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Scientific Article

Resistance of guava accessions to *Meloidogyne enterolobii*

Reação de acessos de goiabeira a *Meloidogyne enterolobii*

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ABSTRACT – Guava is a highly important fruit crop worldwide; however, a severe phytosanitary problem caused by the parasitism of *Meloidogyne enterolobii* has been limiting guava yields in Brazil and in several countries. The incidence of the nematode *M. enterolobii* results in significant decreases in yield, often resulting in plant death in the medium term. Considering a contribution to control tools, the objective of this study was to assess the resistance of 37 accessions of *Psidium guajava* grown in a greenhouse to *M. enterolobii*. Seedlings from seeds of each accession were inoculated with 4,000 eggs of *M. enterolobii* when they were at the three- to six-leaf stage, in two experiments. The root system of each plant was collected at 135 days after inoculation for extracting nematode eggs. The eggs were counted in three aliquots of 1 mL plant^{-1}, and the obtained data were subjected to analysis of variance, which showed significant differences among accessions and among plants of the same accession. Accessions were classified regarding resistance based on the reproduction factor (RF = Pf / Pi = 4,000). The *P. guajava* germplasm studied showed a significant variability in responses to *M. enterolobii*; resistant plants were identified for accessions A08, A15, A26, A13, and A30A in Experiment I and for accessions A31, A11, A16, A30A, GF3, and A08 in Experiment II; the accessions A08 and A30A stood out by presenting resistant plants in both experiments.

Keywords: Root-knot nematode. Screening. *Psidium guajava*.

RESUMO – A goiabeira é uma cultura de grande importância para o mundo, no entanto, um grave problema fitossanitário, causado pelo parasitismo de *Meloidogyne enterolobii*, tem limitado a produtividade da cultura no Brasil e em diversos países. A incidência do nematoide resulta em acentuada queda de produtividade e, na maioria das vezes, as plantas morrem em médio prazo. Visando a contribuir com ferramentas de controle, avaliou-se a reação de 37 acessos de *Psidium guajava* em casa de vegetação quanto à resistência a *M. enterolobii*. As mudas de cada acesso foram produzidas a partir de sementes e, no estádio de três a seis pares de folhas, foram submetidas à inoculação com 4.000 ovos de *M. enterolobii*, em dois experimentos. Cento e trinta e cinco dias após a inoculação, coletou-se o sistema radicular de cada uma das plantas para a extração de ovos. As contagens de ovos foram feitas em três alíquotas de 1 mL/planta e foram submetidas à análise de variância que indicou diferenças significativas entre acessos e entre plantas de mesmo acesso. A classificação dos acessos quanto à resistência foi feita pelo fator de reprodução (FR=Pf/Pi, Pi= 4.000). Pelo estudo do germoplasma de *P. guajava*, observou-se grande variabilidade quanto à reação a *M. enterolobii*, sendo que, no Experimento I, plantas resistentes foram encontradas nos acessos A08, A15, A26, A13 e A30A e, no Experimento II, nos acessos A31, A11, A16, A30A, GF3 e A08, com a detecção de plantas resistentes nos acessos A08 e A30A nos dois experimentos.

Palavras-chave: Nematoide-das-galhas. Screening. *Psidium guajava*.

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INTRODUCTION

Guava (*Psidium guajava* L.) has stood out in agricultural production in Brazil, especially red-fleshed cultivars (PEREIRA et al., 2016). India is the largest guava producing country, followed by Pakistan, while Brazil is the fourth (POMMER; MURAKAMI, 2009; KAREEM et al., 2018).

This is a predominantly outcrossing species (OLIVEIRA et al., 2020), producing fruits containing four times more vitamin C than orange, rich in phenolic compounds responsible for antimutagenic and antioxidant activities (FRANZON et al., 2009; QUINTAL et al., 2017; ZAHIN; AHMAD; AQIL, 2017). These attributes, combined with low production costs, make guava orchards economically important for small, medium, and large-scale growers (RODRÍGUEZ et al., 2010; CAVALCANTE et al., 2019; VITTI; LIMA; MARTINES FILHO, 2020).

However, a severe phytosanitary problem caused by the parasitism by *Meloidogyne enterolobii* Yang & Eisenback (Syn.: *M. mayaguensis* Rammah & Hirschmann) has been limiting guava production in Brazil and worldwide (ALMEIDA; GOMES; SOUZA, 2011; CASTRO et al., 2012; ROSA; WESTERICH; WILCKEN, 2015). The widespread and polyphagous nature of this nematode enables it to parasitize several plant species, including papaya (*Carica papaya* L.), tobacco (*Nicotiana tabacum* L.), coffee (*Coffea* spp.), Barbados cherry (*Malpighia emarginata* DC.), *Psidium* spp., guava, weeds, and several vegetables, making the control of this pathogen challenging

(CAVALCANTE JUNIOR et al., 2021). Additionally, a new race of *M. enterolobii* was recently reported in cotton plantations in western Bahia, Brazil (SOUZA et al., 2022), as the genetic variability of pathogens is a significant challenge in control efforts. The first nematicide for controlling root-knot nematodes in guava orchards was recently approved (AGROFIT, 2021), but it does not ensure success in recovering infested orchards. An aggravating factor that has been reported in studies is a complex disease known as guava decline caused by the interaction of *M. enterolobii* with the fungus *Neocosmospora falciformis* (Carrión) L. Lombard & Crous (Syn.: *Fusarium solani* (Mart.) Sacc.) (GOMES et al., 2013; GOMES et al., 2017; VELOSO; CÂMARA; SOUZA, 2021).

The interaction between these two pathogens in guava orchards has created several difficulties for growers in controlling this disease. Considering that the nematode acts as a facilitator for the fungus infection, the use of rootstocks resistant to *M. enterolobii* is the most suitable tool to minimize losses caused by guava decline. The cultivar BRS Guaraçá was developed by the Brazilian Agricultural Research Corporation (Embrapa Semiarido) through the crossing between *P. guajava* and *P. guineense* to be used as a rootstock for growing guava plants in areas infested by *M. enterolobii* (SOUZA, SANTOS, COSTA, 2018; RIBEIRO et al., 2019).

However, exploring new tools that can assist growers in controlling this serious disease is still necessary. Therefore, studies on genetic diversity within the genus *Psidium* and within the species *P. guajava* can provide information for guava breeding programs. Some studies have shown promising results regarding responses of wild individuals of different *Psidium* species to the reproduction factor of pathogens, denoting variability in plant resistance, which can be interpreted as genetic diversity (COSTA FILHO et al., 2018; OLIVEIRA et al., 2019; SANTOS et al., 2020).

In this context, the objective of this study was to characterize *P. guajava* accessions from a *Psidium* Active Germplasm Bank regarding their resistance to *M. enterolobii* to support guava breeding programs.

MATERIAL AND METHODS

Two experiments were conducted between August and November 2020 in a greenhouse with mean temperature of 28 °C and in the Laboratory of Nematology of the Embrapa Semiarido, in Petrolina, PE, Brazil (09°04'14.0"S and 40° 19'0.03.1"W).

A completely randomized experimental design with eight replications was used, considering one plant per experimental plot. Seeds from 38 *P. guajava* accessions were used, sourced from the Active Germplasm Bank for *Psidium* at the State University of Bahia, Brazil, stored in a cold chamber under temperature of 10 °C and relative humidity of 40% (Table 1). Accessions A30A, A100, A08, A13, and A14 were evaluated in Experiments I and II.

Seeds were distributed in trays with a vermiculite-based substrate generally used for growing vegetable seedlings, in both experiments. The trays were maintained in a greenhouse under 70% light and mean temperature of 28 °C after seeding. The seedlings were transplanted to 4-liter pots containing autoclaved sandy soil (120 °C for 50 minutes) when they exhibited three to six true leaves.

Table 1. Passport data for *Psidium guajava* accessions collected from four states in Brazil and used in Experiments I and II.

Accession	Cultivar	UF	Accession	Species/Cultivar	UF
Experiment I					
A08	Native guava	MG	A30A	Native guava	MG
A13	Native guava	MG	A30B	Native guava	MG
A14	Native guava	MG	A90	Native guava	MG
A15	Native guava	MG	A100	Native guava	MG
A26	Native guava	MG	Paluma	cv. Paluma	BA
Experiment II					
A06	Native guava	MG	GF2	Paluma guava	PE
A08	Native guava	MG	GF3	Paluma guava	PE
A10	Native guava	MG	P01	Paluma guava	BA
A11	Native guava	MG	P02	Paluma guava	BA
A13	Native guava	MG	P03	Paluma guava	BA
A14	Native guava	MG	P04	Paluma guava	BA
A16	Native guava	MG	P05	Paluma guava	BA
A30A	Native guava	MG	P06	Paluma guava	BA
A31	Native guava	MG	P07	Paluma guava	BA
A51	Native guava	MG	P08	Paluma guava	BA
A80	Native guava	MG	P09	Paluma guava	BA
GB	Native guava	PE	P11	Paluma guava	BA
GES	Native guava	ES	Paluma	cv. Paluma	BA

The soil was fertilized through application of 2 g of a 06-24-12 NPK formulation at 30 and 90 days after inoculation. Manual irrigation was performed to maintain soil moisture close to field capacity.

A pure population of *M. enterolobii* was maintained on tomato plants (*Solanum lycopersicum* L. cultivar Rutgers) grown in autoclaved soil. The *M. enterolobii* inoculum was prepared following the methodology proposed by Boneti and Ferraz (1981). After extraction, in counting chambers under an optical microscope, the suspension was adjusted for inoculation of 4,000 eggs + second-stage juveniles (J2) per plant. Inoculation was carried out at 15 days after transplanting by applying the suspension with eggs and J2 in three holes of 2 cm deep equidistant 3 cm from the plant's stem base.

Root fresh weight (g) was determined on a digital scale at 135 days after inoculation, after removing the aerial part of the plants and washing the root system; excess water was removed using paper towels. Root length (cm) was measured with a tape measure, only in the second experiment, to assess the effects of the nematode on root system development. Roots were then processed following the methodology proposed by Boneti and Ferraz (1981) for the extraction of eggs and J2 of *M. enterolobii*, which were counted in counting chambers under an inverted microscope (Nikon® TS100).

The final classification of plants and accessions regarding resistance to the nematode was based on the reproduction factor (RF = Pf/Pi, Pi=4,000) (OOSTENBRINK, 1966).

The programs Genes (CRUZ, 2013) and Sisvar (FERREIRA, 2014) were used for analyses of variance and Scott-Knott test for grouping the variable means, and the Paleontological Statistics Program (PAST 4.03) was used for Principal Component Analysis (PCA) and Pearson correlation test. Root fresh weight data, total number of eggs, and reproduction factor were transformed using the equation $\sqrt{x+1}$.

RESULTS AND DISCUSSION

Experiment I

Significant results were found for the total number of nematode eggs and reproduction factor, whereas no significant results were found for root fresh weight (Table 2). However, the total number of eggs and reproduction factor presented coefficients of variation higher than 40%, indicating that part of this variation can be connected to the response of each plant within the evaluated accessions to the nematode.

Table 2. Analysis of variance for responses of ten guava accessions to parasitism by *Meloidogyne enterolobii* based on root fresh weight (RFW), total number of eggs (TNE), and reproduction factor (RF).

Source of variation	Experiment I			
		Mean square		
	DF	RFW	TNE	RF
Accessions	9	2.48^{ns}	6,549.4*	1.23*
Error	63	1.51	2,538.7	0.52
CV (%)		25.6	50.5	40.8

*Significant at a 5% level by the F-test; ns = not significant; DF = degrees of freedom.

Accessions A15, A30B, A30A, A100, and A13 presented the lowest mean total number of nematode eggs. The highest means were found for accessions A14, A90, A26, and A08 (Table 3), consequently showing the highest nematode reproduction. Accessions A30A, A100, and A13 presented mean total number of eggs and reproduction factor lower than those found for *P. guajava* 'Paluma', which is considered a standard for susceptibility to the nematode. This may be attributed to differences in susceptibility among commercial plant materials, which may express lower resistance to *M. enterolobii* compared to non-domesticated materials. The evaluated accessions in Experiment I presented no significant differences in root fresh weight.

Considering the final root weight is essential in evaluations of nematode variables. Plants with less developed root systems may result in lower reproduction factors due to a significant nematode infestation and the susceptibility of the plant. Accession A30A, for instance, presented a mean reproduction factor of 0.26, indicating resistance according to Oostenbrink (1966). However, this accession had only 1 g of root weight at the end of the test, explaining the low reproduction factor and resistance response found. Accessions A08, A15, A26, A13, and A30A, although classified as susceptible (reproduction factor > 1), were the ones that resulted in the highest numbers of plants classified as resistant. Accession A30A stood out, with five out of eight plants exhibiting resistance.

The range of the reproduction factor for each guava accession showed low values for all evaluated accessions, reinforcing the importance of considering variation between and within accessions. Studies focused on identifying candidate materials for rootstocks and transferring genes of resistance to *M. enterolobii* have shown this variability (OLIVEIRA et al., 2019). Miranda et al. (2012) assessed the response of *Psidium* spp. accessions, including native guava accessions, and found similar results.

Table 3. Root fresh weight (RFW), total number of eggs (TNE), mean reproduction factor (RF), variation between the maximum and minimum reproduction factors (VRF), number of resistant plants (NRP), and response of ten native and commercial guava accessions to inoculation with *Meloidogyne enterolobii*.

Accessions	Variables					
	RFW[ns]	TNE	RF	VRF	NRP	Response*
A15	12.2	12.200a	1.72b	2.38 – 0.82	01	Susceptible
A14	12.3	12.340a	2.3a	4.0 – 1.07	0	Susceptible
A30B	7.4	7.420b	1.69b	2.0 – 1.39	0	Susceptible
A90	18.0	18.080a	2.45a	4.05 – 1.19	0	Susceptible
A30A	1.0	1.040b	1.08b	2.17 – 0.78	05	Susceptible
Paluma	8.0	8.080b	1.69b	2.85 – 0.77	01	Susceptible
A26	11.9	11.940a	2.98a	4.17 – 0.95	01	Susceptible
A100	4.6	4.680b	1.48b	2.04 – 0.86	01	Susceptible
A08	15.0	15.020a	2.09a	3.32 – 0.94	01	Susceptible
A13	5.1	5.140b	1.55b	3.36 – 0.94	02	Susceptible

[ns] Not significant. Means followed by the same letter in the columns belong to same group by the Scott-Knott test at a 5% significance level. [*]Response to nematode multiplication according to Oostenbrink (1966).

According to the Principal Component Analysis (PCA) conducted using data of root fresh weight, total number of eggs, and reproduction factor, the PC1 explained 86.1%, while the PC2 explained 13.7% of the total variance of the data (Figure 1). An inverse correlation was found between the total number of eggs and root fresh weight for accessions A26, A08, A90, and A15, indicating that these accessions had greater root weight and smaller total number of eggs and reproduction factor, i.e., these accessions could be considered candidates for guava breeding programs focused on incorporating genes that confer resistance to *M. enterolobii*.

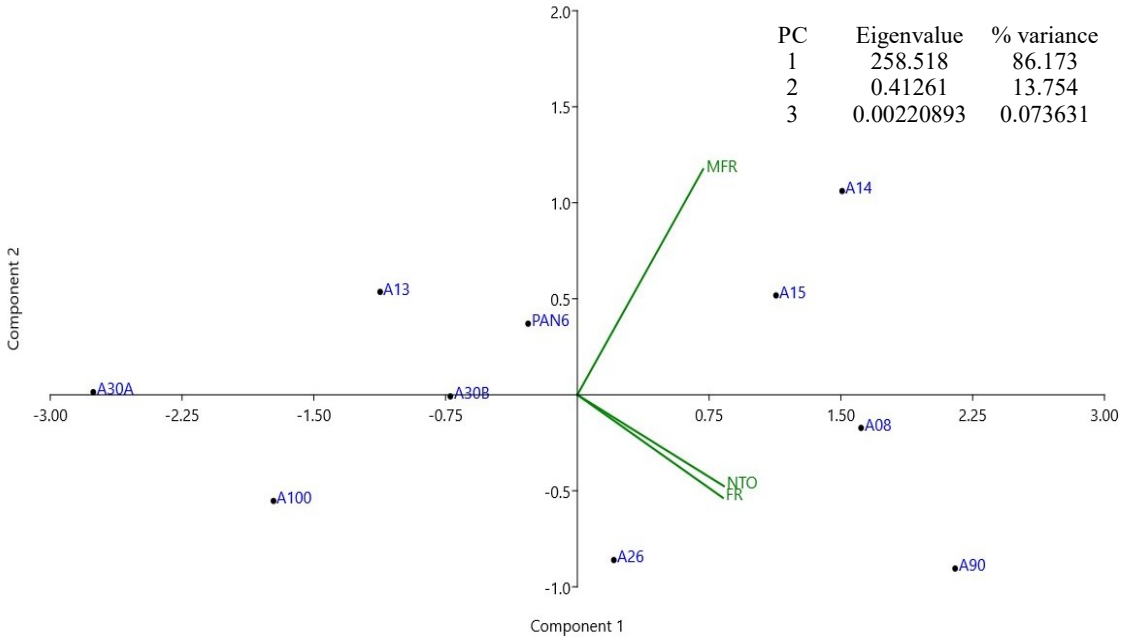

Figure 1. Principal component analysis: correlation among root fresh weight (RFW) of plants from *Psidium guajava* accessions and total number of eggs (TNE) and reproduction factor (RF) of *Meloidogyne enterolobii*.

Experiment II

The responses of 27 guava accessions to *M. enterolobii* were assessed in Experiment II. All evaluated variables were significant at a 1% by the F-test. The total number of nematode eggs and reproduction factor presented coefficients of variation higher than 50% (Table 4), emphasizing the variation in responses to the nematode among plants of different accessions. The total number of eggs and reproduction factor are the most important parameters for identifying *P. guajava* accessions with resistance to *M. enterolobii.* A significant variation in reproduction factor was found among 'Paluma' guava plants, among plants of the same accession, and among accessions ($p<0.01$).

Results for root length are shown in Table 5; the highest means were found for accessions GF2, A14, A13, GF3, A08, P07, and A31.

The greater root fresh weight found for 'Paluma' guava plants may be attributed to their greater vigor in root system development. Accessions P04, P02, P03, P07, P08, GF2, A06, and GF3 showed the greatest root fresh weight all in the same group. Accessions A80, A16, A30A, A31, GB, A11, and GES showed the lowest root fresh weight, also all in the same group (Table 5). This low root development can be explained by the stress caused by the nematode and the wild characteristic of the plant material used.

Table 4. Analysis of variance for responses of 27 guava accessions to parasitism by *Meloidogyne enterolobii* based on root length (RL), root fresh weight (RFW), total number of eggs (TNE), and reproduction factor (RF).

	Experiment II				
	DF	RL	RFW	TNE	RF
Accessions	26	621.48**	1.121.20**	104.455.29**	26.11**
Error	182	62.59	73.40	22.211.33	5.21
CV (%)		34.64	39.23	54.59	51.67

**Significant at a 1% level by the F-test. DF = degrees of freedom

Table 5. Root length (RL), root fresh weight (RFW), total number of eggs (TNE), mean reproduction factor (RF), variation between the maximum and minimum reproduction factors (VRF), number of resistant plants (NRP), and response of 27 native and commercial guava accessions to inoculation with *Meloidogyne enterolobii*.

Accessions	Variables						
	RL	RFW	TNE	RF	VRF	NRP	Response*
A14	35.4a	20.8d	284.978c	48.1b	87.8 – 7.7	0	Susceptible
A31	30.4a	9.1e	169.306a	53.0c	13.7 – 0.4	01	Susceptible
A80	24.9b	11.6e	259.016	23.6c	74.7 – 1.5	0	Susceptible
GB	25.8b	7.9e	201.214c	13.2c	48.7 – 1.7	0	Susceptible
GES	20.8b	5.0e	145.778c	3.9c	12.1 – 1.7	0	Susceptible
A11	23.3b	6.5e	144.889c	5.43c	9.7 – 0.3	01	Susceptible
A16	23.2b	11.1e	176.686c	11.6c	54.3 – 0.1	01	Susceptible
A06	24.0b	13.9b	172.569c	8.1c	19.2 – 3.6	0	Susceptible
A30A	23.4b	9.7e	217.499c	23.0c	68.2 – 0.3	01	Susceptible
A51	27.2b	13.6d	282.256c	11.0c	77.3 – 3.3	0	Susceptible
A10	26.2b	14.5d	269.903c	27.8c	82.9 – 2.2	0	Susceptible
GF3	32.6a	8.1b	17.160b	5.0b	109.7 -0.6	01	Susceptible
A13	34.7a	17.6d	270.762c	11.0c	86.3 – 3.1	0	Susceptible
GF2	36.5a	19.9b	348.991b	34.7b	83.5 – 0.6	0	Susceptible
A08	32.3a	20.3d	199.761c	5.0c	36.1 – 0.08	01	Susceptible
Paluma	18.4b	49.1a	554.425a	84.5a	130.8 – 23.5	0	Susceptible
P01	16.3c	26.1c	352.271b	40.9b	83.6 – 2.0	0	Susceptible
P02	22.7b	37.7b	225.492c	3.9c	32.9 – 2.1	0	Susceptible
P03	22.1b	37.5b	267.625c	3.0c	63.7 – 1.0	0	Susceptible
P04	19.9b	38.2b	403.343b	45.4b	85.7 – 9.3	0	Susceptible
P05	6.12b	25.9c	148.658c	2.0c	11.7 – 1.9	0	Susceptible
P06	21.8b	30.7c	445.931b	34.0a	231.7 – 14.1	0	Susceptible
P07	30.7a	36.2b	519.619a	74.7a	182.2 – 29.6	0	Susceptible
P08	5.0d	29.6c	154.385c	10.0c	24.4 – 1.2	0	Susceptible
P09	8.4d	27.6c	412.400b	23.0b	104.8 – 16.9	0	Susceptible
P10	5.6d	27.2c	219.946c	5.0c	33.4 – 3.7	0	Susceptible
P11	18.4b	31.4c	334.063b	31.5b	62.4 – 4.1	0	Susceptible

Means followed by the same letter in the columns belong to the same group by the Scott-Knott test at a 5% significance level. *Response to nematode multiplication according to Oostenbrink (1966).

The lowest reproduction factors were found for accessions A80, A51, A10, P03, A13, P02, P10, A30A, GB, A16, A06, A31, P08, P05, A11, and GES. Accessions A14, A08, A13, A10, A06, A51, and A16 presented root fresh weight exceeding 10 g (Table 5). This denotes the variability of *M. enterolobii* reproduction in these accessions, as there was a difference in the range of reproduction factor values, despite the high availability infection sites.

Brazil is one of the centers of origin and diversification of plants of *P. guajava* and *P. cattleianum*. These plants predominantly reproduce through allogamy (CHANDRA et al., 2007), as open pollination can interfere with the genetic and epigenetic regulation of the interaction with *M. enterolobii*, leading to diverse responses. These highlights need for further studies.

Plants from accessions A31, A11, A16, A30, GF3, and A08 showed a reproduction factor lower than 1 (RF < 1) and were considered resistant according to Oostenbrink (1966). The mean reproduction factor varied from 231.7 to 0.1 among the evaluated accessions of *P. guajava*, confirming results found by Cavalcante Junior et al. (2021). The reproduction factor is the most essential parameter for measuring responses to nematode parasitism (SILVA et al., 2020). However, according to Miranda et al. (2012), these sources of variation are susceptible to experimental errors, mainly when plants are obtained by cuttings, which did not occur in the present experiment, as the seedlings were obtained from seeds.

Another important factor to consider is that guava decline is a complex disease caused by the interaction of *M. enterolobii* with the fungus *N. falciformis* (GOMES et al., 2013). Thus, further studies are needed to identify materials with characteristics of resistance not only to *M. enterolobii* but also to *N. falciformis.*

According to the PCA conducted for Experiment II using data of root length, root fresh weight, total number of eggs, and reproduction factor, PC1 and PC2 explained more than 80% of the total variance of the data: 50.5% (PC1) and 32.9% (PC2) (Figure 2). Accessions A14, GF2, A10, A51, A08, A80, and A13 showed a positive correlation between the root fresh weight (RFW) and reproduction factor (RF). Thus, these accessions had greater RFW and RF within PC2 and, therefore, may not be candidates for guava breeding programs.

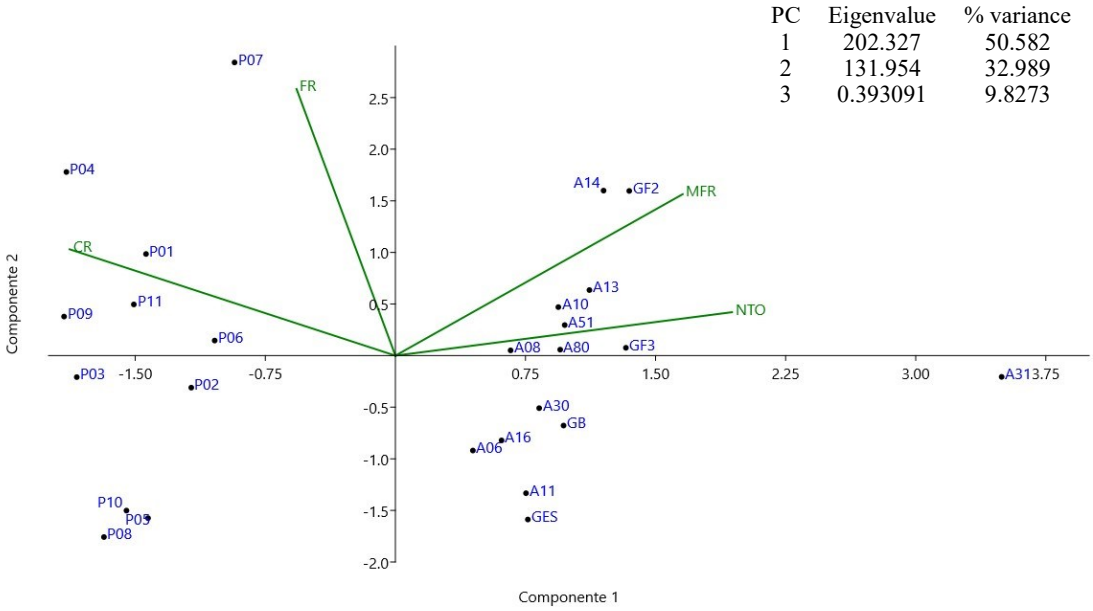

Figure 2. Principal component analysis: correlation among root length (RL) and root fresh weight (RFW) of plants from *Psidium guajava* accessions and total number of eggs (TNE) and reproduction factor (RF) of *Meloidogyne enterolobii*.

The results found showed a significant variability among the studied guava accessions, providing important information for breeding programs focused on obtaining nematode-resistant plants. The guava pollination system has a higher rate of cross-fertilization than self-fertilization, leading to high variability within *P. guajava* species. Oliveira et al. (2020) emphasized that germplasm of trees and shrubs, whether allogamous or autogamous, with a high rate of allogamy, tends to present a high degree of polymorphism.

CONCLUSION

The germplasm of *P. guajava* exhibited variability in responses to *M. enterolobii*, as plants with values of nematode reproduction below 1.0 were found in accessions A08, A11, A13, A15 A16, A26, A30A, A31, and GF3, which can be utilized in guava breeding programs focused on resistance to the nematode.

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