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Inheritance of resistance to *Meloidogyne enterolobii* in *Psidium guajava* x *P. guineense* hybrid

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Abstract Inheritance of the resistance to nematodes has been studied on many different crops, however to our knowledge, no data are available for guava species. The basic genetic resistance parameters to *Meloidogyne enterolobii* are estimated in the current research in order to guide the development of genotypes resistant to the pathogen. The parental plants, F1 and F2 from a *Psidium guajava* x *P. guineense* cross were assessed for the presence or absence of galls and for the number of eggs and juveniles in the root system at the 120th and 240th days after inoculation with 10,000 eggs and juveniles of the nematode. At the age of nine years, the *P. guineense* plant remained without nematode attack symptoms, whereas the maternal plant was destroyed by the pathogen. The F1 generation showed 270 plants with reproduction factor (RF) <0.322, and there were tiny galls in only 16 plants. The segregation for the

presence or absence of galls in the root system in generation F2 was 9:7, wherein the χ^2 values were 0.78 and 2.66, respectively, at the 120th and 240th days after inoculation, whereas the segregation for RF was 15:1, wherein the χ^2 values were 2.76 and 1.18, respectively, at the 120th and 240th days. These results indicate epistatic interaction between two genes: in RF < 1 only one allele sets the resistance to the pathogen. The broad sense heritability of RF, estimated to the two assessments was 0.97, and it also indicates a simple inheritance of resistance to *M. enterolobii*.

Keywords Guava · Nematode · Heritability · Control

Introduction

The guava (*Psidium guajava* L.) is an important cultivated plant worldwide. The species belongs to the family Myrtaceae, which comprises more than 70 genera and 2,800 species (Pereira and Nachtigal 2002). The species has great economic value; it is one of the most important fruits in Brazil, due to its consumption, *in natura* or industrialized. This culture has been facing great decline in its production since the emergence of the nematode *Meloidogyne enterolobii*.

The first report on the occurrence of this pathogen *M. enterolobii* in guava plant roots in Brazil was recorded in São Francisco river valley (Carneiro et al. 2001). This phytopathogen infects all guava root types; it infects from the superficial rootlets to the more lignified taproot, which is located more than 50 cm deep (Reis

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et al. 2011). The practices to control this pathogen, such as the use of nematicides, biological control, nitrogenous fertilizers, non-host plants and integrated handling, have limited or ineffective effect (Freitas et al. 2014).

Sources of resistance to the pathogen were not identified in accessions of *P. guajava* (Castro et al. 2012). There were reports of resistance in wild *Psidium* plant species (Carneiro et al. 2007; Castro et al. 2012; Souza et al. 2014). The use of wild *Psidium* plant species resistant to nematode, such as the rootstock of guava plant cultivars, has been presenting limited or total incompatibility (Freitas et al. 2014; Castro et al. 2012). Robaina et al. (2012) have reported that the grafting of 'Paluma' guava plants with Brazilian guava plants resistant to *M. enterolobii* resulted in tissue welding with the guava plant, but there was no tissue compatibility due to the lack of vascular tissue functionality. Costa et al. (2012) have reported a *P. guajava* x *P. guineense* hybrid resistant to the pathogen and with high compatibility with guava plant species, appointing as an alternative to deal with guava plant nematode.

Costa et al. (2012) have suggested that the resistance to nematode in *Psidium* hybrid comes from the monogenic dominant inheritance, taking in account analyses of the F1 plants from the two crossings of the *P. guajava* and *P. guineense* accessions. There are no reports in the literature about the segregation and the estimated number of genes in F2 populations for resistance to *M. enterolobii*.

The aim of the current study is to estimate the basic genetic resistance parameters to *M. enterolobii*, such as the number of genes, broad heritability and gene action, involved in the resistance to *M. enterolobii*, according to the segregation in F2 populations of a *P. guajava* x *P. guineense* cross. The present research is the first one involving *Psidium* species.

Materials and methods

Plant material

The manual crossing followed the pollination procedure described by Costa et al. (2012). Accessions of guava plants cultivated for five years in the field, and assessed for pathogen reactions were used: guava plants (Gua161 PE), susceptible to the nematode *M. enterolobii*, as maternal plants and the accession of *P. guineense* (Ara138RR), resistant to the pathogen, as paternal plant.

Plants from the crossing between *P. guajava* x *P. guineense* were selfed in the field using properly identified protection paper bags to obtain the F2 population. After 120 days, the fruits were harvested and their seeds prepared for sowing, which was performed in 20-L plastic vases containing one part of artificial substrate (Plantimax®) and one part of soil, at the ratio 1:1, in greenhouse. Irrigation was daily performed according to the need of the material. Seeds germination took place between the 22nd and the 30th days.

DNA extraction and microsatellite markers for genotyping

Young and healthy leaves were collected from the parental and from some F1 plants from the obtained crossings. The leaves were stored at -80°C until the moment of extraction. Protocol CTAB 2 \times , by Doyle and Doyle (1990), was used for DNA extraction, according to the procedure described by Costa and Santos (2013). Microsatellite markers, mPgCI 252 and mPgCI 247, were used to confirm the interspecific hybrids according to the procedure described by Briceño et al. (2010) and Costa and Santos (2013). The PCR amplification was carried out for a final volume of 10 μL , containing 30 ng DNA, 0.2 μL of each primer, 1X Taq DNA polymerase buffer, 2.5 mM MgCl_2 , 0.8 mM dNTPs, and 0.75 U enzyme Taq DNA polymerase. The amplification program consisted of denaturation of the initial cycle at 94°C for 4 min; 30 cycles at 94°C for 45 s, 52°C for 60 s, and 72°C for 60 s; and one stage of final extension at 72°C for 5 min. Half of the volume of the denaturing buffer of 98% formamide (10 mM EDTA, pH 8.0; 1 mg/mL xylene cyanol; and 1 mg/mL bromophenol blue) was added to the PCR mixture, followed by complete denaturation at 94°C for 5 min in a thermocycler. The gels were stained with silver nitrate, as per the procedure described by Creste et al. (2001).

Assessing the resistance to the nematode in F1 and F2 populations

Plants from the F1 and F2 populations were transplanted to polyethylene bags containing autoclaved soil and kept in a greenhouse. When the plants reached 15 to 20 cm tall, they were inoculated with 10,000 eggs and J2 juveniles of *M. enterolobii*. The *M. enterolobii* inoculum was extracted from the guava plant roots collected from commercial areas in Petrolina County – Pernambuco

State. The inoculum was extracted by means of the technique described by Hussey and Barker (1973). The suspension was deposited in two holes in the soil around the plant using a pipette, with 2 mL applied per hole, 1.5 cm from the stem, 2.5 cm deep.

The soil was removed at the 120th day after inoculation; it was followed by a wash in running water in order not to damage the roots. Five g of roots were sampled, which were put in identified plastic bags for further laboratory analysis. After the roots were removed, the plants were put back in plastic bags containing the same soil they were in before.

The roots were ground in a blender using sodium hypochlorite solution (0.5%), for 20 to 30 s, in low speed, to release the eggs, according to the procedure by Hussey & Barker (Hussey and Barker 1973). The roots were processed in order to assess the number of eggs and to further determine the reproduction factor (RF), which was defined by: $RF = \text{final egg population and juvenile/initial population of eggs and juvenile}$. The genotypes with $RF = 0$ were considered immune, the resistant ones showed $RF < 1.00$, and the susceptible ones showed $RF > 1.00$. The F2 population underwent a second assessment 240 days after inoculation.

Statistical analysis

The arrangement of adjustments between the observed and the expected frequencies, according to the hypotheses suggested for segregation in population F2, was done through chi-square test (χ^2) at 5% probability.

An estimate, in broad sense, for the heritability of reproduction factor was obtained through the formula $h^2 = (\sigma_{F_2}^2 - \sigma_{F_1}^2) / \sigma_{F_2}^2$. The estimate using this formula must be considered the maximum heritability,

since it includes variations resulting from the addictive and non-addictive effects.

Results

The adopted procedure to obtain the hybrid was efficient; it was confirmed by the genotyping of two microsatellite loci (Fig. 1). There were five fruits with hybrids out of the 25 manually-handled pollinations and it indicates 20% efficiency in the crossing between *P. guajava* x *P. guineense*. These crossing resulted in 355 seeds with 75% germination rate. The use of SSR markers to confirm the interspecific hybrids have been reported as a safe technique to confirm hybrids.

The female *P. guajava* progenitor presented totally necrotic roots after nine years of cultivation in the field and succumbed to nematode parasitism (Fig. 2) whereas the male *P. guineense* progenitor kept on showing resistance features to the nematode such as absence of the pathogen's eggs and juveniles in the root system, as well as the absence of primary or secondary symptoms in the shoot (Fig. 2). The presence of rare and tiny galls was observed in secondary roots and it indicates that the plant from this *P. guineense* accession is the source of resistance to the assessed nematode.

The F1 generation of *P. guajava* x *P. guineense* showed 242 plants with $RF = 0$; thus, they were considered immune; 28 plants had RF between 0.003 and 0.322; thus, they were considered resistant to the pathogen. There was tiny gall in 16 plants of the hybrid out of the 270 assessed F1 plants (Fig. 3). These results indicate the complete dominance of the male progenitor in the resistance expression to the nematode in F1 plants.

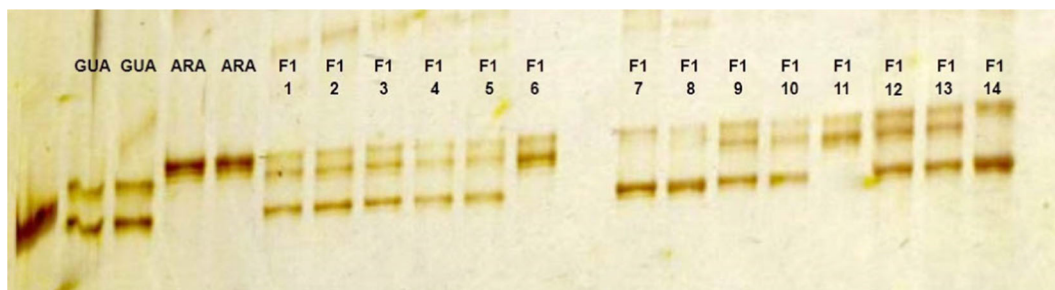


Fig. 1 *Psidium guajava* x *P. guineense* F1 sample: plants 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13 and 14 inherited the second *P. guineense* allele. F1–6 and F1–11 plants inherited the first *P. guineense* allele



Fig. 2 Roots from the maternal progenitor after nine years in the field (**a** and **b**), presenting highly susceptible to *M. enterolobii* and plants roots of the paternal progenitor after nine years in the field (**c** and **d**), presenting no primary and secondary symptoms of the nematode

Within the population of 183 F2 plants of *P. guajava* x *P. guineense*, there were 86 (47%) plants with galls and 97 (53%) with absence of it at the 120th day after inoculation (Fig. 3). A second assessment of this population was conducted at the 240th day after inoculation and it showed galls in 91 (49%) of them and the absence of them in 92 plants (51%). These rates are too close to each other in the two distinct assessments. The segregation for the presence or absence of galls in the root system of F2 plants of the hybrid was 9:7, and the χ^2 test values were 0.78 and 2.66, respectively, at the 120th and 240th days after inoculation (Table 1). These results indicate some epistasis types and the action of alleles of two genes to control the resistance to *M. enterolobii*. The RF varied from 0.0 to 4.13 at the 120th day and from 0.0 to 3.12 at the 240th day after inoculation in the F2 plants. The segregation for the reproduction factor (RF) of F2 plants of the hybrid was 15:1, and the χ^2 test values were 2.76 and 1.18, respectively, at the 120th and 240th days after inoculation (Table 1). These results also

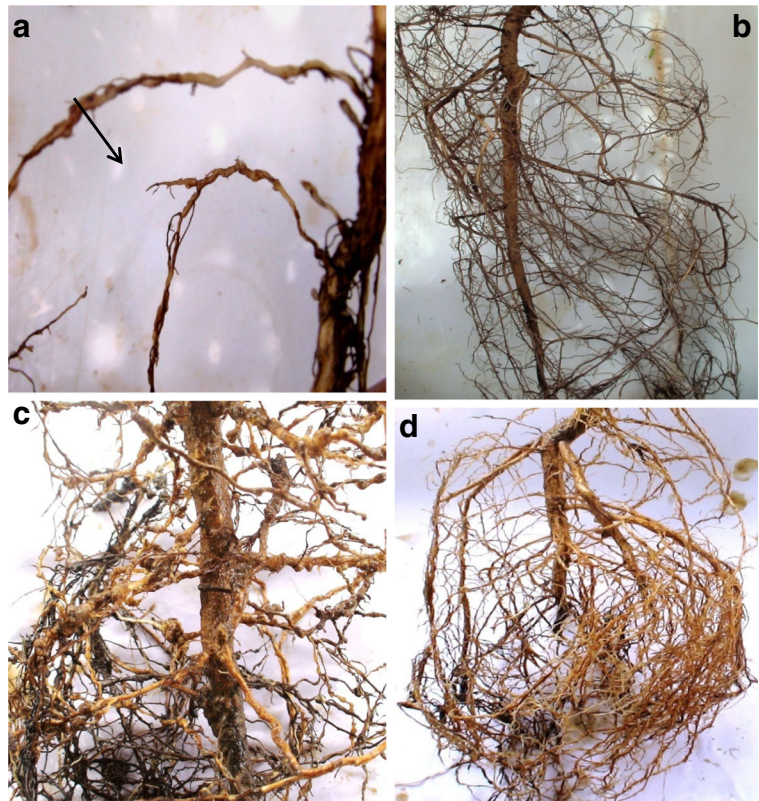
indicate some type of epistasis, and the action of alleles of two genes to control the resistance to *M. enterolobii*.

The reproduction factor heritability, in broad sense, estimated for the assessments conducted at the 120th and 240th days after inoculation was 0.97, and it indicates simple inheritance of resistance to *M. enterolobii* in guava plants. Cervigni et al. (2007) have reported broad heritability of 0.82 for *Pratylenchus* spp. in corn. These high heritability values corroborate the estimates found in the present study.

Discussion

Inheritance of the resistance to nematodes has been studied on many different crops, however to our knowledge, no data are available for guava species. The present research is the first that includes *Psidium* species and like Rubio-Cabetas et al. (1999) in *Prunus cerasifera* it considers the inheritance of resistance to *M. enterolobii*

Fig. 3 Roots of the F1 population from the crossing between *P. guajava* x *P. guineense* with the presence of tiny galls (a) and the absence of galls (b). Roots of the segregating population F2 with the presence of galls (c) and with the absence of galls (d)



(*M. mayeguensis*), a nematode of growing worldwide importance for many plants species.

The use of markers SSR was efficient for confirmation between *P. guajava* x *P. guineense*. Santos et al. (2012) conducted interspecific crossings between *Passiflora* L. species for ornamental purposes; these crossings have confirmed the hybridization through the use of SSR markers.

Costa and Santos (2013), in their study about the similarity between guava and wild *Psidium* species, through SSR markers, have reported 81.4% similarity between *P. guajava* and *P. guineense*. Negi and Rajan (2007) have reported a *P. guajava* x *P. molle* hybrid,

which is resistant to guava wilt an important disease in Asia and Africa. Landrum et al. (1995) have reported the existence of natural hybrids between *P. guajava* x *P. guineense*, based on morphological and chemical characters. Costa et al. (2012) have reported the difficulty to obtain hybrids among *P. guajava* and *P. friedrichstalianiem* and *P. cattleyanum*. These last *Psidium* species have been reported in the literature as resistant to *M. enterolobii* (Freitas et al. 2014). The obtainment of new sources of resistance to the pathogen may be used in the future, as well as may enable new segregation and resistance studies.

Table 1 Chi-square test (χ^2) for the presence (PG), absence (AG) of galls roots and the reproduction factor (RF), wherein FR < 1 and FR > 1 are susceptible in the F2 population of *Psidium guajava* x

P. guineense at the 120th and 240th days after inoculation with 10,000 *Meloidogyne enterolobii* eggs

Assessment	Observed				Expected				Theoretical ratio (R:S)		χ^2	
	PG	AG	FR <1	FR >1	PG	AG	FR <1	FR >1				
At 120	86	97	177	6	102.9	80.1	171.6	11.4	09:07	15:01	0.78	2.76
At 240	91	92	168	15	102.9	80.1	171.6	11.4	09:07	15:01	2.66	1.18

The presence of galls observed in *P. guajava* and *P. guineense* indicates that the paternal plant (*P. guineense*) is the source of resistance to nematodes. In a similar study, Negi and Rajan (2007) have reported that the source of resistance to *P. guajava* x *P. molle* guava plant wilt results from the wild plant species *P. molle*. These results indicate the importance of wild *Psidium* genetic pool as source of resistance to pathogens such as nematodes and bacteria. According to Carneiro et al. (2001), the primary symptom of nematodes in guava plants is the galls (size and amount) formed in the root system and the associated necrosis. It leads to the diminishing of roots and rootlets. The secondary symptom comprises the tanning in the edge of the leaves, which is followed by yellowing. It causes the total defoliation in the shoot and precedes plant death. With regard to the fruits, they lose their smooth and fresh look, ripen prematurely and present size smaller than the expected to commercialization (Moreira et al. 2003).

Nyczepir et al. (2008) have reported smaller number of *M. enterolobii* egg masses, eggs/plant and eggs/dry matter of root (g) in peach plants than in the root system of tomato plants, whereas the presence of galls varied from 3 to 81 in six peach genotypes, in comparison to the 100 genotypes found in tomato plants. According to these authors, the egg reproduction is a better parameter to assess resistance than the presence of galls. Differently from Nyczepir et al. (2008), the present study showed great compliance between the almost complete absence of galls and the presence of eggs and juveniles in the root system of the *Psidium* hybrid.

The segregation for the presence or absence of galls in the root system of F2 plants of the hybrid was 9:7, and the χ^2 test values were 0.78 and 2.66, respectively, at the 120th and 240th days after inoculation (Table 1) and the segregation for the reproduction factor (RF) of F2 plants of the hybrid was 15:1, and the χ^2 test values were 2.76 and 1.18, respectively, at the 120th and 240th days after inoculation (Table 1). These results indicate some epistasis types and the action of alleles of two genes to control the resistance to *M. enterolobii*. Fatobene (2014) selected coffee trees showing multiple resistance to nematodes belonging to genus *Meloidogyne* and reported the resistance of wild coffee trees to *M. paranaenses*, which may be attributed to the expression of a major dominant gene, thus segregating to three resistant ones, one susceptible or two complementary dominant genes, at the ratio of nine

resistant and seven susceptible in F2 generation. Vinholes (2014) reports that the soybean resistance to the nematode *M. javanica* is controlled by two genes that have combined effects, supporting the resistance model controlled by two recessive genes (aabb) with epistatic effect and segregation 12:3:1.

According to such RF data, just one dominant allele, from any of the two genes, is the condition for the resistance to *M. enterolobii* in the present study, since the egg reproduction is a better parameter to assess resistance than the presence of galls.

Conclusion

The segregation results shown in the present study and the heritability estimates, in broad sense, have supported the dominant resistance model controlled by two genes, with epistatic effects. The presence of only one dominant allele is the condition for the resistance of the *P. guajava* x *P. guineense* hybrid to *M. enterolobii*. New *P. guajava* x other wild *Psidium* must be developed and assessed in order to enlarge the sources of resistance to the pathogen, fact that makes it possible having the effective control of such pathogen in commercial areas of guava plants.

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