants in contrast to the low yield or mortality of susceptible plants. In a greenhouse, resistance to *M. incognita* and *M. paranaensis* was tested in F_4 and F_5.

PERFORMANCE

Cultivar IPR 106 is recommended for cultivation in regions of Paraná, Brazil, with mean annual temperatures between 20 and 23 ºC. Coffee yield was evaluated in Paraná in three field trials, initiated in March 2003, in the counties of Itaguajé (alt 410 m asl, lat 22° 40' 41" S, long 51° 57' 55" W), Londrina (alt 588 m asl, lat 23º 21' 40" S, long 51º 09' 51" W), and Mandaguari (alt 653 m asl, lat 23º 30' 29" S, long 51º 42' 51" W), at mean annual temperatures of 23, 21 and 20 ºC, respectively. The annual relative air humidity is between 65 and 70% in Itaguajé and between 75 and 80% in Londrina and Mandaguari. In these three trials, F_5 seeds, of the same F_4 plants that originated F_5 progenies (IAPAR 93166-9-9-16, IAPAR 93166-9-12-18, IAPAR 93166-9-14-19 and IAPAR 93166-9-18-22) were planted for genetic seed stocks.

The trials were planted at spacings of 3.0 x 0.5 m in Itaguajé; and 2.5 x 0.5 m and 3.0 x 0.5 m in Mandaguari and Londrina. In the three trials the nematodes *Meloidogyne exigua*, *M. paranaensis* and *M. incognita* were not found. The trials were arranged in a randomized block design and three replications of seven plants. Chemical control was applied for control insects (e.g. *Leucoptera coffeella* and *Hypothenemus hampei*), mites (e.g. *Oligonychus coffeae* and *Brevipalpus* spp.) and diseases (e.g. *Hemileia vastatrix* and *Cercospora coffeicola*). Fertilization were made according to the soil and foliar analysis, following the recommendations for the coffee crop in Brazil (Matiello et al. 2016). A 2 kg sample of the harvested coffee cherries of each plot was processed to determine green bean weight. The number of plants per hectare was calculated based on plant spacing and the yield estimated in 60kg bags of green coffee. The R software version 3.3.0 (R Core Team 2016) and package agricolae (Mendiburu 2015) were used to perform the ANOVA, Bartlett test of homogeneity of variances, Shapiro-Wilk normality test and Tukey's test at 5% significance.

In the three counties and at different spacings, the yield of cv. IPR 106 was similar to that of Catuaí Vermelho IAC 81 and IAPAR 59 (Table 1). Possibly, the yield of cv. IPR 106 would also be similar when grown at mean annual temperatures

Table 1. Annual mean yield per hectare (bags of green coffee at 60 kg ha^{-1}) in three counties of Paraná state and different row spacings

Cultivar[1]	Locations and spacings[2]						
	Mandaguari[3] (2.50 x 0.5 m)	Mandaguari[3] (3.00 x 0.5 m)	Londrina[4] (2.50 x 0.5 m)	Londrina[4] (3.00 x 0.5 m)	Itaguajé[5] (3.00 x 0.5 m)	Overall mean	(%)[6]
IPR 106	46.06 a	52.16 a	55.08 a	55.46 a	52.17 a	52.19	102.5
IAPAR 59	39.81 a	52.48 a	59.62 a	41.46 a	50.22 a	48.72	95.7
Catuaí V81	42.26 a	45.55 a	60.23 a	57.10 a	49.45 a	50.92	100.0
Overall mean	42.71	50.06	58.31	51.34	50.61	50.61	
CV (%)	10.21	9.43	9.23	12.50	13.19		

[1] Catuaí V81 = Catuaí Vermelho IAC 81, chemically controlled for coffee leaf rust; [2] Means followed by same letters do not differ from each other (α = 0.05) by the Tukey test; [3] Means of 6 years (2007, 2008, 2009, 2010, 2011, 2012); [4] Means of 4 years (2007, 2008, 2009, 2010); [5] Means of 4 years (2006, 2007, 2008, 2009); [6] Relative yield based on Catuaí V81's overall mean.

between 20 and 23 ºC in other coffee regions of Brazil, but regional field trials are needed to confirm this assumption. Cultivar IPR 106 is a dwarf plant, recommended for dense and traditional planting systems.

NEMATODE RESISTANCE

The IPR 106 resistance to *Meloidogyne incognita* and *M. paranaensis* was assessed in a greenhouse of the Instituto de Desenvolvimento Rural do Paraná – IAPAR-EMATER (IDR-Paraná) (alt 575 m asl; lat 23° 21' 20.50" S, long 51° 09' 53.12" W), in Londrina, Paraná, Brazil. Between January and May 2014, two experiments were carried out between January and May 2014, at temperatures between 19.2 ºC and 31.1 ºC. In all experiments, the *Coffea arabica* cv. Mundo Novo IAC 376-4 was used as susceptible control. The experiments were arranged in a completely randomized design, with 20 (experiment with *M. incognita*) or 15 replications (experiment with *M. paranaensis*) of one plant. In both experiments, F_6 seeds of the four F_5 progenies that originated IPR 106 were used.

Nematode populations were collected from coffee plants grown in Altônia, PR, Brazil (*M. incognita*) and in Apucarana, PR (*M. paranaensis*), identified, and each species was cultured from a single egg mass (isolate). The identification of the two species was based on morphological approaches (Hartman and Sasser 1985) and α-esterase phenotypes (Carneiro et al. 2000). The *M. paranaensis* population is registered at the IDR-Paraná Laboratory of Nematology as 98.1. The *M. incognita* population was registered as 39H and identified as race 3 in a test with differential plants (Carneiro and Almeida 2000). In a greenhouse, both populations were multiplied on Mundo Novo IAC 376-4. Approximately 60 days before inoculation, nematodes of the two species were extracted from the Mundo Novo IAC 376-4 roots (Boneti and Ferraz 1981) and inoculated on tomato cv. Santa Clara for multiplication.

Seeds of the IPR 106 and Mundo Novo IAC 376-4 were sown directly in sand-filled germinators. Germinating seedlings (cotyledonary stage) were planted in tubes, left to grow and transplanted (one plant per pot) into 700 cm^3 plastic pots with 600 cm^3 sterilized (at 160 °C for 5 h) substrate (58% sand, 34% clay and 8% silt) when they had 4-6-leaf pairs. Prior to inoculation, the plants were cultivated for one month and watered as required and fertilized once with 3 g Osmocote® Plus (15% N, 12% K_2O, 9% P_2O_5, 2.3% S, 1% Mg, 0.45% Fe, 0.06% Mn, 0.05% Cu, 0.02% Mo).

By using the NaCl method (Boneti and Ferraz 1981), the eggs were extracted from tomato roots, and a suspension of 2 ml with 2.000 eggs (IP = initial population), was poured into two holes, 3.5-4.5 cm deep, located 1.5 cm away from the root collar of each coffee seedling.

The plants were left to grow for 130 days with *M. incognita* and 121 days with *M. paranaensis*, and then the coffee roots were washed and dried on absorbent paper. After this, the root fresh weight was determined and nematodes were extracted as described by Boneti and Ferraz (1981). The final population (FP) was estimated by counting the second-stage juveniles (J2) and eggs in a suspension obtained by extracting the nematodes from the entire soil-free root system. Subsequently, the reproduction factor (RF = FP/IP) was determined (Oostenbrink 1966) and the amount of nematodes per gram of roots (NGR) calculated for each replication.

The ANOVA F test (R Core Team 2016) was used to test the hypothesis of the existence of significant differences in the variables RF and NGR between the cultivars IPR 106 and Mundo Novo. The NGR and RF data were $\sqrt{x+1}$ -transformed. Cultivars with RF > 1.0 were classified as susceptible and when RF ≤ 1.0, as resistant.

The high RF values (30.8 for *M. incognita* and 29.09 for *M. paranaensis*) of the Mundo Novo IAC 376-4 (control cultivar) in both experiments confirmed the inoculum viability and appropriate experimental conditions. Cultivar IPR 106 differed statistically from cv. Mundo Novo, in both variables RF and NGR. Based on RF values, cv. IPR 106 was resistant to *M. paranaensis* (0.08) and *M. incognita* (0.65). The NGR data of both experiments were coherent with the RF values.

The resistance to both nematodes observed in this study confirmed previous findings of the simultaneous resistance of this cultivar against the nematodes *M. incognita* and *M. paranaensis* (Ito et al. 2008). Another histopathological study reported that the resistance of IPR 106 is apparently due to hypersensitive-like response and was detected at 30 days after inoculation with population 98.1 of *M. paranaensis*, when abnormal giant host cells were observed and malformed nematode females (Shigueoka et al. 2019). IPR 106 has not yet been tested for all known *M. exigua* and *M. incognita* races.

PERFORMANCE IN NEMATODE-INFESTED AREA

Nematode resistance was also evaluated in an area infested simultaneously with *M. incognita* and *M. paranaensis*, in the county of São Jorge do Patrocínio (lat 23° 42' 32" S, long 53° 54' 15" W; alt 360 m asl), Paraná, Brazil, where the mean annual temperatures is 23 ºC.

In July 2007 and May 2010, 16 random 1-L soil samples were collected and used to for the identification of the nematode species and population densities in the experiment. Using the Baermann Funnel methodology (Baermann 1917), a 50-cm^3 soil sub-sample was processed to extract the free nematodes, in the IDR-Paraná Laboratory of Nematology. By means of dichotomous keys for morphological and morphometric traits, the genus *Meloidogyne* was identified, in 37.5% of the samples collected in 2007 and in 56.0% of the samples of 2010. In each of the 50 cm^3 soil samples collected in 2007 and 2010, respectively, an average 61 and 172 individuals were found, indicating an increase in the population density during this period.

The remaining soil not used in the Baerman Funnel test was used to fill 1-L plastic containers, in which tomatoes were grown for nematode multiplication. Three months later, females were collected from the roots to identify *Meloidogyne* species by morphological approaches (Hartman and Sasser 1985) and α-esterase phenotypes (Carneiro et al. 2000). In the samples of 2007 and 2010 with presence of *Meloidogyne* spp., a mixture of *M. incognita* and *M. paranaensis* was identified on 82% and 71% of the tomato roots, respectively. In the other samples, *M. incognita* or *M. paranaensis* were found separately.

In April 2003, the field experiment was planted at a spacing 2.5 x 0.5 m, in a randomized block design, with eight replications of seven plants. The cultivars Catuaí Vermelho IAC 81, IAPAR 59, IPR 103, IPR 100 and IPR 106 were evaluated. The first three were used as susceptible checks and IPR 100 as resistant check (Sera et al. 2017). The F_5 seeds of the same composite sample used earlier in the performance experiments of cv. IPR 106 were planted. Fungicide was applied for rust control and fertilization according to soil analysis.

Nematode resistance was evaluated indirectly by the following measurements: yield and plant vigor from 2006 to 2010, plant height and canopy diameter in 2010 and percentage of dead plants in 2010.

Yield and plant vigor were evaluated in June, from 2006 to 2010. Yield was evaluated by the same methodology used in the three above experiments to evaluate cultivar performance. The vigor of the coffee plants was assessed on a 1 - 10 scale (Shigueoka et al. 2014), from the most severely attacked plants to those with best development, respectively. These grades were based on the overall vegetative aspect of the plants, using the criteria plant height, canopy diameter, main orthotropic stem diameter, branch diameter, secondary plagiotropic branches, leafiness, and leaf color and thickness.

The R software version 3.3.0 (R Core Team 2016) and package agricolae (Mendiburu 2015) were used to perform the ANOVA, Bartlett test of homogeneity of variances, Shapiro-Wilk normality test and Tukey's test at 5% significance. The $\sqrt{x+1}$ transformation was used for all variables.

As of 2007 (4^{th} year after planting), the yield and vigor of the cultivars IPR 103, IAPAR 59 and Catuaí decreased progressively, and particularly that of the latter two dropped sharply. In 2009 and 2010, the yields of these three susceptible cultivars were almost zero, while the plants were small and stunted, with sparse leafiness and no new branching, very thin branches and yellowish leaves (Table 2). In 2010, the susceptible cultivars had a high percentage of dead plants, and the height and diameter of the surviving were lower than of cvs IPR 106 and IPR 100 (Table 3).

In this area infested with *M. paranaensis* and *M. incognita*, the agronomic performance of cv. IPR 106 was satisfactory, with an average yield of 32.44 bags (60 kg) of green beans per hectare and good plant vigor.

OTHER TRAITS

The maturation cycle of cv. IPR 106 is late, similar to Catuaí. In Brazil, at mean annual temperatures of 22 ºC, 21 ºC and 20 ºC, maturation usually occurs in June, July and August, respectively. Cultivar IPR 106 has very vigorous plants and was selected in regions with low soil fertility in Paraná state. IPR 106 is moderately susceptible to the physiological races of coffee leaf rust found in Paraná (Sera et al. 2010), and chemical control is necessary. According to Sera et al. (2010), the rust severity on cv. IPR 106 was lower than on Catuaí and IPR 100, but greater than on the so-called Sarchimor cultivars

Table 2. Variables of Arabica coffee cultivars assessed between 2006 and 2010 in an area infested with *Meloidogyne paranaensis* and *M. incognita*, in São Jorge do Patrocínio, PR, Brazil

Cultivar	Variables[1, 2]											
	Y06	Y07	Y08	Y09	Y10	MY	V06	V07	V08	V09	V10	MV
IPR 100	34.1 a	25.9 a	76.9 a	61.3 a	15.3 a	42.7 a	9.4 a	8.3 a	8.4 a	7.9 a	7.6 a	8.3 a
IPR 106	21.3 ab	12.8 abc	64.2 ab	58.7 a	5.2 ab	32.4 a	7.7 ab	8.0 a	7.4 ab	6.9 a	6.2 a	7.2 ab
IPR 103	22.9 ab	13.5 ab	36.7 bc	0.0 b	0.7 b	14.8 b	8.4 ab	6.2 ab	5.8 bc	4.4 b	2.9 b	5.6 bc
IAPAR 59	11.3 b	10.4 bc	23.3 c	3.3 b	0.0 b	9.7 b	6.9 b	4.3 b	4.3 cd	3.3 bc	2.5 b	4.2 c
Catuaí V. 81	27.1 ab	3.9 c	32.5 bc	0.0 b	0.0 b	12.7 b	7.1 b	5.4 b	3.8 d	2.6 c	1.5 b	4.0 c
Overall mean	23.4	13.3	46.7	24.7	4.2	22.5	7.9	6.4	5.9	5.0	4.1	5.9
CV%	26.1	31.0	34.6	20.6	68.4	22.1	7.8	11.2	10.3	10.2	19.9	8.8

[1] Y06, Y07, Y08, Y09 and Y10 = yield in bags of 60 kg of green coffee per hectare in 2006, 2007, 2008, 2009 and 2010, respectively; MY = mean yield based on the Y06, Y07, Y08, Y09 and Y10; V06, V07, V08, V09 and V10 = plant vigor evaluated in 2006, 2007, 2008, 2009 and 2010, respectively; MV = mean plant vigor based on the V06, V07, V08, V09 and V10; [2] Means followed by same letters do not differ from each other (α = 0.05) by the Tukey test.

Table 3. Plant height, canopy diameter and percentage of dead plants of Arabica coffee cultivars in São Jorge do Patrocínio, PR, Brazil

Cultivar	Plant height[1] (m)	Canopy diameter[1] (m)	% of dead plants[1]
IPR 100	1.96 a	1.84 a	1.79 b
IPR 106	2.09 a	1.87 a	5.36 b
IPR 103	1.07 b	0.96 b	26.79 ab
IAPAR 59	1.11 b	0.82 b	35.71 ab
Catuaí V. 81	1.02 b	0.55 b	44.66 a
Overall mean	1.45	1.21	22.86
CV (%)	12.24	10.43	78.12

[1] Means followed by same letters do not differ from each other (α = 0.05) by the Tukey test.

IPR 97, IPR 98, IPR 104 and IAPAR 59. IPR 106 has moderately resistance to *Pseudomonas syringae* under a natural occurrence of the pathovars *garcae* and *tabaci*, on field conditions in adult plants (Fernandes et al. 2020). The fruits of cv. IPR 106 are very large, oblong, yellow, and the mean sieve size of the grains is 18. The fruits can be harvested easily, both manually and mechanically. The canopy architecture is cylindrical, and the young leaves are green or bronze. The cup quality of cv. IPR 106 is excellent, usually better than that of other Arabica coffee cultivars.

SEED MAINTENANCE AND DISTRIBUTION

In Brazil, cultivar IPR 106 was registered by the National Cultivar Registry (RNC) of the Ministry of Agriculture, Livestock and Food Supply (MAPA), under no. 09942. In January 2015, IPR 106 was protected by the National Cultivar Protection Service (SNPC), in Brazil (nº 20150128). The IDR-Paraná is in charge of the genetic and basic seed stock. Seed growers of private companies registered by MAPA are responsible for certified seed production.

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