

RESEARCH/INVESTIGACIÓN

SELECTION OF *MUSA* GENOTYPES FOR RESISTANCE TO *RADOPHOLUS SIMILIS* COBB

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ABSTRACT

Santos, J. R. P., M. A. Teixeira, D. C. Costa, S. O. Silva, F. G. Faleiro, and J. E. Cares. 2013. Selection of *Musa* genotypes for resistance to *Radopholus similis* Cobb. *Nematropica* 43:1-8.

Damage from nematodes are among the main constraints affecting banana plantations. Losses caused by these parasites can reach 100%, when not properly controlled. The use of resistant cultivars to control pests and diseases and adaptation to adverse environmental conditions is an appealing approach as far as profitability and environmental preservation are concerned. The objective of this work was to evaluate banana genotypes for resistance to the burrowing nematode (*Radopholus similis*) under greenhouse conditions. Diploid (AA), triploid (AAA and AAB) and tetraploid (AAAB) genotypes were inoculated with a suspension of 100 *R. similis* (juveniles, males and females) from a population isolated in Pernambuco State, Brazil. Genotypes were assessed 120 days after inoculation for the number of nematodes per gram of root, nematodes in roots, nematodes in soil, total numbers of nematodes (roots + soil) and the reproduction factor (Rf). The Rf comparison among genotypes indicated that Borneo, Grande Naine and 1304-06 were susceptible and 4249-05, 0337-02, 0323-03, and 4279-06 were resistant to *R. similis*. Results also indicated that in the future these potentially resistant genotypes can lead to cultivars resistant to the burrowing nematode.

Key words: banana, burrowing nematode, genetic breeding, resistance.

RESUMO

Santos, J. R. P., M. A. Teixeira, D. C. Costa, S. O. Silva, F. G. Faleiro, and J. E. Cares. 2013. Seleção de genótipos de *Musa* para resistência a *Radopholus similis* Cobb. *Nematropica* 43:1-8.

Os danos causados por fitonematoides estão entre os principais problemas fitossanitários da bananicultura brasileira, sendo que perdas causadas por tais parasitas podem chegar a 100% quando o seu controle não é efetuado corretamente. O uso de cultivares resistentes a pragas, doenças e a condições adversas do ambiente é a estratégia ideal do ponto de vista econômico e de preservação do meio ambiente. O objetivo deste trabalho foi estudar a reação de clones de bananeira em relação ao nematoide cavernícola, *Radopholus similis*, sob condições de casa de vegetação. As plantas foram inoculadas com uma suspensão de 100 juvenis, machos e fêmeas do nematoide. O delineamento experimental foi inteiramente casualizado com quatro repetições. Os genótipos foram avaliados após 120 dias da inoculação. Foram avaliados número de nematoides por grama de raiz, nematoides na raiz, nematoides no solo, número total de nematoides (solo + raiz) e o fator de reprodução (Rf). Comparando o fator de reprodução entre os genótipos, os clones Borneo, Grande Naine e 1304-06 se comportaram como suscetíveis e os genótipos 4249-05, 0337-02, 0323-03 e 4279-06 como resistentes a essa população de *R. similis*. Estes genótipos mostraram-se com potencial para serem utilizados em programas de melhoramento visando obter cultivares com resistência ao nematoide cavernícola.

Palabras clave: banana, melhoramento genético, nematoide cavernícola, resistência.

INTRODUCTION

Worldwide, banana plantations have been threatened by serious pathogens and pests. In addition to fungal, bacterial and viral diseases, banana is also susceptible to the attack by plant-parasitic nematodes. Nematodes infect roots and rhizomes, causing severe losses in banana plantations (Gowen *et al.*, 2005).

According to Gowen and Quénéhervé (1990), 146 species distributed in 43 genera of nematodes had been reported associated with banana plants. In Brazil, several species of these genera have been reported, both in roots and in the rhizosphere of banana plants (Cavalcante *et al.*, 2005; Ritzinger *et al.*, 2007). The burrowing nematode, *Radopholus similis* (Cobb, 1893) Thorne, 1949, considered to be the most economically important species (Fallas and Marbán-Mendoza, 1994), has a worldwide distribution and is capable of causing extensive necrosis and galleries in the root, therefore it is commonly called as “the burrowing nematode”. Other plant-parasitic species attacking banana include *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, *Pratylenchus coffeae* (Zimmermann, 1898) Filipjev and Schuurmans Stekhoven, 1941, *Meloidogyne* spp., and *Rotylenchulus reniformis* Linford and Oliveira, 1940; which occur frequently and cause extensive damage in banana plantations (Ritzinger *et al.*, 2007).

Damage caused by nematodes is among the main production constraints in banana plantations. Losses due to these parasites can reach 100% when nematode control practices are not followed (Silva *et al.*, 2001). Under tropical conditions, damage is more severe due to the absence of strong climate changes for regulation of nematode populations. Banana monocropping results in the dominance of specific plant-parasitic nematodes, allowing them to multiply to economically harmful levels (Araya *et al.*, 1995). Nematodes impair absorption and transport of water and nutrients by the root system, leading to plant uprooting and predisposition to the attack by other soil microorganisms (Dias and Ribeiro Júnior, 2001).

There are several recommended control practices for suppressing the population of nematodes in banana fields, but the method of choice has been the use of chemical nematicides. However, this has a high cost and may compromise environment quality. Currently, the nonchemical management practices for nematode management on banana crops include sanitary practices as the use of *in vitro* propagated plants, fallow, and crop rotation (Quénéhervé, 2008).

Among the measures to control *R. similis*, the possibility of resistant cultivars has shown promise (Quénéhervé *et al.*, 2009a; Quénéhervé *et al.*, 2009b; Santos *et al.*, 2010). The use of cultivars resistant to pests, diseases and adverse environmental conditions is the optimal strategy in terms of profitability and environmental preservation, especially for banana growing regions under low levels of technology

adoption, and low economic returns.

Scarcity of reports on genetic resistance to nematodes on bananas is probably due to the difficulty of finding sources of resistance to migratory endoparasitic nematodes. A few sources of resistance have been reported in some genotypes, such as ‘Pisang Jari Buaya’ (*Musa* AA) (Pinochet and Rowe, 1978), Yangambi Km5 (*Musa* AAA Ibiota) (Fogain and Gowen, 1997), and a diploid clone Kunnan (*Musa* AB) (Collingborne and Gowen, 1997). In addition to these sources of resistance, several others have been reported (Wehunt *et al.*, 1978; Fallas and Marbán-Mendoza, 1994, Costa, 2004; Kalorizou *et al.*, 2007; Quénéhervé *et al.*, 2009a, Quénéhervé *et al.*, 2009b).

The objective of this work was to evaluate 26 banana genotypes consisting of diploids (AA), triploids (AAA, AAB) and tetraploid (AAAB) from the Germplasm Bank of Embrapa Mandioca e Fruticultura (Embrapa - CNPMF) for resistance to *R. similis* under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted in the Biology Experimental Station at University of Brasilia, from April to August 2006, under greenhouse conditions with temperatures ranging from 10 °C to 30 °C, and relative humidity from 60 to 100%.

The genotypes (Table 1) came from the Banana Germplasm Bank of Embrapa Mandioca e Fruticultura, where the plantlets were obtained by micro propagation in tissue culture medium, and acclimated during three weeks in a growth chamber (27-28 °C) calibrated for 12 hours photoperiod. The seedlings were transplanted to 1.5 L plastic pots containing an autoclaved mixture of red latosol and sand in a 3:1 ratio. After transplanting, the seedlings were kept in a greenhouse for 30 days. Fifteen days after transplanting, the plants were fertilized with 8.3 g of NPK (4:14:8) per pot and watered as needed.

A population of *R. similis* isolated from banana roots cv. Pacovan in the State of Pernambuco, Brazil, was maintained on banana plants cv. Grande Naine under greenhouse conditions. This population was selected because it was previously identified as the most aggressive among all the other populations of *R. similis* assayed in 2004 (Costa *et al.*, 2008).

The nematodes were extracted from the roots, which were fragmented in a blender and incubated for about 24 hours in modified Baermann funnel under aeration. The specimens recovered from the Baermann funnel were then collected on 38 µm sieve. The extracted nematodes were then increased in carrot tissue culture.

Plants were inoculated with a suspension of *R. similis* 30 days after transplanting. The inoculum was calibrated to 100 nematodes/mL. Two-holes 5-6 cm deep were opened through the soil surface around the plantlets exposing the roots, and the suspension of nematodes (1 mL) inoculated on the roots with a pipette.

The study was terminated 120 days after inoculation

Table 1. Banana genotypes studied to determine resistance or susceptibility to *Radopholus similis*, from the Germplasm Bank of Embrapa Mandioca e Fruticultura.

Genotypes	INIBAP's code	Genomic group	Genetic classification	Parental cross	Outstanding features	Source
0323-03 ^z	AA	Hybrid	Calcutta 4 X S/N 2	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
0337-02 ^z	AA	Hybrid	Calcutta 4 X Galeo-ITC0259	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
1304-04 ^z	AA	Hybrid	Malaccensis-FHIA X Madang-001252	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
1304-06 ^z	AA	Hybrid	Malaccensis-FHIA X Madang-001252	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
1318-01 ^z	AA	Hybrid	Malaccensis-FHIA X Sinwobogi-002089	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
1319-01 ^z	AA	Hybrid	Malaccensis-FHIA X Tjau Lagada	Resistant to YS and FOC	Silva <i>et al.</i> , 2001	
4223-06 ^z	AA	Hybrid	M53 X S/N 2	Resistant to YS and BS	Silva <i>et al.</i> , 2001	
4249-05 ^z	AA	Hybrid	M53 X M48	Resistant to YS	Silva <i>et al.</i> , 2001	
4252-03 ^z	AA	Hybrid	M53 X Kumburg-002747	Resistant to YS	Silva <i>et al.</i> , 2001	
4279-06 ^z	AA	Hybrid	M53 X (Tuu Gia-001848 x Calcutta 4)	Resistant to YS	Silva <i>et al.</i> , 2001	
4285-02 ^z	AA	Hybrid	M53 X (Pisang Madu-002054 x Calcutta 4)	Resistant to YS	Silva <i>et al.</i> , 2001	
5854-03 ^z	AA	Hybrid	(Calcutta 4 x Pahang-001228) X (Borneo x Madang)	Resistant to YS	Silva <i>et al.</i> , 2001	
8694-15 ^z	AA	Hybrid	(Calcutta 4 x Galeo) X SH-3263			
Birmanie	AA	Simple diploid				
Borneo	ITC0253	AA	Simple diploid	Partially resistant to BS	Fouré (1990)	
Calcutta 4	ITC0249	AA	Simple diploid	Resistant to BS	Jones (2000)	
N118	AA	Simple diploid				
Pa Rayong	PRAY	AA	Simple diploid	Resistant YS	Matos <i>et al.</i> , 2001	
Pisang Nangka	1279	AAAB	Cultivar			
Pisang Jaran	ITC0678	AA	Cultivar			
Pisang Pipit	ITC0685	AA	Cultivar			
Tjau Lagada	ITC0090	AA	Cultivar	Resistant to FOC; Partially resistant BS	Dantas <i>et al.</i> , 1993	
Grande Naine	ITC0180	AAA	Cultivar	Resistant to FOC		
Yangambi Km5	ITC1123	AAA	Cultivar	Resistant to BS, YS and FOC	Silva <i>et al.</i> , 2001; Silva <i>et al.</i> , 2004	
Thap Maeo	ITC1301	AAB	Cultivar	Resistant to YS, BS and FOC	Gasparoto <i>et al.</i> , 2006	
Vitória	AAAB	Cultivar	Pacovan X M53			

^zHybrids crossed by Embrapa Mandioca e Fruticultura

at which time the nematodes were extracted from the soil and banana roots using the modified methods of Jenkins (1964), and Coolen and D'Herde (1972), respectively and counted. Treatments were distributed in a completely randomized design with four replications on benches in a greenhouse.

Host susceptibility of banana plants to *R. similis* was determined according to the rating system for percentage reduction on the nematode reproduction factor (Rf: final population / initial population) (Seinhorst, 1967) in each banana genotype (Table 2) (Sasser *et al.*, 1987).

The cultivar that scored the highest rating for nematode reproduction was considered the standard of susceptibility (Moura and Régis, 1987). Variables such as number of nematodes per gram of root, number of nematodes per root system, number of nematodes in soil and the total number of nematodes were also evaluated.

Tests for homogeneity of variances were applied according to Cochran and Cox (1957). For performing analysis of variance, the data were transformed to $\log_{10}(x + 1)$ and means grouped by the Scott-Knott test ($P < 0.01$), with aid of the Genes software: quantitative genetics and experimental statistics, version 2006.4.1 (Cruz, 2006). The data are reported as untransformed means.

RESULTS

The average number of nematodes per gram of root (Table 3) ranged from 0.01 in the diploid 4249-05 to 18.26 in the simple diploid Borneo. These genotypes also had respectively, the lowest and the highest numbers of nematodes in the root system; nematodes in the soil; and total nematode counts as well. Means clustered into four groups (a, b, c, and d) by the Scott-Knott test ($P < 0.01$). However, grouping based on other variables evaluated was not equivalent, except for 'Borneo', 'Grande Naine', and 'Pisang Pipit' that grouped together regardless of the variable evaluated.

The genotypes with lower numbers of nematodes

in roots were 4249-05, 0337-02, 4279-06, and Pisang Jaran, contrarily to Borneo, Grande Naine, 1304-06, 4252-03, 8694-15, and Pisang Pipit. The average numbers of nematodes found in the roots ranged from 0.75 (4249-05) to 1842.50 (Borneo). This variable distinguished four groups of genotypes by the Scott-Knott test ($P < 0.01$).

Lower nematode populations in soil were observed in diploids 4249-05, 0337-02, 0323-03, and 4279-06 (0, 7.5, 26.25, and 37.50 nematodes per pot, respectively). This trend was also observed for total number of nematodes found in each genotype. The largest quantities of nematodes found in soil were recovered from the genotypes 1304-06, 5854-03, and Borneo (450, 412.5, and 288.75, respectively). The number of nematodes in soil could be grouped by genotypes into three groups.

Total number of nematodes clustered by genotypes into four groups (a, b, c, and d) by the same test (Table 3). This variable did not follow the trend observed for the number of nematodes in soil, since the genotypes Borneo, Grande Naine and 1304-06 scored highest among all genotypes evaluated.

The highest factor of reproduction (Table 3) was observed for the simple diploid Borneo (21.31), followed by cv. Grande Naine (11.30), and the hybrid 1304-06 (11.00). The lowest reproduction factors were found in the diploids 4249-05 (0.01), 0337-02 (0.11), 0323-03 (0.40), and 4279-06 (0.41).

Based on the percentage reduction of the nematode reproduction factor, out of 26 genotypes, one was classified as highly susceptible (Borneo), two were susceptible (Grande Naine and 1304-06), three with low resistance (4252-03, 8694-15, and 1304-04), 16 as partially resistant (Table 3), three resistant (0337-02, 0323-03, and 4279-06), and one highly resistant (4249-05).

According to Pearson's correlation analysis, the variables showing the strongest correlations were nematodes in the roots vs. total number of nematodes (0.916), and nematodes in the roots vs. nematodes per gram of root (0.903). The lowest correlation found was between nematodes per gram of root vs. nematodes in the soil (0.548) (Table 4).

DISCUSSION

The genotypes Borneo, Grande Naine, and 1304-06 with higher Rf values were characterized as being highly susceptible or susceptible plants. These three genotypes also allowed higher total nematode count, with most of the nematodes in the roots and fewer in the soil. Although, the diploid 1304-06 did not group in the same cluster with Borneo and Grande Naine with respect to the number of nematodes per gram of root, these three genotypes have emerged as susceptible hosts to *R. similis*. In a previous study by Costa *et al.* (1998), the genotype 1304-06 expressed reaction of susceptibility

Table 2. Host reaction based on the percentage reduction of *Radopholus similis* reproduction factor (Rf) related to the most susceptible genotype (Sasser *et al.*, 1987).

Percent Rf reduction	Genotype reaction
0 – 25	Highly susceptible (HS)
26 – 50	Susceptible (S)
51 – 75	Low resistance (LR)
76 – 95	Partially resistant (PR)
96 – 99	Resistant (R)
100	Highly resistant (HR) or Immune (I)

Table 3. Populations and reproduction of *Radopholus similis* on 26 banana genotypes under greenhouse 120 days after nematode inoculation.

Genotypes	Nematodes/g root ^x	Total nematodes ^x	Factor of reproduction (Rf)	Reduction on Rf (%)	Reaction ^y
Borneo	18.3 a	2131.3 a	21.3	0.0	HS
Grande Naine	18.1 a	1130.3 a	11.3	47.0	S
1304-06	6.1 b	1099.8 a	11.0	48.4	S
4252-03	7.2 b	772.3 a	7.7	63.8	LR
8694-15	5.0 b	759.0 a	7.6	64.4	LR
1304-04	3.4 b	610.8 a	6.1	71.3	LR
Pisang Pipit	9.7a	492.0 a	4.9	76.9	PR
5854-03	1.1 c	446.8 a	4.5	79.0	PR
1318-01	2.1 c	373.3 a	3.7	82.5	PR
4285-02	2.6 c	347.8 b	3.5	83.7	PR
N118	4.7 b	325.8 b	3.3	84.7	PR
Tjau Lagada	4.8 b	324.5 b	3.3	84.8	PR
Calcutta 4	4.2 b	315.5 b	3.2	85.2	PR
1319-01	2.9 b	249.3 b	2.5	88.3	PR
Pa Rayong	1.0 c	244.5 b	2.5	88.5	PR
Birmanie	8.3 b	239.8 b	2.4	88.8	PR
Vitória	1.4 c	230.0 b	2.3	89.2	PR
Pisang Nangka	2.7 b	180.8 b	1.8	91.5	PR
Thap Maeo	0.7 d	152.3 b	1.5	92.9	PR
4223-06	0.5 d	149.0 b	1.5	93.0	PR
Pisang Jaran	0.3 d	109.3 b	1.1	94.9	PR
Yangambi Km5	0.6 d	105.5 b	1.1	95.1	PR
4279-06	0.1 d	41.3 c	0.4	98.1	R
0323-03	0.2 d	39.8 c	0.4	98.1	R
0337-02	0.1 d	10.8 c	0.1	99.5	R
4249-05	0.0 d	0.8 d	0.0	100.0	HR
CV% ^z	38.2 d	17.1			
Fcal. ^z	13.6 d **	13.1 **			

^x Data are means of four replications. For analysis original data were converted into $\log_{10}(x + 1)$. Means in columns followed by the same letter are not different according to the test of Scott-Knott. $P < 0.01(**)$. Untransformed means are presented in columns;

^y Reaction: (HS) highly susceptible; (S) susceptible; (LR) low resistance; (PR) partially resistant; (R) resistant. and (HR) highly resistant.

^z (CV %) coefficient of variation; (Fcal.) value of F in the analysis.

Table 4. Pearson's correlation analysis between variables for *Radopholus similis* population density in soil and banana roots.

	Nematode/g root	Nematodes in roots	Nematodes in soil	Total nematodes
Nematodes/g root	1**			
Nematodes in roots	0.9032**	1		
Nematodes in soil	0.5477**	0.6853**	1	
Total nematodes	0.7962**	0.9162**	0.9008**	1

**Significant ($P < 0.01$)

when challenged with the same nematode with a Rf of 28.54, higher than that found in this study (11.00), although the reaction to the nematode was the same. In the case of cv. Grande Naine, it is known that its roots can be highly affected by the nematode. According to Fallas and Marbán-Mendoza (1994), Grande Naine reacted with high levels of root lesions contributing directly to the reduction of fresh root weight. Wehunt *et al.* (1978) and Quénéhervé *et al.* (2009b) reported the wild species Borneo (*Musa acuminata microcarpa*) as a good host for *R. similis*. Similar host behavior was observed in this current study.

Previously genotype 1304-04, reported by Costa *et al.* (1998) as susceptible (Rf = 1.53), but was classified as having a low resistance rating (Rf = 6.11) in this study. This difference may be related to environmental factors, time for evaluation, difference in the amount of inoculum (170 specimens in previous work), aggressiveness of the nematode populations, and possible experimental errors.

Among 26 genotypes evaluated in the current study, we have identified 16 as being partially resistant to the nematode. However, only future work under field conditions can attest the value of this partial resistance to *R. similis*, or tolerance when present. We also call attention to the accessions Yangambi Km5, N118, Thap Maeo, 1318-01, 1319-01, and 4223-06 which were previously reported as partially resistant to *R. similis* (Costa *et al.*, 1998; Costa, 2004).

Wehunt *et al.* (1978) ranked cv. Tjau Lagada as susceptible based on the criterion of the degree of resistance, in contrast to the results of this work where this diploid reacted with partial resistance based on the percentage reduction on the factor of reproduction.

The hybrid 1318-01 was classified as a partially resistant to *R. similis*. Probably this moderate resistance was inherited from the parental genotype *Musa acuminata malaccensis* which was classified as resistant by Wehunt *et al.* (1978), and the same may have occurred with the accession 1319-01.

Gowen (1976) found that the genotypes Pahang and M48 were neither good hosts of *R. similis*, nor of

Helicotylenchus multicinctus. Therefore, it is possible that the genotype M48 has transferred resistance genes to the hybrid 4249-05, which showed the highest level of resistance in this assay. The same may have occurred with the partially resistant genotype 5854-03, which has in its genome background, contribution from the genotype Pahang.

The genotypes M48 and M53 are hybrids from Jamaica that have been used as a source of resistance to various pathogens (Silva *et al.*, 1998). Two of the

genotypes considered highly resistant and resistant (4249-05 and 4279-06, respectively) have these hybrids as parents and may have inherited resistance genes. Hybrid 4249-05 had the highest reduction on reproduction in relation to Borneo. The hybrid 4279-06 also considered resistant, besides having the accession M53 as parent, also has as ancestor, the genotype Calcutta 4 classified as partially resistant in this study. Carlier *et al.* (2003) described the diploid Calcutta 4 as a source of resistance to plant pathogens, including nematodes. This fact may explain the resistance observed in current work for the genotypes 0337-02 and 0323-03 which possess Calcutta 4 as one of their parents. These genotypes were classified by Costa *et al.* (1998) as highly susceptible and susceptible, respectively. These authors evaluated these genotypes based on the susceptible cv. Nanicão and in this study Borneo was used as standard susceptible.

It should be noted that the diploid 0323-03 was statistically different from the other resistant genotypes in the number of nematodes present in the roots. This fact indicates that this diploid may not be regarded as a poor host for *R. similis*.

The diploid 4249-05 stood out statistically with the least amount of total nematodes. Classified as resistant, the accessions 4249-05, 0337-02, 0323-03 and 4279-06, together with the genotypes Yangambi Km5, Pisang Jaran, 4223-06, and Thap Maeo, with lower numbers of nematodes per gram of root deserve to be considered in future work towards resistance to the burrowing nematode.

This study showed the potential use of banana genotypes from the Germplasm Collection of Embrapa Mandioca e Fruticultura for breeding programs to control *R. similis*, as it confirmed a high percentage of genotypes bearing resistance to the parasite. Among 26 genotypes evaluated in this study, 76.94 % behaved as resistant or partially resistant to the nematode. The variables number of nematodes per gram of root, number of nematodes in the root system, number of nematodes in soil, and total number of nematodes provided consistent results with the factor of reproduction of

the nematode when evaluating banana genotypes for resistance to *R. similis*.

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