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SHORT COMMUNICATION / *COMUNICAÇÃO*

Resistance to *Meloidogyne paranaensis* in wild *Coffea arabica*

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ABSTRACT

Nine accessions of wild *Coffea arabica* from Ethiopia were evaluated for resistance to *Meloidogyne paranaensis.* Two well-characterized susceptible and resistant cultivars were used as comparative controls. The experiments were conducted in a growth chamber using a clonal population of *M. paranaensis* (esterase phenotype P1) originating from Brazil. Resistance and susceptibility to the nematode were evaluated using the number of nematodes (eggs and J2) per plant, number of nematodes per gram of root and the reproduction factor (RF). All wild coffee accessions expressed a resistance response to *M. paranaensis* similar to that of the resistant control Nemaya (RF < 1.0). These results provide coffee breeders with material whose resistance can be transferred into commercial cultivars.
Keywords: coffee, root-knot nematode, pathogenicity.

RESUMO

Avaliação da resistência de cafeeiros silvestres (*Coffea arabica*) a *Meloidogyne paranaensis*

Foram avaliados quanto à resistência a *Meloidogyne paranaensis*, nove acessos de cafeeiros silvestres incluindo dois cultivares bem caracterizados como testemunhas de suscetibilidade e resistência. Os experimentos foram realizados sob condições controladas em câmara de crescimento, utilizando uma população clonal de *M. paranaensis* (fenótipo de esterase P1), proveniente do Brasil. A resistência e a suscetibilidade ao nematóide foram avaliadas com base no número total de nematóides por planta (ovos + J2) e por grama de raiz e no fator de reprodução (FR). Todos os acessos mostraram resposta à infecção por *M. paranaensis* similar à da testemunha resistente (FR < 1,0). Com esses resultados, novos materiais, cuja resistência pode ser transferida aos cultivares comerciais, ficam disponíveis para os fitomelhoristas.
Palavras-chave: café, nematóide das galhas, patogenicidade.

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint on coffee production in most countries worldwide (Campos & Villain, 2005; Campos & Silva, 2008). Seventeen species of *Meloidogyne* are acknowledged as pathogens to coffee (Carneiro & Cofcewicz, 2008). Economic losses due to root-knot nematodes vary considerably depending on the species involved and its distribution. Some *Meloidogyne* species induce numerous galls but only cause a 10 to 20% drop in yield (Bertrand et al., 1997). Other species cause serious damage in plantations, destroying up to 80% of the root system within five years of planting (Bertrand & Anthony, 2008).

Meloidogyne paranaensis (Carneiro et al*.,* 1996) is one of the most destructive root-knot species on coffee. This species induces foliar necrosis, reduces growth, causes leaf drop and a general plant decline, and can even cause plant death (Campos & Villain, 2005). *M. paranaensis* is widely distributed in Brazil and Guatemala, where coffee represents an important source of income and employment. *M. paranaensis* was mistaken for *M. incognita* for more than 20 years (Carneiro et al*.*, 1996). Consequently, publications prior to 1996, and a few since, refer to an isolate collected in Guatemala at the beginning of the 1990s as *Meloidogyne* sp. or *M. incognita*, when it was in fact identified as *M. paranaensis* (Carneiro et al*.*, 2004). Although the populations of *M. paranaensis* from Brazil and from Guatemala presented different esterase phenotypes Est P1 and Est P2, respectively, they are very closely related in molecular and morphological approaches (Carneiro et al., 2004). Nevertheless, these two populations presented different physiological behavior in relation to resistant tomato with Mi gene: P2 parasitized the resistant tomato and P1 did not (Carneiro, R.M.D.G., pers. inform.). Among the non-chemical methods available to control root-knot nematodes in coffee, the use of resistant cultivars is considered one of the most effective and environmentally safe alternatives.

Work on coffee resistance to *M. paranaensis* (Est P2) began in Guatemala and resistant accessions were identified among cultivars of *Coffea canephora* Pierre ex. Froehn. (Anzueto et al., 2001; Bertrand et al., 2000). In *C. arabica* L., all cultivars were susceptible to *M. paranaensis* (Est P2), whereas some wild coffee accessions from Ethiopia, the center of origin of the species, were resistant (Anzueto et al., 2001). These findings have increased the interest in wild coffee for breeding purposes, which have greater genetic diversity than commercial cultivars (Anthony et al., 2001) and also resistance to *M. arabicida* López & Salazar,1989 whose distribution is restricted to Costa Rica (Bertrand et al., 2002b). The objective of this paper was to screen wild *C. arabica* for resistance to *M. paranaensis* (Est P1) from Brazil.

The study was conducted in the Nematology laboratory of the French Agricultural Research Centre for International Development (CIRAD) at Montpellier, France. The plant material consisted of seven wild coffee accessions (Table 1) from Ethiopia (Guillaumet & Hallé, 1978) and two well-characterized coffee cultivars (Caturra and Nemaya), used as controls. Seven accessions had been reported as being resistant to *M. paranaensis* isolate from Guatemala (Anzueto et al., 2001). The commercial cultivar Caturra (Carvalho et al., 1991) served as the susceptible *M. paranaensis* control (Anzueto et al., 2001). The rootstock cultivar Nemaya served as the *M. paranaensis* resistant control (Bertrand et al., 2002a). Seedlings of Nemaya were produced in seed gardens in the Coffee Research Centre of Guatemala (ANACAFE) (Bertrand et al., 2002a). Seedlings were used because *C. arabica* is not polymorphic (Anthony et al., 2001; 2002) and is self-fertile (Carvalho et al., 1991). Seedlings of the wild accessions and Caturra were provided by the Tropical Agricultural Research and Higher Education Centre (CATIE) in Costa Rica. Cuttings of two Ethiopian accessions (Ar 57, Ar 59) were also prepared from a coffee genebank maintained by the IRD in Montpellier. These accessions have a common origin in Ethiopia with the Et-57 and Et-59 accessions conserved in the field genebank of CATIE. Seedlings and cuttings were cultivated in 300 cm^3 plastic pots containing 4:1 (v/v) sterilized soil and fine sand in a growth chamber at 25°C (± 1°C) and 70% relative humidity. Five plants of each accession were evaluated. A clonal population of *M. paranaensis* was established from a single egg mass of nematodes originally collected from coffee in Apucarana (Paraná State, Brazil) and then maintained in culture by Embrapa Recursos Genéticos e Biotecnologia. A clonal population ensures repeatability of the evaluations (Bertrand & Anthony, 2008). This clonal population was cultured in a growth chamber on Caturra coffee plants. When the plants were three months old (two pairs of fully-expanded leaves), single plants were inoculated with about 750 eggs and second-stage juveniles (J2) into 1-cm deep holes around the collar region. Four months later, plants were removed carefully from the pots. The root systems were gently washed with tap water and weighed. Nematodes were extracted by maceration, centrifugation and flotation (Coolen & d'Herde, 1972). The final population density (Pf = number of eggs + J2) was quantified using a Peter slide under LM and nematode reproduction factors (RF = Pf/750) were calculated. RFs were characterized as showing resistance (RF < 1.0) or susceptibility (RF > 1.0) (Oostenbrink, 1966). Data of root weight and nematode population were analyzed by one-way analyses of variance (ANOVA) using Statistica version 7.1 software (© StatSoft, Inc.). Mean values were compared between accessions and controls with Duncan's test at P=0.01.

TABLE 1 - Origin of the wild coffee accessions collected in Ethiopia by ORSTOM in 1966 (Guillaumet and Hallé, 1978). The "Et" accessions are conserved in the CATIE field genebank at Turrialba and the "Ar" accessions in the IRD greenhouse at Montpellier

	Ethiopian collection site		Material	Previous
Accession	Village	Province	evaluated	Evaluation*
Et-15	Gimma-Goré	Kefa	Seedling	Resistant
Et-25	Tippi	Illubabor	Seedling	Resistant
Et-25B	Tippi	Illubabor	Seedling	Resistant
Et-32B	Tippi	Illubabor	Seedling	
Et-52	Bonga	Kefa	Seedling	
Et-57	Bonga	Kefa	Seedling	Resistant
Ar 57	Bonga	Kefa	Cutting	Resistant
Et-59	Bonga	Kefa	Seedling	Resistant
Ar 59	Bonga	Kefa	Cutting	Resistant

* using a *Meloidogyne paranaensis* isolate Est P2 from Guatemala (Anzueto et al., 2001)

ANOVA revealed that the root weights were different (P = 0.015) among the material evaluated, and the means formed two groups according to Duncan's test (Table 2). This justified the use of the number of nematodes per gram of root additionally to the total number of nematodes extracted per plant (final population = FP). Differences were shown by analyses of variance for the FP (P < 0.021) and the number of nematodes per gram of root (P < 0.0008). The FP of the resistant 'Nemaya' was 233 and 6,700 on the susceptible 'Caturra' (Table 2). On average, the resistant control contained about 30 times fewer nematodes than the susceptible plant. Nematode per gram of root had a lower coefficient of variation than the FP (26.1% vs. 42.7% respectively). The wild accessions had FP from 67 to 477 (Table 2). The accessions' response to *M. paranaensis* was similar to 'Nemaya', limiting nematode reproduction to low levels.

At least one nematode was extracted from each plant, indicating that all the accessions supported reproduction of *M. paranaensis*. Similar residual populations have been observed in coffee inoculated with *M. exigua* (Gonçalves & Pereira, 1998; Anthony et al., 2005) and *M. incognita*

TABLE 2 - Evaluation of resistance to *Meloidogyne paranaensis* (Est P1) from Brazil in wild coffee accessions as compared to the susceptible 'Caturra' and resistant 'Nemaya' cultivars. Values in a column with the same letter are not different (P=0.01) by the Duncan test

Cultivar or accession	Root weight (g)	Nematodes / plant	Nematodes / g root	Reproduction Factor
Caturra	6.4[ab]	6,700[a]	1,013[a]	8.9[a]
Nemaya	5.0[b]	233[b]	58[b]	0.3[b]
Et-15	7.3[ab]	477[b]	61[b]	0.6[b]
Et-25	5.6[b]	167[b]	31[b]	0.2[b]
Et-25B	4.8[b]	200[b]	50b	0.3[b]
Et-32B	4.8[b]	180[b]	36[b]	0.2[b]
Et-52	4.7[b]	100[b]	21[b]	0.1[b]
Et-57	5.9[ab]	67[b]	12[b]	0.1[b]
Et-59	6.3[ab]	167[b]	27[b]	0.2[b]
Ar 57	8.9[ab]	320[b]	37[b]	0.4[b]
Ar 59	10.6[a]	88[b]	8[b]	0.1[b]

(Gonçalves et al., 1996). This suggests an underlying mechanism of defense response after infection, which allows reproduction of a small number of nematodes.

These results confirmed a previous screening of resistance in wild coffee from Ethiopia using a *M. paranaensis* Est P2 isolate from Guatemala (Anzueto et al., 2001). New sources of resistance (Et-32B and Et-52) were identified. This identification has increased the set of resistant genotypes available to coffee breeding programs. Accessions resistant to *M. paranaensis* were collected in the provinces of Kefa and Illubabor, which constitute the center of diversity for *C. arabica*. Root-knot nematode resistance may be a common trait in wild coffee and widely distributed among wild plants. The coffee genetic resources of the study are conserved in several coffee genebanks worldwide (Brazil, Colombia, Ivory Coast, Cameroon, Tanzania, Kenya and Madagascar) and are thus available for the scientific community (Anthony et al., 2007).

Resistance to *M. paranaensis* can be easily transferred into susceptible cultivars by making controlled hybridizations (Bertrand & Anthony, 2008). All the accessions from the study except one (Et-25B) have been used as male parents and crossed with commercial cultivars Caturra and Catuai in order to produce F_1 hybrids (Bertrand et al., 2005). Breeders should be able to produce coffee hybrids that have resistance genes to *M. paranaensis* and to *M. arabicida* from the wild coffee genitor, and to *M. exigua* from an introgressed line like Iapar 59, which has inherited the resistance gene *Mex-1* from *C. canephora* (Bertrand et al., 2001). In a few years, it may be possible to offer coffee growers cultivars that are resistant to a range of root-knot nematodes, greatly reducing yield losses and nematicide usage.

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