Resistance of *Malpighia emarginata* DC. genotypes to *Meloidogyne enterolobii* parasitism

ABSTRACT

Aims: Considering the inexistence of *M. emarginata* cultivars resistant to *M. enterolobii* available for cultivation, and the scarcity of information about the severity of its parasitism in *M. emarginata*, the present study investigated the response of genotypes from the active germplasm bank of Universidade Federal Rural de Pernambuco to *M. enterolobii* parasitism, aiming the selection of resistant genotypes for use as rootstocks for commercial varieties.
Study design: The experimental design was completely randomized, with 21 genotypes and one independent matrix (control), with six replicates each. The experimental unit was represented by one plant per plot.
Place and Duration of Study: Department of Agronomy, Universidade Federal Rural de Pernambuco – UFRPE - Brazil between June 2013 and July 2014.
Methods: In the experiment, completely randomized design was adopted, with 21 genotypes from the AGB and one as a control for susceptibility. The *M. emarginata* cuttings were inoculated with 10,000 nematode eggs, and after 150 days, they were evaluated for the following parameters: egg mass index, gall index, reproduction factor, number of eggs per gram of root, number of eggs per root system.
Results: Twenty out of the twenty-two genotypes analyzed were susceptible. The genotypes 021-CMF and 037-CMF were considered resistant. To our knowledge, this is the first identification of *M. emarginata* genotypes resistant to *M. enterolobii*.
Conclusions: These results are of great importance for the breeding and cultivation of the species since these two genotypes can be indicated for use as rootstocks and for breeding programs aimed at transferring resistance to other cultivars with desirable production characteristics that are susceptible to the phytonematode.

Keywords: *Acerola, Brabados cherry, root-knot nematode, rootstocks, parasitism*

1. INTRODUCTION

Malpighia emarginata DC. also known as Barbados cherry and Acerola, has been gaining space in the fruit-growing sector due to its high amount of vitamin C. Its cultivation has shown a great potential for expansion, whether for fresh consumption, juice industry or for the pharmaceutical industry [1]. Although it is a rustic plant, *M. emarginata* is sensitive to root-knot nematodes, which are its main limiting factor, negatively influencing the production and the quality of fruits. In the last years, orchards have shown a considerable decrease in production due to these phytonematodes [2]. One of the main species that has been shown to be very harmful to the crop is the *Meloidogyne enterolobii* Yang & Eisenback, described in China in 1983 [3,4].
The main symptoms caused by the attack of this phytonematodes are the small, deformed and yellowing leaves; delay and reduction in the seedlings development, and in cases of high infestations, poor plant development and declining production may occur [5-8].
A survey carried out in several irrigated perimeters in the São Francisco Valley Region, in northwestern Brazil, revealed a high percentage of infected *M. emarginata* trees, raising the concern that this phytonematode may turn the cultivation unfeasible, as it has happened to guava orchards in that same region [7-9]. Genotypes with resistance or tolerance to phytonematodes may be used as rootstocks, as a low cost and sustainable alternative to chemical control methods and, can easily be adopted by growers without environmental and sanitary risks [9-11]. The use of resistant rootstocks could provide effective control and

significantly reduce the damages caused by *M. enterolobii*, allowing the recovery of infested areas[12]. The knowledge of how *M. emarginata* genotypes respond to *M. enterolobii* infection is also of great importance since in perennial crops the management of these organisms is even more difficult. Therefore, for new orchards, it is essential to choose non-infested areas or the use of resistant genotypes [2]. Thus, in this work we evaluated 21 different *M. emarginata* genotypes from the active germplasm bank of UFRPE, aiming at the selection and indication of genotypes resistant to the phytonematode *M. enterolobii*.

2. MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at the Agronomy Department of the Universidade Federal Rural de Pernambuco – UFRPE - Brazil. Twenty-two *M. emarginata* genotypes were utilized, of which twenty-one belong to the Active Bank of Germplasm (AGB) of the Carpina Sugarcane Experimental Station (E.E.C.A.C./UFRPE), located in the municipality of Carpina – PE and the variety BRS Sertaneja, which was selected due to its susceptibility to *M. enterolobii* [2,11].

2.1 Collection and propagation of the plant material

Semi-woody cuttings with three nodes and two pairs of leaves were obtained from the evaluated genotypes which were between thirteen and fifteen years old. The cuttings were planted in a mini-tunnel containing commercial Brasplant® substrate; the depth of planting was 1/3 of the length of the stake. In order to maintain humidity, the mini tunnel was covered with transparent white plastic and a 50% shade was used for shading. Irrigation was performed daily early in the morning and late in the afternoon.

2.2 Inoculum source

M. enterolobii inoculums were obtained from Embrapa Semiárido - CPATSA - Petrolina, PE, and kept in tomato and multiplied in tomato plants (*Solanum lycopersicon* L.*)*, lineage 684, known as resistant to *M. incognita and M. javanica* [7]. Two months after the inoculation, the tomato roots were carefully removed from the substrate, then washed and cut into 1-2cm segments. Eggs were then extracted according to the technique described by [13]. After obtaining the nematode egg suspension, 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.

2.3 Evaluation of genotypes for resistance to *M. enterolobii*

Sixty-day seedlings were transplanted to 10 L plastic pots containing commercial Brasplant® substrate and placed in a greenhouse. During the conduction of the experiment, the average temperature was 26 ± 2 ° C and relative humidity of 65 ± 5%. Water irrigation was performed daily in the early morning and late afternoon; irrigation and fertilization with were done weekly [14].

After 120 days of planting, inoculation was done at a concentration of 10,000 eggs/plant. The egg suspension was deposited in three small holes in the soil around the plant's neck, with an automatic graduated pipette. 150 days after inoculation, the root system of each genotype was carefully washed and evaluated according to the following parameters: gall index (GI) and egg mass index (EMI), both determined according to the scale proposed by [15].

Subsequently, the eggs were extracted following the methodology described by Hussey and Barker (1973) [13]. The number of eggs per root system (NER) was estimated with a photonic microscope. In addition, the number of eggs per gram of root (NEGR) and reproduction factor (RF), obtained by the ratio of the final number of eggs to the initial number of inoculated eggs, were also estimated [16].

With the RF, the highest value was taken as the susceptibility standard and, from this, the percentages of RF reduction was obtained by the formula: (RRF) = Frp-Frt / Frp x 100 were calculated, where: Frp = reduction in the reproduction factor in the standard and Frt = reproduction factor in the treatment [16]. According to Moura and Regis (1987)[17], it is possible to classify genotypes for resistance or susceptibility by considering RRF values. Thus, RRF = 0-

25 (Highly Susceptible-HS); RFR = 26-50 (Susceptible-S); RFR = 51-75 (Little Resistant-LR); RRF = 76-95 (Moderately Resistant-RM); RRF = 96-99 (Resistant-R); RRF = 100 (Highly Resistant-HR or Immune-I). The relative weight of the shoots (RWS), the relative weight of the roots (RWR), as well as the shoots dry biomass (SDB) were also calculated.

2.4 Experimental Design

The experimental design was completely randomized, with 21 genotypes and one independent matrix (control), with six replicates each. The experimental unit was represented by one plant per plot.
Analysis of variance was performed using the Sisvar software. The data was transformed into $\log_x$ for the variable number of eggs per root grass, in the square root of x, for a number of eggs per root system and the reproduction factor, the other variables did not undergo any transformation. Subsequently, the means were compared by the Scott-Knott test, at 5% probability.

3. RESULTS

Five months after inoculation with *M. enterolobii,* root galls and egg masses were detected in all inoculated plants. Significant differences based on the analysis of variance were found by the Scott-Knott test to the following variables: GI, EMI, RF, NER, NEGR, RWS, RWR and SDB (Table 1).

Table 1. Analysis of variance for the resistance indexes of *M. emarginata* to the parasitism of *M. enterolobii*

	DF	MS							
		GI	EMI	RF	NER	NEGR	RWS	RWR	SDB
Genotypes	21	1.14**	1.68**	4.05**	1928.40**	728.28**	425.04**	787.57**	146.56**
Residual	110	0.11	0.14	0.78	70.99	204.77	48.97	94.72	10.02
CV (%)		17.65	21.75	48.21	48.14	49.43	16.23	24.30	18.75

*DF: degrees of freedom; MS: mean square; GI: gall index; EMI: egg mass index; RF: reproduction factor; NER: number of eggs per root system; NEGR: Number of eggs per root grain; RWS: Relative weight of the shoots; RWR: The relative weight of the roots; SDB: Shoots dry biomass; Cv: coefficient of variation. ** p <0,05 by the scott-knott test*

The lowest values for the GI and EMI variables and according to the criterion of Sasser (1980) [18] were observed in 018-CMF and 37-CMF genotypes (Table 2).

Table 2. *M. emarginata* genotypes response in relation to the parasitism of *M. enterolobii*

Genotypes	GI	EMI	SR	RF	RRF	DR
SERTANEJA	4,7[1]	4,2[1]	S[1]	8,50[1]	41,13	S
002-SPE	5	5	S	10,34	28,39	S
016-CMF	4,2	4	S	1,78	87,67	MR
017-CMF	4	3,8	S	3,42	76,32	MR
018-CMF	1,3	0,8	R	1,30	91,00	MR
021-CMF	2,7	2	S	0,56	96,12	R

022-CMF	3	2,7	S	4,56	68,42	LR
023-CMF	5	4,5	S	2,38	83,52	MR
024-CMF	2,8	1,8	S	1,34	90,72	MR
025-CMF	3,8	2,8	S	2,46	82,96	MR
028-CMF	4,3	4,3	S	2,89	79,99	MR
033-CMF	5	5	S	14,44	-	S
036-CMF	4,8	4,3	S	2,04	85,87	MR
037-CMF	1	0,4	R	0,50	96,54	R
038-CMF	4,8	4,3	S	5,97	58,66	LR
039-CMF	4,6	3,8	S	2,77	80,82	MR
040-CMF	3	2	S	2,43	83,17	MR
041-CMF	4,4	3,4	S	6,98	51,66	LR
042-CMF	4,7	4,5	S	7,27	49,65	S
043-UFRPE	4,2	2,8	S	2,30	84,07	MR
044-APE	5	4,7	S	10,50	27,29	S
045-APE	5	5	S	7,77	46,19	S

[1]mean value for the six replicates; [2] negative values compared to the control; GI= 0 to 5 according to Sasser (1980); EMI= Egg Mass Index (0-5); SR= Susceptibility reaction: S= susceptible (IG≥3); R= resistant (IG≤3); RF= Reproduction; RRF = reduction in the reproductive factor compared to the control DR= Differential Reaction: HS= highly susceptible; S = susceptible; LR= low resistance; MR= moderately resistant.

The observed RF values ranged from 0.50 (037-CMF) to 14.44 (033-CMF) (Table 2). The variable RFR represents how much each genotype differed in its RF in relation to the most susceptible genotype. The 033-CMF was the most susceptible genotype observed, with RF even greater than the susceptibility control, the Sertaneja cultivar. The highest percentages were obtained by the genotypes 21 and 37 which obtained a RRF of 96.12 and 96.54, respectively, and they were classified as resistant (R) (Table 2).

Regarding the number of eggs per root gram (ERG) and the amount of eggs per root system (ERS), the genotypes 21 and 37 were characterized by the lowest values for (15.55 and 11.77) and (64.73 and 64.6), resulting in promising genotypes regarding resistance (Table 3).

Table 3. Reaction of *M. emarginata* genotypes to *M. enterolobii*, for the indicator variables of susceptibility, evaluated

Genotypes	NEGR [1]	NER [2]	RWR (g)	RWS (g)	SDB (g)
Sertaneja	41.19 b	263.74 b	39.01 b	42.00 b	16.89 b
002-SPE	46.31 b	331.19 b	47.00 c	46.66 b	19.66 c
016-CMF	17.86 a	124.63 a	48.94 c	41.33 a	15.66 b
017-CMF	27.81 a	175.68 a	42.54 c	56.66 d	25.34 d
018-CMF	19.18 a	93.71 a	21.97 a	36.66 a	11.94 a

021-CMF	15.55 a	64.73 a	25.66 a	37.66 a	11.66 a
022-CMF	38.54 a	201.69 b	29.22 a	33.33 a	12.95 a
023-CMF	24.92 a	149.52 a	39.10 b	45.74 b	15.86 b
024-CMF	20.51 a	109.45 a	30.66 a	37.33 a	11.33 a
025-CMF	18.48 a	130.57 a	48.28 c	46.66 b	19.72 c
028-CMF	27.38 a	161.67 a	34.66 b	40.66 a	15.33 b
033-CMF	42.13 b	357.32 b	73.00 d	61.66 d	23.00 c
036-CMF	23.82 a	136.87 a	32.54 b	51.24 c	24.35 d
037-CMF	11.77 a	64.6 a	25.11 a	37.83 a	15.81 b
038-CMF	37.63 b	223.86 b	33.85 b	36.33 a	11.66 a
039-CMF	17.84 a	143.37 a	55.94 c	36.60 a	14.51 b
040-CMF	20.12 a	137.69 a	42.29 c	48.00 b	21.64 c
041-CMF	36.05 b	247.94 b	45.65 c	59.33 d	28.01 d
042-CMF	39.79 b	235.71 b	37.66 b	38.33 a	14.00 b
043-UFRPE	18.91 a	130.98 a	46.60 c	40.00 a	17.27 b
044-APE	44.13 b	297.23 b	45.33 c	39.00 a	12.00 a
045-APE	46.88 b	276.33 b	36.01 b	36.77 a	12.79 a

NEGR= Number of eggs per grain of root and NER= number of eggs per root system; RWR= Relative weight of the roots; RWS = Relative weight of the shoots; SDB= Shoots dry biomass. [1]logx turned variables , [2]√x turned variables . [x] average values followed by the same letter in the columns do not differ by the scott-knott test at the 5% probability.

4. DISCUSSION

The concept of resistance used in plant nematology describes the ability of a given plant to suppress the development and reproduction, or even the infection process of a nematode [19]. To select the root-knot nematodes, the symptoms can be evaluated with ease, but it is common for symptoms caused by these parasites to be confused with physiological problems such as nutritional deficiency and hydric stress, or even with other pests and diseases [20], and is also common for some plant species the absence of any apparent symptoms, despite the infection of its roots, therefore, the term resistance is also used to describe the capacity of a host to suppress the disease [19, 21]. The Gall index and the degree of galling may be used to measure the ability of a plant to lessen or overcome the attack by the root-knot nematode. However, these indexes do not indicate the occurrence of nematode reproduction directly, while the reproduction factor, is a variable that allows the direct measurement of the nematode’s reproductive capacity in the host [22]. The GI is usually used in germplasm tests to address the type of host reaction and the percentage of reduction of the parasite's reproduction rate in relation to the most susceptible cultivar, allowing the epidemiological characterization of the nematode-host interaction [2, 17, 23].

Considering only the GI criterion, 018-CMF and 37-CMF genotypes could be classified as resistant (GI < 2), but the evaluation of nematode parasitism resistance based solely the

development of galls may lead to inaccurate results due to the potential subjectivity and empiricism of the counting methodology [24]. In this study, the genotypes exhibited an expressive variation in their susceptibility to the phytonematode considering the RF criterion.
Genotypes 21-CMF and 37-CMF, in addition to exhibiting low values in relation to the GI, EMI, RWS and RWR parameters, presented RF <1, with values of 0.56 and 0.50 respectively (Table 1), therefore being indicated as rootstocks resistant to *M. enterolobii* parasitism. The genotype 018-CMF despite being considered resistant by the GI criteria, exhibited a RF of 1.3 and RFR of 91, and therefore classified only as moderately resistant.
Regarding the RWS and RWR variables (Table 3), it was verified that there was a significant difference between the studied genotypes. The genotypes 017-CMF and 033-CMF exhibited the higher RWS values in relation to the others showing good development of the shoots even when parasite by *M. enterolobii*. The genotype 033-CMF showed a higher value of RWR, and also the higher GI and RF values, being the most susceptible of the observed genotypes. [11] evaluated the responses of eleven UFRPE-AGB *M. emarginata* genotypes to the parasitism of *M. enterolobii.* Regarding the variables RWS and RWR, the authors verified a significant difference only for the 028-CMF genotype, which exhibited the higher shoot and roots development of the evaluated genotypes and was classified as moderately resistant. For the SDB variable, the highest values were observed for genotypes 017-CMF, 036-CMF and 041-CMF, which were classified little or moderately resistant to *M. enterolobii*. Considering another species of the *Meloidogyne* genus, [10] did not find a significant difference for the parameters RWR and RWS, in *M. emarginata* UFRPE AGB genotypes parasited by *M. incognita*.
The evaluation of RWS and RWR may contribute to the selection of tolerant genotypes, to root-knot nematodes, since the absorption and distribution of the nutrients are highly related to the growth rate of the plants, and may be impaired by parasites in the root system [25], but only the observation of developmental characteristics are not sufficient to the determination of resistance or long term tolerance to these parasites.

5. CONCLUSIONS

1. The genotypes 021-CMF and 037-CMF were resistant to *M. enterolobii* and could be indicated as rootstocks.
2. The genotype 033-CMF is indicated as susceptibility control to *M. enterolobii* parasitism, exhibiting higher values of RF than the commercial variety Sertaneja.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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