

1 Resistance of *Malpighia emarginata* genotypes to

2 *Meloidogyne enterolobii* parasitism

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5 ABSTRACT

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7 **Objective:** Considering the inexistence of *M. emarginata* cultivars resistant to *M. enterolobii*
8 available for cultivation, and the scarcity of information about the severity of its parasitism in *M.*
9 *emarginata*, the present study investigated the response of genotypes from the active
10 germplasm bank of Universidade Federal Rural de Pernambuco to *M. enterolobii* parasitism,
11 aiming the selection of resistant genotypes for use as rootstocks for commercial varieties.

12 **Study design:** The experimental design was completely randomized, with 21 genotypes and
13 one independent matrix (control), with six replicates each. The experimental unit was
14 represented by one plant per plot.

15 **Place and Duration of Study:** Department of Agronomy, Universidade Federal Rural de
16 Pernambuco – UFRPE - Brazil between June 2013 and July 2014.

17 **Methods:** In the experiment a completely randomized design was adopted, with 21 genotypes
18 from the AGB and one as a control for susceptibility. The *M. emarginata* cuttings were
19 inoculated with 10,000 nematode eggs, and after 150 days, they were evaluated for the
20 following parameters: egg mass index, gall index, reproduction factor, number of eggs per gram
21 of root, number of eggs per root system.

22 **Results:** Twenty out of the twenty-two genotypes analyzed were susceptible. The genotypes
23 021-CMF and 037-CMF were considered resistant. To our knowledge, this is the first
24 identification of *M. emarginata* genotypes resistant to *M. enterolobii*.

25 **Conclusions:** These results are of great importance for the breeding and cultivation of the
26 species, since these two genotypes can be indicated for use as rootstocks and for breeding
27 programs aimed at transferring resistance to other cultivars with desirable production
28 characteristics that are susceptible to the phytonematode.

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

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33 1. INTRODUCTION

34 *Malpighia emarginata*, also known as Barbados cherry and Acerola, has been gaining space in
35 the fruit-growing sector due to its high amount of vitamin C. Its cultivation has shown a great
36 potential for expansion, whether for fresh consumption, juice industry or for the pharmaceutical
37 industry [1]. Although it is a rustic plant, *M. emarginata* is sensitive to root-knot nematodes,
38 which are its main limiting factor, negatively influencing the production and the quality of fruits.
39 In the last years, orchards have shown a considerable decrease in production due to these
40 phytonematodes [2]. One of the main species that has been shown to be very harmful to the
41 crop is the *Meloidogyne enterolobii* Yang & Eisenback, described in China in 1983 [3,4].

42 The main symptoms caused by the attack of this phytonematodes are the small, deformed and
43 yellowing leaves; delay and reduction in the seedlings development, and in cases of high
44 infestations, poor plant development and declining production may occur [5-8].

45 A survey carried out in several irrigated perimeters in the São Francisco Valley Region, in
46 northwestern Brazil, revealed a high percentage of infected *M. emarginata* trees, raising the
47 concern that this phytonematode may turn the cultivation unfeasible, as it has happened to
48 guava orchards in that same region [7-9]. Genotypes with resistance or tolerance to
49 phytonematodes may be used as rootstocks, as a low cost and sustainable alternative to
50 chemical control methods and, can easily be adopted by growers without environmental and
51 sanitary risks [9-11]. The use of resistant rootstocks could provide an effective control and
52 significantly reduce the damages caused by *M. enterolobii*, allowing the recovery of infested

53 areas[12]. The knowledge of how *M. emarginata* genotypes respond to *M. enterolobii* infection
54 is also of great importance, since in perennial crops the management of these organisms is
55 even more difficult. Therefore, for new orchards, it is essential to choose non-infested areas or
56 the use of resistant genotypes [2]. Thus, in this work we evaluated 21 different *M. emarginata*
57 genotypes from the active germplasm bank of UFRPE, aiming at the selection and indication of
58 genotypes resistant to the phytonematode *M. enterolobii*.
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60 2. MATERIAL AND METHODS

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

67 2.1 Collection and propagation of the plant material

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

74 2.2 Inoculum source

75 *M. enterolobii* inoculums were obtained from Embrapa Semiárido - CPATSA - Petrolina, PE,
76 and kept in tomato and multiplied in tomato plants (*Solanum lycopersicon* L.), lineage 684,
77 known as resistant to *M. incognita* and *M. javanica* [7]. Two months after the inoculation, the
78 tomato roots were carefully removed from the substrate, then washed and cut into 1-2cm
79 segments. Eggs were then extracted according to the technique described by [13]. After
80 obtaining the nematode egg suspension, eggs were counted from 1 mL samples on photon
81 microscopes and the concentration of the suspension was adjusted to 1000 eggs/mL using
82 distilled water.
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88 2.3 Evaluation of genotypes for resistance to *M. enterolobii*

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90 Sixty-day seedlings were transplanted to 10 L plastic pots containing commercial Brasplant®
91 substrate and placed in a greenhouse. During the conduction of the experiment the average
92 temperature was 26 ± 2 ° C and relative humidity of $65 \pm 5\%$. Water irrigation was performed
93 daily in the early morning and late afternoon; irrigation and fertilization with was done weekly
94 [14].

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

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

105 With the RF, the highest value was taken as the susceptibility standard and, from this, the
106 percentages of RF reduction was obtained by the formula: $(RRF) = Frp-Frt / Frp \times 100$ were

107 calculated, where: Frp = reduction in the reproduction factor in the standard and Frt =
 108 reproduction factor in the treatment [16]. According to Moura and Regis (1987)[17], it is possible
 109 to classify genotypes for resistance or susceptibility by considering RRF values. Thus, RRF = 0-
 110 25 (Highly Susceptible-HS); RFR = 26-50 (Susceptible-S); RFR = 51-75 (Little Resistant-LR);
 111 RRF = 76-95 (Moderately Resistant-RM); RRF = 96-99 (Resistant-R); RRF = 100 (Highly
 112 Resistant-HR or Immune-I). Relative weight of the shoots (RWS), the relative weight of the roots
 113 (RWR), as well as the shoots dry biomass (SDB) were also calculated.

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115 2.4 Experimental Design

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117 The experimental design was completely randomized, with 21 genotypes and one independent
 118 matrix (control), with six replicates each. The experimental unit was represented by one plant
 119 per plot.

120 Analysis of variance was performed using the Sisvar software. The data was transformed into
 121 log_x for the variable number of eggs per root grass, in square root of x, for number of eggs per
 122 root system and the reproduction factor, the other variables did not undergo any transformation.
 123 Subsequently, the means were compared by the Scott-Knott test, at 5% probability.

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125 3. RESULTS

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

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131 **Table 1. Analysis of variance for the resistance indexes of *M. emarginata* to the parasitism of *M.***
 132 ***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

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

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137 The lowest values for the GI and EMI variables and according to the criterion of Sasser (1980)
 138 [18] were observed in 018-CMF and 37-CMF genotypes (Table 2).

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142 **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

143 ¹mean value for the six replicates; ² negative values compared to the control; GI= 0 to 5 according to
144 Sasser (1980); EMI= Egg Mass Index (0-5); SR= Susceptibility reaction: S= susceptible (IG \geq 3); R=
145 resistant (IG \leq 3); RF= Reproduction; RRF = reduction in the reproductive factor compared to the control
146 DR= Differential Reaction: HS= highly susceptible; S = susceptible; LR= low resistance; MR= moderately
147 resistant.
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149 The observed RF values ranged from 0.50 (037-CMF) to 14.44 (033-CMF) (Table 2). The
150 variable RFR represents how much each genotype differed in its RF in relation to the most
151 susceptible genotype. The 033-CMF was the most susceptible genotype observed, with RF
152 even greater than the susceptibility control, the Sertaneja cultivar. The highest percentages
153 were obtained by the genotypes 21 and 37 which obtained a RRF of 96.12 and 96.54,
154 respectively, and they were classified as resistant (R) (Table 2).

155 Regarding the amount of eggs per root gram (ERG) and the amount of eggs per root system
156 (ERS), the genotypes 21 and 37 were characterized by the lowest values for (15.55 and
157 11.77) and (64.73 and 64.6), resulting in promising genotypes regarding resistance (Table
158 3).
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160 **Table 3. Reaction of *M. emarginata* genotypes to *M. enterolobii*, for the indicator variables of**
161 **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

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

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4. DISCUSSION

169 The concept of resistance used in plant nematology describes the ability of a given plant to
170 suppress the development and reproduction, or even the infection process of a nematode [19].
171 To detect the root-knot nematodes, the symptoms can be evaluated with ease, but it is common
172 for symptoms caused by these parasites to be confused with physiological problems such as
173 nutritional deficiency and hydric stress, or even with other pests and diseases [20], and is also
174 common for some plant species the absence of any apparent symptoms, despite the infection of
175 its roots, therefore, the term resistance is also used to describe the capacity of a host to
176 suppress the disease [19, 21]. The Gall index and the degree of galling may be used to
177 measure the ability of a plant to lessen or overcome the attack by the root-knot nematode.
178 However, these indexes do not indicate the occurrence of nematode reproduction directly, while
179 the reproduction factor, is a variable that allows the direct measurement of the nematode's
180 reproductive capacity in the host [22]. The GI is usually used in germplasm tests to address the
181 type of host reaction and the percentage of reduction of the parasite's reproduction rate in
182 relation to the most susceptible cultivar, allowing the epidemiological characterization of the
183 nematode-host interaction [2, 17, 23].
184 Considering only the GI criterion, 018-CMF and 37-CMF genotypes could be classified as
185 resistant (GI < 2), but the evaluation of nematode parasitism resistance based solely the
186 development of galls may lead to inaccurate results due to the potential subjectivity and

187 empiricism of the counting methodology [24]. In this study, the genotypes exhibited an
188 expressive variation in their susceptibility to the phytonematode considering the RF criterion.
189 Genotypes 21-CMF and 37-CMF, in addition to exhibiting low values in relation to the GI, EMI,
190 RWS and RWR parameters, presented RF <1, with values of 0.56 and 0.50 respectively (Table
191 1), therefore being indicated as rootstocks resistant to *M. enterolobii* parasitism. The genotype
192 018-CMF despite being considered resistant by the GI criteria, exhibited a RF of 1.3 and RFR of
193 91, and therefore classified only as moderately resistant.
194 Regarding the RWS and RWR variables (Table 3), it was verified that there was a significant
195 difference between the studied genotypes. The genotypes 017-CMF and 033-CMF exhibited the
196 higher RWS values in relation to the others showing good development of the shoots even
197 when parasite by *M. enterolobii*. The genotype 033-CMF showed the higher value of RWR, and
198 also the higher GI and RF values, being the most susceptible of the observed genotypes. [11]
199 evaluated the responses of eleven UFRPE-AGB *M. emarginata* genotypes to the parasitism of
200 *M. enterolobii*. Regarding the variables RWS and RWR, the authors verified a significant
201 difference only for the 028-CMF genotype, which exhibited the higher shoot and roots
202 development of the evaluated genotypes and was classified as moderately resistant. For the
203 SDB variable, the highest values were observed for genotypes 017-CMF, 036-CMF and 041-
204 CMF, which were classified little or moderately resistant to *M. enterolobii*. Considering another
205 species of the *Meloidogyne* genus, [10] did not find significant difference for the parameters
206 RWR and RWS, in *M. emarginata* UFRPE AGB genotypes parasited by *M. incognita*.
207 The evaluation of RWS and RWR may contribute to the selection of tolerant genotypes, to root-
208 knot nematodes, since the absorption and distribution of the nutrients are highly related to the
209 growth rate of the plants, and may be impaired by parasites in the root system [25], but only the
210 observation of developmental characteristics are not sufficient to the determination of resistance
211 or long term tolerance to these parasites.
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213 5. CONCLUSIONS

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

218 219 COMPETING INTERESTS

220 Authors have declared that no competing interests exist.
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223 224 REFERENCES

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