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Response of *Malpighia emarginata* active germplasm bank accessions to *Meloidogyne enterolobii* parasitism

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ABSTRACT. *Malpighia emarginata* is cultivated in almost all Brazil and is considered an important agricultural crop. The root-knot nematode *Meloidogyne enterolobii* has been described as a major threat to this crop, causing great production losses. Due to the scarcity of information about the severity of this parasite in *M. emarginata* plants in Brazil, this study investigated *M. enterolobii* resistance of ten *M. emarginata* genotypes from the active germplasm bank of Universidade Federal Rural de Pernambuco. The experiment was conducted adopting a completely randomized design in a factorial arrangement of 11 x 2 x 5, where *M. emarginata* cuttings were inoculated with 10,000 eggs in

a greenhouse. After 150 days, plants were evaluated for the following parameters: gall index, egg mass index, number of eggs per root system, number of eggs per gram of root, and reproduction factor. The accessions showed different responses depending on host x pathogen interaction, from susceptibility to moderate tolerance. Accessions 027-CMF and 031-CMF were considered tolerant to the nematode and could be of great value in new breeding programs for resistance to *M. enterolobii* infection.

Key words: Acerola; Barbados cherry; Root-knot nematode

INTRODUCTION

Malpighia emarginata DC is a shrub found naturally in Central America including parts of Amazonia and the Caribbean islands. Commonly known in Brazil as "Acerola", the species was introduced to Brazil in the late 1950s, via seeds brought from the Antilles and the United States. This crop established itself as an agricultural crop of economic importance due to the high content of ascorbic acid found in its fruits, and its high industrial yield in the production of pulp (Junqueira et al., 2002; Lima et al., 2003; Freire et al., 2008). The strong demand on the international market triggered an indiscriminate expansion of this crop throughout Brazil. However, there are few varieties of *M. emarginata* available to Brazilian producers. Orchards are often established by materials of unknown origin, resulting in poor uniformity of trees and fruits. These and some other factors, such as nematode infections, limit the development of this crop (Salla et al., 2002; Bueno et al., 2007; Moraes Filho et al., 2013).

Nematodes represent the largest source of biotic stress experienced by plants and can cause stunting, early senescence, and, in severe cases, total crop loss (Bird, 2004; Perry and Moens, 2011). Second-stage juvenile parasites penetrate the roots of host plants. There they induce dramatic changes in selected root vascular cells forming elaborate feeding cells to permanently supply nutrients that enable the nematodes to develop into reproductive adults (Williamson and Gleason, 2003; Davis et al., 2008; Perry and Moens, 2011).

Many nematodes have been detected in association with *M. emarginata*, but due to their pathogenicity, *Meloidogyne* parasites are the most important. Known commonly as root-knot nematodes, *Meloidogyne* species are obligate sedentary endoparasites that infect over 2000 plant species (Sasser, 1980). There are reports of occurrence of *M. incognita*, *M. javanica*, *M. arenaria*, and, more recently, *M. enterolobii* (also known as *M. mayaguensis*) causing major damage to this crop (Franco and Ponte, 1989; Holanda et al., 1997; Souza et al., 2006; Bueno et al., 2007; Dias-Arieira et al., 2010). Plants affected by *M. enterolobii* develop symptoms such as small and deformed leaves, accompanied by large amounts of galls on the roots that may lead to reduction of their productivity (Castro et al., 2009; Castellano et al., 2011). Despite its easy diagnosis, it is common for symptoms caused by root-knot nematodes to be confused with physiological problems such as nutritional deficiency and hydric stress, or even with other pests and diseases (Ritzinger and Ritzinger, 2002).

Thus, the use of resistant rootstocks that are tolerant to these soil pathogens has become a necessity. Resistant rootstocks have low-cost and low environmental impact; however, any identified nematode-resistant sources are currently understudied (Rossiter, 2007; Pinheiro, 2012; Souza et al., 2015). Much of the genetic variability of *M. emarginata* in

Brazil is represented in an active germplasm bank (AGB), belonging to Universidade Federal Rural de Pernambuco (UFRPE). This AGB contains 42 accessions from different regions and was implemented with the aim to preserve the genetic variability of *M. emarginata*, as well as to provide evaluation and identification of promising genotypes (Lima et al., 2003; Moraes Filho et al., 2013). The present study aimed to evaluate the accessions of the *M. emarginata* AGB for resistance to *M. enterolobii*.

MATERIAL AND METHODS

This study was conducted in the Agronomy Department of the UFRPE. We evaluated ten *M. emarginata* genotypes from the UFRPE *M. emarginata* AGB, and an independent matrix, Sertaneja BRS, for *M. enterolobii* susceptibility. Cuttings were obtained from the matrices in April 2012, and planted in mini-tunnels on sterile substrate. A shade cloth was placed over the mini-tunnels at 1.2 m above ground, reducing brightness by 50%. Irrigation was carried out daily by nebulization, and by the use of a gutter for water storage to maintain a constant humidity at 80%. After 60 days, the cuttings that showed roots were transplanted to plastic pots with a 10-L capacity, containing sterilized substrate.

M. enterolobii inoculums were obtained from Embrapa Semi-Arid - CPATSA - Petrolina, PE, and kept in tomato (*Solanum lycopersicum* L.), strain 684, recognized as resistant to *M. incognita* and *M. javanica*. Two months after the inoculation the roots of tomato plants were carefully removed from the substrate, washed and cut into small segments of 1-2 cm. Eggs were then extracted according to the technique described by Hussey and Barker (1973). The suspension was immediately passed through "US Standard Series" sieves of 200 and 500 meshes. The eggs that were retained in the latter sieve were washed in running water to remove sodium hypochlorite residues and transferred to 50-mL plastic bottles. Eggs were counted from 1 mL samples on photon microscopes and the concentration of the suspension was adjusted to 1000 eggs/mL using distilled water. The experiment was conducted adopting a completely randomized design in a factorial arrangement of 11 x 2 x 5 corresponding to 11 genotypes and two inoculation levels (with and without infestation *M. enterolobii*) with five replicates each. Each plot contained one plant. Soil infestation was carried out 60 days after the planting. This was done through application of 10,000 eggs per plant, applied in four 2-cm deep holes around the plant stem, using a pipette. The plants were watered daily with Hoagland solution (Hoagland and Arnon, 1950). After 150 days of infection, examination of the root system of the genotypes was carried out. The gall (GI) and egg mass (EI) indices were evaluated under a stereomicroscope using the International *Meloidogyne* Project (IMP) rating scale (Taylor and Sasser, 1978). To evaluate nematode reproduction, egg extractions were performed according to Hussey and Barker (1973). We calculated number of eggs per root system (NER), number of eggs per gram of root (NEG), and the reproduction factor (RF), obtained by the ratio between the final and initial nematode population. The highest RF value obtained was established as susceptibility standard for the calculation of the reduction percentage (RFR). The results of each genotype were analyzed according to the methodology of Moura and Régis (1987), by using the following percentages: 0-25 = highly susceptible, 26-50 = susceptible, 51-75 = little resistant, 76-95 = moderately resistant, 96-99 = resistant, 100 = highly resistant or immune. At the same time, we estimated relative fresh weight of shoots (RWS) and relative fresh biomass of root system (RWR).

For the statistical analysis, stabilizing transformation of variance was performed using the square root for the RWS and RWR. Log (x + 1) transformation was used for all other parameters. Parametric analysis of variance was performed for all sources of variation. The post-ANOVA procedure used was the Tukey test, using 5% probability for the variables RWS, RWR, NER, and NEG. To evaluate the correlations between variables, we used Pearson's correlation coefficient in the SAEG software.

RESULTS AND DISCUSSION

Based on GI, the 031-CMF genotype was considered resistant (GI < 3). All other genotypes were susceptible to the parasite, exhibiting GI values between 3 and 5 (Table 1). Likewise, based on the EI, the 031-CMF genotype was considered resistant (EI < 3), whereas all other genotypes were considered susceptible.

Table 1. Reaction of *Malpighia emarginata* genotypes to *Meloidogyne enterolobii* parasitism.

Accession	EI	GI	SR	FR	RFR	GR
Sertaneja-BRS	5.0	4.1	S	24.08	-	-
002-SPE	4.0	3.8	S	6.72	72.09	LR
015-CPA	4.2	4.8	S	10.31	57.18	LR
026-CMF	4.2	3.6	S	8.8	63.46	LR
027-CMF	4.6	4.6	S	4.56	81.06	MR
028-CMF	3.4	3.8	S	9.28	61.46	LR
029-CMF	3.6	3.4	S	7.28	69.77	LR
030-CMF	3.8	4.2	S	11.84	50.83	S
031-CMF	2.6	2.8	R	4.96	79.40	MR
033-CMF	3.0	3.2	S	9.52	60.47	LR
035-CMF	4.2	4.0	S	16.32	32.23	S

EI: egg mass index (0-5); GI: gall index (0-5); SR: susceptibility reaction according to Taylor and Sasser (1978) [S = susceptible (IG ≥ 3), R = resistant (IG ≤ 3)]; FR: reproduction factor; RFR: reduction of the reproduction factor; GR: genotype reaction (S = susceptible, LR = little resistant; MR = moderately resistant).

Measurement of nematode parasitism resistance based solely on GI and EI may lead to inaccurate results due to the potential subjectivity and empiricism of the counting methodology. Natural root bulges can be mistaken for early-stage galls, and colored wastes may be similar to egg masses accumulated in the roots, leading to either over- or underestimated counts (Costa Filho, 2012). Considering the reduction in the RF compared to the standard RFR, the 027-CMF and 031-CMF genotypes were considered moderately resistant. All other genotypes were little resistant or susceptible. From a parasitological point of view, all the plants were good hosts (RF > 1).

Even though important from an epidemiological point of view, moderately resistant genotypes are not recommended for control of *M. enterolobii*. The use of these genotypes, both for fruit production or as rootstock, is inadequate because the parasite population level can be significantly higher under field conditions, affecting the longevity and health of the orchard (Maranhão et al., 2003). However, most *M. emarginata* cultivars are highly susceptible to *M. enterolobii* (Castellano et al., 2011; Cavichioli et al., 2014), and tolerant varieties could be an alternative for fruit production.

The life cycle of *Meloidogyne* species varies with both host culture and temperature. It lasts approximately 25 days at 27°C, becoming longer at lower or higher temperatures (Agrios, 2005). During the 150 days of the infection period, the temperatures were close to 24.5°C.

While the exact number of days for the completion of the nematode cycle was not precisely quantified, the formation of galls on the superficial roots was not observed in the first 60 days; they were detected only from the fourth month. However, after 150 days, we observed that the cycle was completed without disturbance, based on the high rate of egg masses observed in all accessions.

Based on ANOVA, significant ($P \leq 0.05$) differences were observed by the Tukey test for the variables: RF, NER, RWS, RWR, GI, and EI (Table 2).

Table 2. Analysis of variance for the resistance indexes of *Malpighia emarginata* to *Meloidogyne enterolobii* parasitism.

Source of variation	d.f.	MS					
		RF	NER	RWS	RWR	GI	EI
Accession	10	157.89**	0.16**	0.34**	0.55**	0.17**	0.24**
Residual	40	67.08	0.67	0.02	0.06	0.66	0.10
CV (%)		31.42	7.47	9.77	14.97	12.15	15.09

d.f.: degrees of freedom; MS: mean square; RF: reproduction factor; NER: number of eggs per root system; RWS: relative weight of shoots; RWR: relative weight of the roots; CV: coefficient of variation. **P < 0.05 by the Tukey test.

In the evaluation of the effects of *M. enterolobii* parasitism for RWS and RWR (Table 3), a significant difference was observed only for accession 028-CMF, which was less affected by the presence of the parasite. Thus, this parameter may contribute to the selection of genetically tolerant material *M. enterolobii*. The highest average NER was observed for Sertaneja-BRS, which was statistically higher than the average for accessions 027-CMF and 031-CMF, which showed the lowest NER values.

For the GI, we observed that accessions 015-CMF and 027-CMF had the highest GI values. In contrast, accession 031-CMF showed the lowest GI value and was significantly lower compared to all other accessions. Accession Sertaneja-BRS had the highest EI value, which was significantly higher compared to accession 031-CMF, which exhibited the lowest EI value. The 033-CMF accession showed an average EI = 3, which is the maximum grade to be considered resistant according to Sasser (1980). However, this value did not differ statistically from the other accessions considered susceptible.

Table 3. Comparison of means of development variables of *Malpighia emarginata* in response to *Meloidogyne enterolobii* parasitism.

Accession	RWS	RWR	NER	GI	EI
Sertaneja-BRS	1.42^{a}	1.64^{a}	240.80^{a}	4.10^{ab}	5.00^{a}
002-SPE	1.33^{a}	1.40^{a}	67.20^{ab}	3.80^{ab}	4.00^{ab}
015-CPA	1.34^{a}	1.36^{a}	103.10^{ab}	4.80^{a}	4.20^{ab}
026-CMF	1.44^{a}	1.56^{a}	88.00^{ab}	3.60^{ab}	4.20^{ab}
027-CMF	1.24^{a}	1.45^{a}	45.60^{b}	4.60^{a}	4.60^{ab}
028-CMF	2.22^{b}	2.55^{b}	92.80a^{b}	3.80^{ab}	3.40^{ab}
029-CMF	1.40^{a}	1.53^{a}	72.80^{ab}	3.40^{ab}	3.60^{ab}
030-CMF	1.45^{a}	1.53^{a}	118.40^{ab}	4.20^{ab}	3.80^{ab}
031-CMF	1.50^{a}	1.77^{a}	49.60^{b}	2.80^{b}	2.60^{b}
033-CMF	1.40^{a}	1.52^{a}	95.20^{ab}	3.20^{ab}	3.00^{ab}
035-CMF	1.41^{a}	1.52^{a}	163.20^{ab}	4.00^{ab}	4.20^{ab}
CV (%)	9.77	14.97	7.48	12.15	15.10

Averages followed by the same superscript letter do not differ significantly ($P < 0.05$) from each other, based on the Tukey test. RWS: relative weight of shoots; RWR: relative weight of the roots; NER: number of eggs per root system; GI: gall index; EI: egg mass index.

To conclude, we found that most of the evaluated accessions were susceptible to *M. enterolobii*. Accession 031-CMF was classified as moderately resistant and tolerant to *M. enterolobii*. This accession showed less effect on the root system compared to other genotypes. Accession 027-CMF, despite displaying susceptibility based on the Sasser (1980) criteria, had the lowest RF among all accessions analyzed. It was thus classified as tolerant. Both accessions are considered promising for future breeding programs for resistance to nematode *M. enterolobii*.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Agrios GN (2005). Plant pathology. 5th edn. Elsevier Academic Press, Burlington.

Bird DM (2004). Signaling between nematodes and plants. *Curr. Opin. Plant Biol.* 7: 372-376. http://dx.doi.org/10.1016/j.pbi.2004.05.005

Bueno PRR, Guerreiro JC, Brass FEB and Cervigni G (2007). Primeiro relato de ocorrência do nematóide *Meloidogyne mayaguensis* em acerola, na região de Garça-SP. *Rev. Cienc. Eletron. Agron.* 12.

Castellano G, Quijada O, Jiménez N, Crozzoli R, et al. (2011). Reacción de cultivares de cerecita (*Malpighia glabra*) a *Meloidogyne enterolobii* (Nematoda: Meloidogynidae). *Fitopatol. Venez.* 24: 25-27.

Castro JMC, Santana MLMP and Barbosa NML (2009). Nematoides-das-galhas (*Meloidogyne* spp.) em aceroleira e recomendações de manejo. *Embrapa Semiárido. Instruções Técnicas* 87.

Cavichioli JC, Garcia MJM, Brida AL and Wilcken SRS (2014). Reação de aceroleira (*Malpighia emarginata* D.C.) à *Meloidogyne enterolobii*. *Rev. Bras. Frutic.* 36: 156-160. http://dx.doi.org/10.1590/0100-2945-429/13

Costa Filho JH (2012). Coleta e reação de acessos de melancia a *Meloidogyne enterolobii*. Master's thesis (Fitotecnia), Universidade Federal Rural do Semi-Árido, Mossoró.

Davis EL, Hussey RS, Mitchum MG and Baum TJ (2008). Parasitism proteins in nematode-plant interactions. *Curr. Opin. Plant Biol.* 11: 360-366. http://dx.doi.org/10.1016/j.pbi.2008.04.003

Dias-Arieira CR, Furlanetto C, Santana SM, Oliveira Barizão DA, et al. (2010). Fitonematoides associados a frutíferas na região Noroeste do Paraná, Brasil. *Rev. Bras. Frutic.* 32: 1064-1071. http://dx.doi.org/10.1590/S0100-29452010005000119

Franco A and Ponte JJ (1989). Acerola, *Malpighia glabra* L, um novo hospedeiro de nematoides das galhas. *Nematol. Bras.* 13: 181-183.

Freire JLO, Lima AN, Freira ALO, Marinus JVML, et al. (2008). Avaliações biométricas de aceroleira (*Malpighia ermaginata* D.C.) e caracterização dos atributos externos e internos dos frutos. *Eng. Ambiental* 5: 41-52.

Hoagland DR and Arnon DI (1950). The water-culture method for growing plants without soil. University of California, Berkeley.

Holanda YCA, Ponte JJ and Silveira FJ (1997). Disease of the Barbados cherry plant (*Malpighia glabra*) in the State of Ceará, Brazil. *Fitopatol. Bras.* 22: 453.

Hussey RS and Barker KR (1973). Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57: 1025-1028.

Junqueira KP, Pio R, Vale MR and Ramos JD (2002). Cultura da Aceroleira. UFLA, Lavras.

Lima AG, Melo EA, Maciel MIS and Lima DES (2003). Avaliação do teor de antocianinas em polpa de acerola congelada proveniente de frutos de 12 diferentes aceroleiras (*Malpighia emarginata* D.C.). *Cienc. Tecnol. Alim.* 23: 101-103.

http://dx.doi.org/10.1590/S0101-20612003000100021

Maranhão SRVL, Moura RM and Pedrosa EMR (2003). Reação de indivíduos segregantes de Araçazeiro a *Meloidogyne incognita* Raça 1, *M. javanica* e *M. enterolobii*. *Nematol. Bras.* 27: 173-178.

Moraes Filho RM, Martins LS, Musser RS, Montarroyos AV, et al. (2013). Genetic variability in accessions of the acerola germplasm bank of Universidade Federal Rural de Pernambuco, Brazil. *Genet. Mol. Res.* 12: 5145-5151. http://dx.doi.org/10.4238/2013.October.29.8

Moura RM and Régis EMO (1987). Reações de cultivares de feijoeiro comum (*Phaseolus vulgaris*) em relação ao parasitismo de *Meloidogyne javanica* e *M. incognita* (Nematoda: Heteroderidae). *Nematol. Bras.* 11: 215-225.

Perry RN and Moens N (2011). Introduction to plant-parasitic nematodes: modes of parasitism. In: Genomics and molecular genetics of plant-nematode interactions (Jones J, Gheysen G and Fenoll C, eds.). Springer, Dordrecht, 3-20.

Pinheiro JB (2012). Os desafios atuais da nematologia no contexto da olericultura: *Meloidogyne enterolobii* (sin. *M. mayaguensis*) em hortaliças. Embrapa Hortaliças, 12.

Ritzinger CH and Ritzinger R (2002). Nematoides em acerola. Acerola em foco. Embrapa Mandioca e Fruticultura, Cruz das Almas.

Rossiter JGA (2007). Potencialidades dos genótipos de aceroleira (*Malpighia emarginata* D.C.) quanto ao enraizamento e resistência a nematoide visando a obtenção de porta-enxerto. Master's thesis (Agronomia: Melhoramento Genético de Plantas), Universidade Federal Rural de Pernambuco, Recife.

Salla MFS, Ruas CF, Ruas PMA and Carpentieri-Pípolo V (2002). Uso de marcadores moleculares na análise da variabilidade genética em acerola (*Malpighia emarginata* D.C.). *Rev. Bras. Frutic.* 24: 15-22. http://dx.doi.org/10.1590/S0100-29452002000100005

Sasser JN (1980). Root-knot nematodes: a global menace to crop production. *Plant Dis.* 64: 36-41. http://dx.doi.org/10.1094/PD-64-36

Souza DC, Botelho FB, Rodrigues CS, Furtini IV, et al. (2015). Resistance of upland-rice lines to root-knot nematode, *Meloidogyne incognita*. *Genet. Mol. Res.* 14: 17384-17390. http://dx.doi.org/10.4238/2015.December.21.7

Souza RM, Nogueira MS, Lima IM, Melarato M, et al. (2006). Manejo do nematóide das galhas da goiabeira em São João da Barra (RJ) e relato de novo hospedeiro. *Nematol. Bras.* 30: 165-169.

Taylor AL and Sasser JN (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). North Carolina State University and U.S.A.I.D., N.C. Graphics, Raleigh.

Williamson VM and Gleason CA (2003). Plant-nematode interactions. *Curr. Opin. Plant Biol.* 6: 327-333. http://dx.doi.org/10.1016/S1369-5266(03)00059-1