Pathogenicity and Genetic Variability of *Radopholus similis* Populations in Bananas (*Musa acuminata* AAA and AA) based on RAPD Analysis*

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*This article is part of the DSc. thesis of senior author - Graduate Program in Plant Pathology, University of Brasília, with scholarship and financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Edital Universal (019 / 2003)

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Received for publication on April 6, 2006. Accepted on July 29, 2008
Edited by Claudio Marcelo Oliveira and Regina Carneiro

Summary - Costa, D.C., F.G. Faleiro, J.E. Cares & A.C. Gomes. 2008. Pathogenicity and genetic variability of *Radopholus similis* populations in bananas (*Musa acuminata* AAA and AA) based on RAPD analysis.

The burrowing nematode (*Radopholus similis*) is considered the most economically important nematode to banana production worldwide. In Brazil, yield losses can reach up to 100 % among Cavendish bananas. Although no information is available on genetic and biological variability of the nematode in Brazil in bananas and other plants, observations have suggested the occurrence of several biotypes of the nematode. The objective of this study was to observe genetic variability of 12 populations of *R. similis* using RAPD markers, in relation to geographic origin and aggressiveness on diploid (AA) and triploid (AAA) banana genotypes. This study showed that all populations of *R. similis* reproduced in all banana genotypes, and higher values for the reproduction factor occurred on Grand Naine, Pisang Jari Buaya, and Yangambi km 5. Differences in aggressiveness were reflected on plant height and root weight. Populations from Bahia (BA_1 and BA_2), Minas Gerais (MG_1 and MG_2), Pernambuco (PE), Cuba (CUB), and Costa Rica (CR) were more aggressive on banana plants, in particular those from Pernambuco and Bahia. By contrast, populations from Rio de Janeiro (RJ), São Paulo (SP_1 and SP_2), Santa Catarina (SC), and Australia (AUS) were less aggressive. Through RAPD markers at the relative genetic distance of 0.45, the populations of *R. similis* were separated in five similarity groups. No correlation between geographic proximity and genetic similarity was observed among *R. similis* populations, except for the populations from Bahia (BA_1 and BA_2). In this study it was evidenced a close association between the level of aggressiveness on bananas and a short genetic distance, except for the populations from Rio de Janeiro (RJ) and Australia (AUS).

Key words: pathogenicity, aggressiveness, genetic variability, burrowing nematode, banana, Brazil.

Resumo - Costa, D.C., F.G. Faleiro, J.E. Cares & A.C. Gomes. 2008. Variabilidade patogênica e genética de populações de *Radopholus similis* em bananeiras (*Musa acuminata* AAA e AA.) baseada em marcadores RAPD.

O nematóide cavernícola (*Radopholus similis*) é o nematóide de maior importância econômica em bananeiras em todo mundo. No Brasil as perdas na produção atingem 100 % entre as bananeiras do subgrupo Cavendish. Embora nenhuma informação tenha sido divulgada quanto à variabilidade genética e biológica das populações de *R. similis* associadas com bananeiras e a outras hospedeiras no Brasil, observações têm sugerido a existência de vários biótipos do nematóide. O objetivo desse estudo foi observar a variabilidade genética de 12 populações de *R. similis* usando marcadores RAPD, em relação à origem geográfica e agressividade sobre genótipos diplóides (AA) e triplóides (AAA) de banana. Este estudo mostrou que todas as populações reproduziram-se

em todas as cultivares de banana e, que valores elevados de fator de reprodução ocorreram nas cultivares Grande Naine, Pisang Jari Buaya e Yangambi km 5. Diferenças de agressividade foram refletidas na altura das plantas e massa das raízes. As populações da Bahia (BA_1 e BA_2), de Minas Gerais (MG_1 e MG_2), de Pernambuco (PE), de Cuba (CUB) e da Costa Rica (CR) foram mais agressivas sobre as bananeiras, especialmente a de Pernambuco e as da Bahia. Ao contrário, as populações do Rio de Janeiro (RJ), de São Paulo (SP_1 e SP_2), de Santa Catarina (SC), e da Austrália (AUS) foram menos agressivas. Através de marcadores RAPD, a uma distância genética relativa de 0,45, as populações de *R. similis* foram separadas em cinco grupos. Não houve correlação entre proximidade geográfica e similaridade genética entre as populações, exceto para as populações da Bahia (BA_1 e BA_2). No presente estudo, foi evidenciada uma associação entre o grau de agressividade em bananeiras e menor distância genética entre as populações de *R. similis*, exceto para as populações do Rio de Janeiro (RJ) e da Austrália (AUS).

Palavras-chaves: patogenicidade, agressividade, variabilidade genética, nematóide cavernícola, banana, Brasil.

Introduction

The nematode *Radopholus similis* has been known since 1890, when Cobb detected it parasitizing banana roots in the Fiji Islands. The nematode was described as *Tylenchus similis* Cobb, 1893. In 1949, Thorne transferred it to the genus *Radopholus*, with this species renamed as *R. similis* (Cobb, 1893) Thorne, 1949. This nematode has a worldwide distribution, and is able to cause extensive root necroses, and is therefore commonly named as the burrowing nematode. Lesions are progressive towards the point of root emission, reaching the rhizome, where extensive dark lesions surround the rhizome. The decay of anchoring roots is responsible for plant uprooting. In addition to reduction in plant stand, parasitism by this nematode reflects negatively on fruit yield, due to late flowering, lack of uniformity in bunch development at harvest time, and reduction in bunch weight (Jaehn, 1993).

This species probably is originated from Australia and New Zealand (Sher, 1968), then disseminated to banana plantations throughout the world due to frequent exchange of infected rhizomes. *Radopholus similis* was first reported in Brazil in banana plantations of Juquiá, State of São Paulo by Carvalho (1959). Thereafter, the nematode was reported in other municipalities in the region of Vale do Ribeira, State of São Paulo, including Registro, Eldorado Paulista, Miracatu, Pedro de Toledo, Itariri, Pariquera-Açu, Iguape, Peruíbe and Itanhaem. Zem & Lordello (1983) and Lima & Goulart (1986) reported the occurrence of *R. similis* in banana plantations in the states of Bahia, Ceará, Espírito Santo, Goiás, Distrito Federal, Maranhão, Mato Grosso do Sul, Paraíba, Pernambuco, Rio Grande do Norte, Rio de Janeiro, São Paulo, Santa Catarina and Minas Gerais. There were additional reports on other hosts in the State of Rio Grande do Sul (Sperandio & Monteiro, 1993).

Several factors may affect taxonomic identification of nematodes. Identification is mainly based on the analysis of morphologic and morphometric characteristics. However, for closely related species, morphology of individuals is not sufficient for a precise distinction. Therefore, physiological, cytogenetic, sorological and molecular characteristics can establish complementary means for the identification of morphologically similar taxa. Molecular techniques based on PCR (RFLP, RAPD and sequencing of the ITS regions) have been used to identify and to differentiate among plant-parasitic nematodes, as well as for establishing phylogenetic relationships, to evaluate intraspecific genetic variation, and to associate biological profile with nematode virulence (Allen *et al.*, 1997; Thiéry *et al.*, 1997; Blaxter *et al.*, 1998; Subbotin *et al.*, 1999; Elbadri *et al.*, 2002; Wishart *et al.*, 2002; Handoo *et al.*, 2004; 2005).

Du Charme & Birchfield (1956) demonstrated the existence of two races in *R. similis*: one attacking bananas and the other banana and citrus plants. Afterwards, studies of behavior, sexual attraction (Huettel *et al.,* 1982), biochemical (Huettel *et al.,* 1983a,b), and kariological (Huettel & Dickson, 1981; Huettel *et al.,* 1984a), separated both races into two species, *R. similis* and *R. citrophilus*, the latter restricted

to the State of Florida (Huettel *et al.,* 1984b). Later, Siddiqui (1996) suggested *R. citrophilus* as a sub-species of *R. similis* (*R. similis citrophilus* and *R. similis similis*). The possibility of the existence physiological races or biotypes in *R. similis* has increased interest in genetic variation at the intraspecific level (Kaplan & Gottwald, 1992; Hahn *et al.,* 1994) since knowledge of genetic variation within the species *R. similis* is essential for breeding programs to resistance to this nematode.

In Brazil, banana plantations have been expanding into different regions, contributing for making the country the second largest banana producer worldwide (FAO, 2007). No information is available on the biology and genetic variability among populations of *R. similis* associated with bananas in Brazil. The objective of this study was to observe genetic variability of 12 populations of *R. similis* using RAPD markers, in relation to geographic origin and aggressiveness on diploid (AA) and triploid (AAA) banana genotypes.

Materials and Methods

Pathogenicity of *Radopholus similis* populations on bananas. The experiment was conducted from October to December, 2002 at the Biological Station of the University of Brasilia. The experiment design was completely randomized, with four replications, in a factorial arrangement (12 *x* 3) of twelve populations of *R. similis* (Table 1) and three banana cultivars with different levels of resistance to the nematode (Table 2). These banana cultivars were selected taking in account the importance of Grand Naine to the Brazilian banana market, as well as a standard of susceptibility to *R. similis*; Yangambi km 5 as a recently released cultivar, named as Caipira by Embrapa Mandioca e Fruticultura to the Brazilian growers; while Pisang Jari Buaya had been chosen based on previous information assuring resistance of this genotype to *R. similis*. The populations of *R. similis* for this study were extracted from roots and rhizomes of banana (*Musa* AAB), collected in different banana plantations from Brazil. For comparison, three foreign populations of *R. similis* from Cuba (Villa Clara), Costa Rica (Talamanca) and Australia (Queensland) were also included. These populations were introduced from CIRAD (Montpellier, France), considering that similar studies on aggressiveness had been accomplished with them (Fallas *et al.*, 1995; Stoffelen *et al.*, 1999; Elbadri, 2000). Micro-propagated banana plantlets, approximately 10 cm high, were inoculated with 100 nematodes (adults and juveniles) from one of the populations of *R. similis* multiplied on tissue culture of carrot (*Daucus carota* L.) discs (O'Bannon & Taylor, 1968; Fallas & Sarah, 1994). Non inoculated plants of each cultivar served as control. After inoculation, plants were maintained for 120 days in a growth chamber at approx. 28 °C and 12 h daily light. Plants were uprooted and differences of the aggressiveness of *R. similis* populations were compared on the basis of plant height, fresh root weight, percentage of

Table 1 - *Radopholus similis* populations evaluated in the present study.

Code	Host plant	Locality, state, country
MG_1	*Musa* AAB 'Prata Anã'	[1]Janaúba, Minas Gerais, Brazil
MG_2	*Musa* AAB 'Prata Anã'	[1]Jaíba, Minas Gerais, Brazil
BA_1	*Musa* AAA 'Yangambi km 5'	[1]Cruz das Almas, Bahia, Brazil
BA_2	*Musa* AAA 'Nanicão'	[1]Bom Jesus da Lapa, Bahia, Brazil
PE	*Musa* AAB 'Pacovan'	[1]Petrolina, Pernambuco, Brazil
RJ	*Musa* AA 'Ouro'	[1]Rio de Janeiro, Rio de Janeiro, Brazil
SP_1	*Musa* AAA 'Grande Naine'	[1]Jacupiranga, São Paulo, Brazil
SP_2	*Musa* AAA 'Grande Naine'	[1]Cajati, São Paulo, Brazil
SC	*Musa* AAA 'Nanicão'	[1]Luís Alves, Santa Catarina, Brazil
AUS	*Musa* AAA	[2]Queensland, Australia
CUB	*Musa* sp.	[2]Villa Clara, Cuba
CR	*Musa* AAA 'Valery'	[2]Talamanca, Costa Rica

[1]Roots and rhizomes collected in banana plantations from Brazil.
[2]Culture on carrot disk maintained at CIRAD, Montpellier, France.

Table 2 - Banana cultivars used in pathogenicity experiment with different levels of resistance to *Radopholus similis*.

Cultivar	Genome	Host status	Reference
Grand Naine	AAA	Highly susceptible	Elsen *et al.* 2002
Yangambi km 5	AAA	Partially resistant	Fogain & Gowen (1998) and Fallas & Marbán- Mendonza (1994)
Pisang Jari Buaya	AA	Resistant	Wehunt *et al.* (1978), Pinochet & Rowe (1979) and Elsen *et al.* (2002)

healthy and dead roots, and percentage of root and rhizome necrosis according to a score scale in Figure 1 (modified from Bridge, 1988; Speijer & De Waele, 1997). The population levels of *R. similis* in roots, rhizome and in soil, and the reproduction factor (RF) were evaluated after nematode extraction, through the methods modified from Coolen & D'Herde (1972) and from Jenkins (1964), respectively.

For analysis of variance, original data (from percentages of lesions in roots and in rhizomes, number of nematodes per gram of root, and final population of nematodes) were converted into log (x +1). For all variables the means were compared by Tukey's test ($P \leq 0.05$).

Genetic variability based on RAPD markers. The twelve populations of *R. similis* obtained from different banana plantations from Brazil, Australia, Costa Rica and Cuba (Table 1) were analyzed for genetic differences based upon RAPD markers. *Scutellonema bradys* (Steiner & Le Hew, 1933) Andrassy, 1958, obtained from yam tubers cultivated in Cruz das Almas, Bahia, Brazil was included as an outgroup. The populations of *R. similis* were multiplied in carrot disks in tissue culture.

DNA extraction. A pre-selection test was performed to determine the number of nematodes (1,000; 2,000; 3,000 or 4,000) necessary to extract a satisfactory amount of DNA for use in PCR. Based on this test, a suspension of 1,000 nematodes (juveniles and adults) of each population was placed, into a 0.5 m*l* microcentrifuge tube and centrifuged at 2,000 g for 3 minutes. Afterwards, the supernatant was discarded and the precipitate used for DNA extraction. An extraction buffer (Blacke *et al.,* 1992) containing Tris-HCl 10 mM (pH 8.0), EDTA 1 mM, Nonidet P-40 1% and Proteinase K (0,1 µg / µ*l*) was prepared. Aliquots of 7 µ*l* of the buffer were added to each of the tubes containing juveniles and adults of the populations of *R. similis* and *S. bradys*. Maceration and DNA extraction were carried out according to Fallas *et al.* (1996) with minor modifications. The amount of DNA extracted from each sample was estimated by spectrophotometry at 260 nm (Sambrook *et al.,* 1989). The A_{260} / A_{280} ratio was used to evaluate DNA purity. After quantification, DNA samples from each population were diluted to 5 ng / µ*l* and stored at – 80 ºC.

RAPD primers selection. For the selection of decameron primers that generated clear and polymorphic products, DNA from five *R. similis* populations collected from Bahia and from Minas Gerais (Brazil), Cuba and Costa Rica , and the population of *S. bradys* (Sb) (outgroup), were amplified by PCR. Selected primers were used in 13 µ*l* volume reactions, containing Tris-HCl 10 mM (pH 8,3), KCl 50 mM, $MgCl_2$ 3 mM, 100 µM of each of the desoxyribonucleotides, 0.4 µM of primer, one unit of *Taq* polimerase enzyme (BRL / Life Technologies) and approximately 15 ng of DNA. Amplifications were carried out on an MJ Research thermocycler with 40 cycles at 94 ºCfor 15 seconds, 35 ºC for 30 seconds and 72 ºC for 90 seconds, after an initial DNA denaturation step at 94 ºC for 2 minutes. After 40 cycles, an extention step of the amplified chains was carried out at 72 ºC for 6 minutes. These samples were applied in agarose gel (1.2%), stained with ethydium bromide and immersed in TBE buffer (Tris-Borate 90 mM, EDTA 1 mM). The eletrophoretic separation was conducted for four hours at 90 volts. Following migration, gels were photographed under ultraviolet light.

A total of 44 primers belonging to the OPD, OPE, OPF, OPG e OPH (Operon Technologies Inc., Alameda, CA, EUA) kits were tested, and 18 that generated clear polymorphic bands were selected (Table 3). Although the RAPD technique is relatively simple and requires a small amount of DNA for sharp band visualization, in some cases it carries the

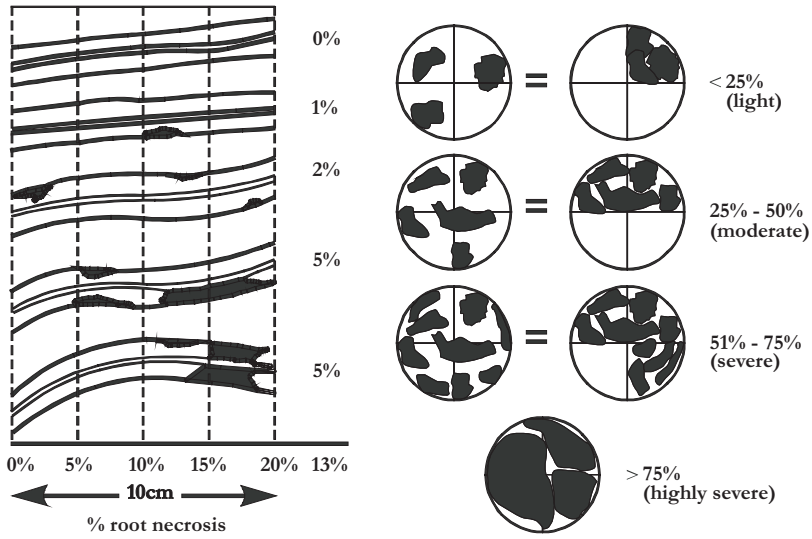

Figure 1 - Scales for evaluation of the percentage necrosis in roots - according to Speijer & De Waele, 1997 **(a)**, and rhizomes - modified from Bridge (1988) **(b)**, applied to quantify damage caused by 12 populations of *Radopholus similis* on three banana genotypes under growth chamber conditions.

Table 3 - Oligonucleotide primers utilized in generation of RAPD markers in 12 populations of *Radopholus similis* and one population of *Scutellonema bradys*.

Primers	5′ → 3′ sequence
OPD-04	TCTGGTGAGG
OPD-05	TGAGCGGACA
OPD-07	TTGGCACGGG
OPD-08	GTGTGCCCCA
OPD-16	AGGGCGTAAG
OPD-20	ACCCGGTCAC
OPE-18	GGACTGCAGA
OPF-04	GGTGATCAGG
OPF-05	CCGAATTCCC
OPF-06	GGGAATTCGG
OPF-10	GGAAGCTTGG
OPF-12	ACGGTACCAG
OPG-02	GGCACTGAGG
OPG-03	GAGCCCTCCA
OPG-04	AGCGTGTCTG
OPG-13	CTCTCCGCCA
OPG-16	AGCGTCCTCC
OPG-17	ACGACCGACA

disadvantage of not allowing band reproducibility. Taking into account the possibility of variations in band patterns, two replicates of each population were compared during the phase of primer pre-selection, with no significant variation observed.

RAPD marker analysis. The RAPD markers generated were converted into binary data matrices. Genetic distances between different populations were generated from this matrix based on the Nei & Li (1979) similarity coefficient using the Genes software (Cruz, 1997). A matrix of genetic distance was used for cluster analysis using the hierarchic method (dendrogram) using the UPGMA (Unweighted pair-group arithmetic average) clustering method and the

Statistica software (Statsoft, 1999). Cluster stability was measured by bootstrap analysis with 1,000 replications using the Winboot software (Yap & Nelson, 1996).

Results

Pathogenicity of *Radopholus similis* populations on bananas. Twelve populations of *R. similis* reproduced in different ratios on the banana genotypes in this study (Table 4). Lower values of the nematode RF were observed on Yangambi km 5 (1.5 – 39.7), followed by Pisang Jari Buaya (12.0 – 73.5), while higher reproduction factors were on Grand Naine (32.7 – 98.5).

The populations PE, BA_2, BA_1, MG_2, MG_1 and CUB were more aggressive as compared with CR, RJ, SP_2, SC, SP_1 and AUS on Grand Naine, with respect to plant height and root weight (Table 4). A positive correlation was detected between higher numbers of nematodes per gram of roots with more severe effects on the reduction of plant growth and on the root weight, when compared with control plants. Pernambuco (PE) population was the most aggressive one on Grand Naine, with the highest number of nematodes leading to the lowest root weight.

On Yangambi km 5, the populations CUB, MG_1, SP_2, BA_2, BA_1 and PE expressed higher aggressiveness for the variable root weight. With the exception of SP_1 population, no difference in damages to plant height was observed, when compared with control plants (Table 4). The population BA_2 was responsible for the poorest plant growth. Generally, lower values of root weight matched with higher numbers of nematodes per gram of roots, except for plants inoculated with the BA_1 population. The populations CUB, MG_1 and SP_2 were the most aggressive on Yangambi km 5, with the lowest root weight and a positive correlation with higher number of nematodes.

The cultivar Pisang Jari Buaya, considered as resistant to *R. similis*, did not behave in such a way. This cultivar allowed reproduction of all populations of *R. similis* in this study (Table 4). The populations PE, CR, BA_2 and BA_1 expressed higher aggressiveness towards root weight, and except for CR, these populations greatly reduced plant height. Mostly, there was a negative correlation between values of root weight and numbers of nematodes per gram of roots. Except for the populations CR and PE, lowest values of root weight matched with higher numbers of nematodes. For the populations SP_1, MG_1, MG_2, CUB and SC, higher numbers of nematodes per gram of roots were associated with higher values of root

Table 4 - Variables evaluated 120 days after inoculation of three banana cultivars with 100 nematodes of 12 populations of *Radopholus similis* [Pernambuco (PE), Bahia (BA_1 e BA_2), Minas Gerais (MG_1 e MG_2), Rio de Janeiro (RJ), São Paulo (SP_1 e SP_2), Santa Catarina (SC), Cuba (CUB), Costa Rica (CR) and Australia (AUS)], under growth chamber conditions. Control = plants non inoculated, N/G = nematodes per g root, RF = reproduction factor, PH = plant height, RW = root weight.

Populations	Grand Naine				Yangambi km 5				*Pisang Jari Buaya*			
	N/G	RF	PH (cm)	RW (g)	N/G	RF	PH (cm)	RW (g)	N/G	RF	PH (cm)	RW (g)
Control	-	-	23.0 a	19.7 a	-	-	31.7 a	25.2 a	-	-	31.0 a	15.6 a
SP_1	360 bc	48.5 b	19.2 bc	8.4 bc	69 bcd	9.0 bc	29.2 ab	12.7 bc	905 a	73.5 a	26.7 b	7.8 bcd
SP_2	457 bc	48.0 b	19.6 ab	8.1 bcd	764 a	24.0ab	26.2 bc	3.1 g	48 bcd	13.3 b	26.0 b	8.7 bcd
SC	287 c	47.7 b	19.0 bcd	8.6 bc	8 d	1.5 c	26.2 bc	9.2 cdef	444 a	45.0 ab	25.7 b	10.6 abc
RJ	285 c	49.0 b	18.8 bcd	8.1 bcd	74 bcd	11.2 b	25.9 bc	15.2 b	28 d	10.4 b	25.5 bc	11.1 ab
AUS	317 c	32.7 b	18.6 bcd	8.9 b	313 a	29.5 ab	25.2 cd	9.4 cde	39 cd	12.0 b	25.2 bc	10.1 bcd
CR	337 bc	48.0 b	17.8 bcd	6.7 bcd	39 cd	3.7 c	25.7 bc	9.6 cde	749 a	44.2 ab	25.2 bc	5.2 de
CUB	1256 abc	65.0 ab	16.5 bcde	4.7 bcde	551 ab	15.0 b	25.2 cd	2.5 g	387 ab	37.8 ab	25.1 bcd	9.7 bcd
MG_2	965 abc	42.6 b	16.0 bcde	3.7 cde	26 cd	6.0 bc	25.2 cd	10.0 cd	295 abc	30.7 b	24.5 bcd	8.8 bcd
MG_1	1,137 abc	63.0 ab	15.8 cde	5.4 bcde	453 ab	15.0 b	24.2 cd	3.0 g	548 a	39.0 ab	24.2 bcd	8.0 bcd
PE	3,207 a	53.5 b	15.8 cde	1.1 e	680 a	38.0 a	23.7 cd	5.6 defg	716 a	19.5 b	22.0 cde	2.0 e
BA_1	1,224 abc	44.2 b	15.3 de	3.2 de	210 abc	10.0 b	23.2 cd	4.8 efg	311 abc	18.0 b	20.5 e	5.8 cde
BA_2	2,780 ab	98.5 a	15.0 e	3.1 de	945 a	39.7 a	22.0 d	4.1 fg	356 ab	22.5 b	19.5 e	5.7 cde
CV (%)	15.7	17.8	6.9	17.4	15.7	17.8	6.9	17.4	15.7	17.8	6.9	17.4

Means of four replications, means followed by the same letter in the column did not differ by Tukey's test ($P \leq 0.05$), original data were converted into log ($x + 1$).

weight, especially in plants inoculated with SP_1. Regardless of the nematode population, the numbers of *R. similis* on Pisang Jari Buaya were higher than on Yangambi km 5, and lower than on Grand Naine.

The differences between cultivars with respect to percentage of healthy and dead roots confirm Grand Naine as the most susceptible to *R. similis*, with higher percentage of dead roots as compared with Yangambi km 5, whereas Pisang Jari Buaya remained in an intermediary position (Figure 2). In addition, regardless of banana genotype, according to Tukey's test ($P \leq 0.05$), the percentage of necrotic area in banana roots, the populations MG_2, BA_1 and PE were the most aggressive, followed by CUB, MG_1, BA_2 and CR (Figure 3), whereas AUS, SC, RJ, SP_1, and SP_2 were responsible for lower percentages of root necrotics. For the percentage of necrotic area on the rhizome surface, PE was the most damaging population, followed by BA_2, BA_1, CR, MG_2, MG_1 and CUB. The rhizomes were less affected by SP_2, SP_1, RJ, SC and AUS populations (Figure 3 and 4).

Genetic variability *Radopholus similis* based on RAPD markers. A total of 341 markers were obtained, with 314 (92 %) polymorphic and 27 (8 %) monomorphic. The average number of observed markers per primer was 18.9. The shortest genetic distance (0.219) was obtained between the two *R. similis* populations from Bahia (BA_1 and BA_2) and between those from Pernambuco and Costa Rica. The largest distance between populations of *R. similis*

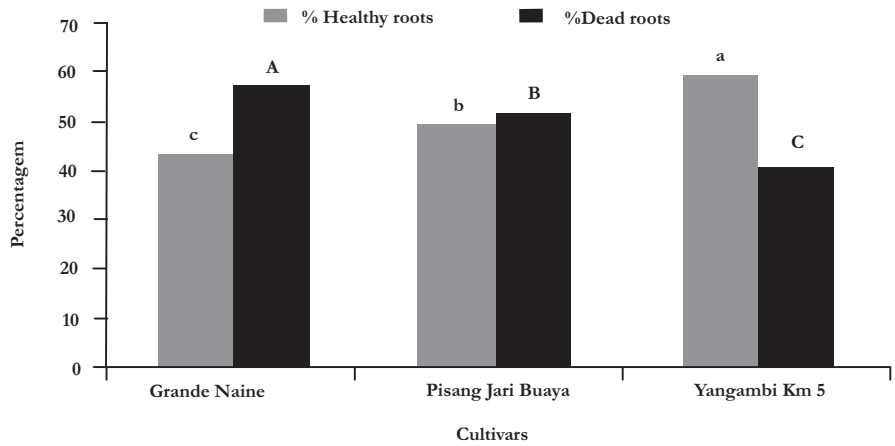

Figure 2 - Percentages of healthy and dead roots in three banana genotypes by 12 populations of *Radopholus similis*, 120 days after inoculation with 100 nematodes per plant, under growth chamber conditions.

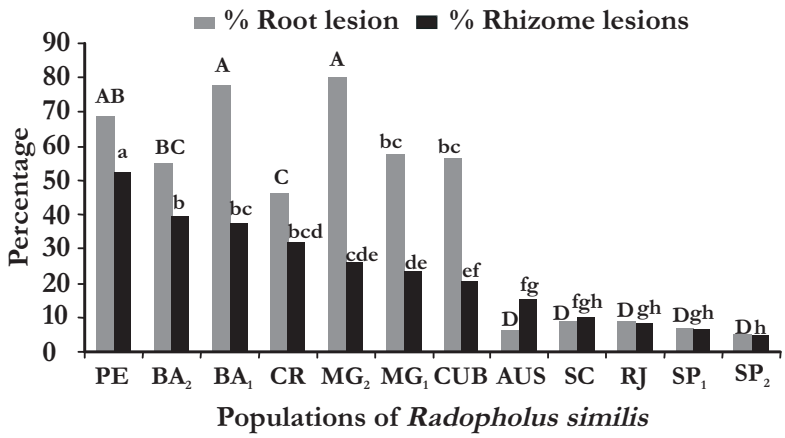

Figure 3 - Percentages of necrotic area in roots and in rhizomes of plants of banana 'Grand Naine', 'Pisang Jari Buaya' and 'Yangambi km 5' by 12 populations of *Radopholus similis* [Pernambuco (PE), Bahia (BA_1 e BA_2) Minas Gerais (MG_1 e MG_2), Rio de Janeiro (RJ), São Paulo (SP_1 e SP_2), Santa Catarina (SC), Cuba (CUB), Costa Rica (CR) and Australia (AUS)], 120 days after inoculation with 100 nematodes per plant, under growth chamber conditions.

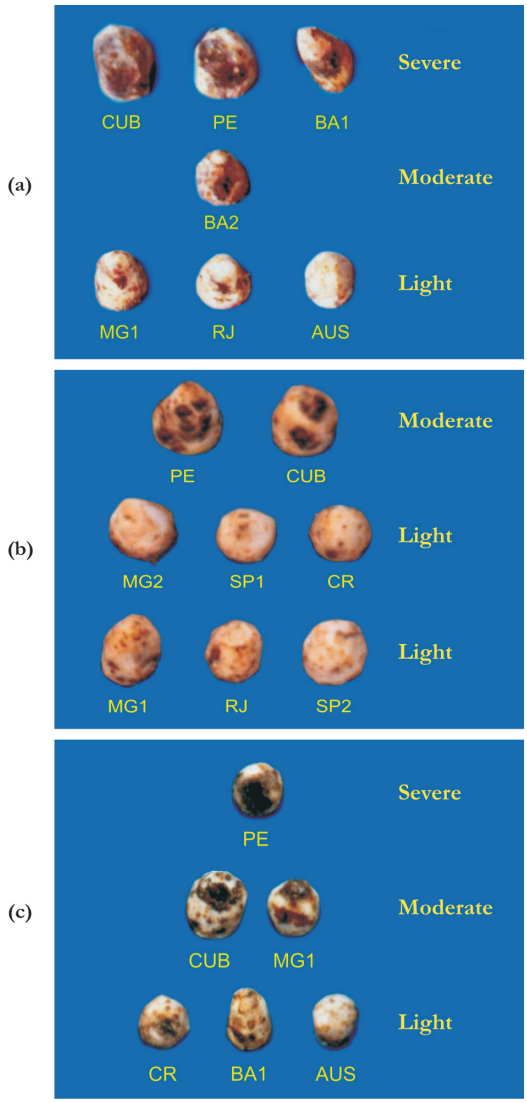

Figure 4 - Rhizome necrosis caused by 12 populations of *Radopholus similis* [Pernambuco(PE), Bahia (BA_1 e BA_2), Minas Gerais (MG_1 e MG_2), Rio de Janeiro (RJ), São Paulo (SP_1 e SP_2), Santa Catarina (SC), Cuba (CUB), Costa Rica (CR) and Australia (AUS)], 120 days after inoculation of 100 nematodes per banana plant, under growth chamber conditions: **(a)** Grand Naine, **(b)** Yangambi km 5, **(c)** Pisang Jari Buaya.

(0.404) was obtained between those from Costa Rica and Minas Gerais (MG_1) (Table 5).

Cluster analysis enabled the separation between *R. similis* and the outgroup *S. bradys* populations (Figure 5). The 12 populations of *R.simlis* were divided into 5 groups at a relative genetic distance of 0.45. The three foreign populations and the one from Pernambuco were clustered in the first group; both populations from Bahia (BA_1 and BA_2), one from Minas Gerais (MG_2), and the one from Rio de Janeiro (RJ) were clustered in a second group; the third group was formed by Santa Catarina (SC) and São Paulo (SP_1) populations, closely related to SP_2, whereas MG_1 populations did not cluster with any other populations. Repeatability (%) of clustering above 50 % for 1,000 bootstrap cycles occurred in only four groups [SC, SP_1 and SP_2 (80 %); RJ and MG_2 (58 %); BA_1 and BA_2 (64 %); PE and CR (60 %)], revealing that such clusters are the most consistent (Figure 5).

Discussion

Present results show remarkable differences

Table 5 - Distance matrix between 12 populations of *Radopholus similis* [Pernambuco (PE), Bahia (BA1 e BA2), Minas Gerais (MG1 e MG2), Rio de Janeiro (RJ), São Paulo (SP1 e SP2), Santa Catarina (SC), Cuba (CUB), Costa Rica (CR) and Australia (AUS)] and one population of *Scutellonema bradys* (Sb), calculated using the Nei & Li (1979) coefficient based on 341 RAPD markers.

Populations		1	2	3	4	5	6	7	8	9	10	11	12	13
1	SC	0												
2	SP 1	0.269	0											
3	SP 2	0.321	0.313	0										
4	RJ	0.339	0.283	0.282	0									
5	MG 1	0.379	0.290	0.382	0.318	0								
6	MG 2	0.358	0.333	0.304	0.245	0.340	0							
7	BA 1	0.336	0.367	0.324	0.311	0.349	0.242	0						
8	BA 2	0.304	0.341	0.312	0.297	0.349	0.274	0.219	0					
9	PE	0.331	0.279	0.336	0.292	0.289	0.278	0.301	0.273	0				
10	CR	0.352	0.314	0.387	0.291	0.404	0.272	0.308	0.315	0.219	0			
11	AUS	0.382	0.326	0.383	0.286	0.367	0.252	0.331	0.347	0.291	0.252	0		
12	CUB	0.365	0.273	0.357	0.294	0.348	0.285	0.331	0.331	0.271	0.248	0.232	0	
13	Sb	0.481	0.453	0.471	0.447	0.532	0.438	0.460	0.452	0.445	0.409	0.452	0.425	0

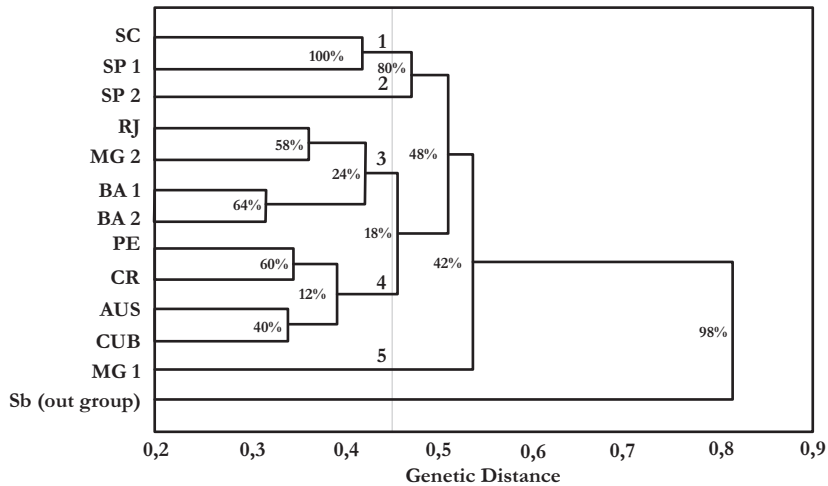

Figure 5 - UPGMA derived dendrogram for 341 RAPD markers from 12 populations of *Radopholus similis* [Pernambuco (PE), Bahia (BA_1 e BA_2), Minas Gerais (MG_1 e MG_2), Rio de Janeiro (RJ), São Paulo (SP_1 e SP_2), Santa Catarina (SC), Cuba (CUB), Costa Rica (CR) and Australia (AUS)] and one of *Scutellonema bradys* (Sb). Genetic distances were calculated using the Nei & Li (1979) coefficient.

between *R. similis* populations in relation to capacity for reproduction and level of damage towards the three banana genotypes. Among the variables evaluated, the highest level of significance was observed for reduction in root weight, which indicates this variable as the most reliable to evaluate degrees of aggressiveness between populations of *R. similis* on banana plants. Population density has been demonstrated as a suitable variable for determining relations between reduction in plant growth and nematode aggressiveness (Sarah *et al.,* 1993; Fallas & Marbán-Mendoza, 1994; Hahn *et al.,* 1996). In this study, 120 days after nematode inoculation, there were some cases where severe damage on banana plants did not correlated with high nematode densities, which can be explained by population dynamics. Costa *et al.*(2003) observed that the Cuban population of *R. similis* reached a high population density in 'Grand Naine' banana roots 60 days after inoculation, whereas the Brazilian and the Costa Rican populations of the nematode reached equivalent levels, 90 and 120 days after inoculation, respectively. Similar results, with the

same Cuban population (Villa Clara) used in this study, were obtained on carrot tissue culture by Stoffelen *et al.*(1999) over an eight week period of evaluation.This Cuban population reached high levels of nematode density four weeks before two other *R. similis* populations reached equivalent levels. This information confirms that faster growing populations initiate greater damage earlier than slow growing populations. Generally there was a positive correlation between final nematode density and reduction in plant growth and root weight. Decreases in plant height and root weight, and higher percentages of roots and rhizome necrosis were attributed to higher nematode densities per gram of roots, mainly in the cultivars Grand Naine and Yangambi km 5.

In this work, differences in aggressiveness were significant between nematode populations. The population from Costa Rica, with intermediate aggressiveness on Grand Naine, was responsible for a significant decrease in plant height and root weight in Pisang Jari Buaya. Similarly, the population from São Paulo (SP_2), with low aggressiveness on Grande Naine, reduced drastically root weight on Yangambi km 5. The partially resistant reaction of Yangambi km 5 to *R. similis* reported in previous studies (Fallas & Marbán-Mendoza, 1994; Fogain & Gowen, 1998) was confirmed for some *R. similis* populations. Only minor plant damage was attributed to the populations from Rio de Janeiro, São Paulo (SP_1), Minas Gerais (MG_2), Costa Rica, Australia, and Santa Catarina on this cultivar. These results indicate that Yangambi km 5, although allowing fast multiplication of some populations of *R. similis*, leading to severe plant damages, is able to reduce reproduction in other populations to levels such that they are unable to cause significant damage. Another point to consider is that although there were roots and rhizome necrosis, they were of moderate extension, indicating that these variables are not suitable for resistance evaluation under field conditions. The results presented showed that the levels of damage due to *R. similis* on banana plants, depend not only on the size of nematode population, but also on the nematodes capacity to elicit defense reactions.

Costa *et al.* (1998) observed that Pisang Jari Buaya behaved as susceptible to BA_1 population. The present results also confirm Pisang Jari Buaya as susceptible to *R. similis*, comparably to Grand Naine. Similar results were reported by Elbadri (2000) in relation to five populations of *R. similis* on Pisang Jari Buaya. Differences in the levels of resistance observed in propagative materials of Pisang Jari Buaya multiplied from tissue culture, suggest that these differences are due to somaclonal variations and to environmental influences. Banana 'FHIA-3' plants were less susceptible to *R. similis* when produced from culture tissue. In contrast, FHIA-1, FHIA-18 and FHIA-23 became more susceptible when propagated from tissue culture (Viaene *et al.,* 1998). The susceptibility of Pisang Jari Buaya observed in this work may be attributed to the fact that the plants used in this experiment came from a tissue culture selection, such as that obtained by Nguyet (1999) in Costa Rica, when the author selected the clone Pisang Jari Buaya ITC-312, susceptible to *R. similis*, from the same origin as Pisang Jari Buaya III-116 resistant to *R. similis* in Honduras (Pinochet & Rowe, 1979).

The different levels of aggressiveness observed between *R. similis* populations from Cuba, Costa Rica and Australia in this work were consistent with the findings by Fallas *et al.* (1995) and by Elbadri (2000) with the same populations on Grand Naine. The distinction between the Brazilian populations with different levels of aggressiveness did not reflect the pathogenic variablility of *R. similis* in the geographic regions represented in this work, since the populations in this study did not represent the entire area of banana production in the country. More detailed studies on different *R. similis* populations are needed for a better understanding of the nematode behavior across the country. More aggressive populations collected in the states of Bahia, Pernambuco and Minas Gerais, justify high population densities in banana plantations, and yield losses up to 100 % in such localities. The twelve populations of *R. similis* in this study failed to reproduce on *Citrus latifolia* (data not shown), what make to believe that all populations evaluated belong to the banana race of the burrowing nematode.

The genetic similarity encountered in this work with respect to the foreign populations are in agreement with the results obtained by Fallas *et*

al.(1996) and by Elbadri (2002), considering RAPD and RFLP analyses of the populations from Australia (Queensland), Cuba (Villa Clara) and Costa Rica (Talamanca).

Present results demonstrated to certain point correlation between genetic distance and pathogenicity of *R. similis* populations on bananas, since the populations with low reproductive capacity and low aggressiveness on the three banana cultivars evaluated, clustered in the same similarity group, for example SC and SP_1 populations. Other cluster groups included populations of greater aggressiveness, such as Bahia (BA_1 and BA_2) and Minas Gerais (MG_2) and the populations from Pernambuco, Costa Rica and Cuba. By contrast, the populations from Rio de Janeiro and Australia, of low aggressiveness, demonstrated short genetic distances compared to more aggressive populations, such as the ones from Bahia (Ba_1 and BA_2), Minas Gerais (MG2), Pernambuco, Cuba and Costa Rica. These results are in agreement with those by Fallas *et al.* (1996), which clustered in the same group populations of *R. similis* with different levels of aggressiveness on bananas. Additionally, Elbadri *et al.* (2002) observed that populations of *R. similis* with low capacity of reproduction on Grand Naine grouped in the same cluster based on RFLP analysis. Kaplan *et al.* (1996) reported the presence of RAPD bands associated with parasitism of some *R. similis* populations on citrus plants in Florida.

With the exception of the populations from Bahia (BA_1 e BA_2), an interesting fact was the absence of correlation between geographic proximity and genetic similarity. For example, PE population was genetically closer to the foreign populations compared with other Brazilian populations. Another example was the greater genetic distance between the populations from Minas Gerais. Similar results were reported by Hahn *et al.* (1994) based on RAPD markers that distinguished one genetically different population of *R. similis* from two other populations, although these three populations had been collected from tea plants in Sri Lanka. The fact that MG_1 and MG_2 populations, with different molecular phenotypes, were collected in banana plantations in Minas Gerais, confirms the possibility of occurrence of genetic variability of the pathogen in a same agricultural region. The genetic divergence of the MG_1 population to the other *R. similis* populations can be hypothesized as reflecting the level of pathogenicity and / or host range. Nematode populations that differ in molecular markers, host range and on this basis of morphology, can be interpreted as races, physiological races, biotypes and pathotypes (Du Charme & Birchfield, 1956; Pinochet, 1979; Gnanapragasan *et al.,* 1991). Therefore, studies on the virulence in different hosts would be important to complement the characterization of MG_1 population.

Amphimitic reproduction is important to the diversity of natural populations. DNA polymorphism within nematode species has been frequently detected, and polymorphic DNA bands can be related to parasitism capacity or to biochemical differences (Huettel *et al.,* 1983a;b), however, it seems more probable that dissemination at long and short distances of *R. similis* in propagative material contributes for the apparent similarity, since there is evidence that worldwide dispersion of *R. similis* is relatively recent (early 19th Century) (Gowen & Quénéhervé, 1990). Therefore, knowledge on the geographic origin can contribute to the understanding of the relationship between genetic similarity and dissemination of nematodes through propagative material. Although only one population from Santa Catarina State was evaluated, the genetic proximity of this population with those from São Paulo supports the hypothesis of the dissemination of *R. similis* from the State of São Paulo through infected plantlets to Santa Catarina and other states. With the expansion of the banana growing area along the irrigated valley of the São Francisco river, trading of banana planting materials, without adequate observation of their origin and sanitary conditions, has contributed to the dissemination of *R. similis* to regions with different types of soils and climatic conditions. The genetic proximity of MG_2, RJ, BA_1 and BA_2 populations may possibly be justified by the intense flow of planting materials from the State of Rio de Janeiro to the regions of Janaúba (northern Minas Gerais) and Bom Jesus da Lapa (western Bahia).

The genetic variability observed among the Brazilian populations indicates this may be the result of more than one introduction of *R. similis* in Brazil.

Historically, according to Zem (1982), *R. similis* was probably introduced into Brazil through imported plantlets from Central America, or from Africa to the coast of the State of São Paulo, from where it quickly disseminated to the State of Rio de Janeiro. Its occurrence in other regions of the country could be mostly limited to collections of banana cultivars introduced for research in experimental areas of several research centers (Sharma, 1974). Kaplan *et al.* (1996) commented that the variability of *R. similis* can be greater in Africa than in Hawaii, Florida and Central America. According to Fallas *et al.* (1996) greater genetic variability and aggressiveness occur among African populations. These reports strongly support the hypothesis proposed by Zem (1982) considering that the introduction of *R. similis* into Brazil occurred through propagative material from Africa, which consequently resulted in greater genetic variability and agressiveness among the Brazilian populations of the burrowing nematode as demonstrated in this study.

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