

Inheritance of resistance to *Meloidogyne enterolobii* and individual selection in segregating populations of *Psidium* spp

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Abstract Interaction between the phytonematode *Meloidogyne enterolobii* and the fungus *Fusarium solani* has caused direct and indirect losses in the entire guava production chain and consequent extermination of guava plantations throughout Brazil. The combined action of these two pathogens is known as “guava decline”. In order to obtain and assess *Psidium* spp. interspecific hybrids for resistance to the nematode *M. enterolobii*, interspecific crosses of *P. guineense* (susceptible araçá) x *P. cattleianum* (resistant araçá); *P. guineense* (susceptible araçá) x *P. guajava* (susceptible guava) and *P. cattleianum* (resistant araçá) x *P. guajava* (susceptible guava) were conducted. These crosses resulted in hybrid immune, susceptible and resistant to *Meloidogyne enterolobii*. The chi-square test rejected the hypothesis of monogenic inheritance with incomplete dominance, which corroborates that this trait has polygenic action. Predictions of genetic values and parameters were obtained by the REML / BLUP procedure, at individual level. Finally, the 30 selected individuals (immune and resistant) were obtained, which will be backcrossed with guava for the recovery of the

agronomic traits desired and subsequent release of a new cultivar.

Keywords Mixed models · Genetic breeding · Interspecific hybrids · *Fusarium solani* · *Psidium guajava* · Guava decline · Root-knot nematodea

Introduction

Brazil is one of the world largest producers of red guava (*Psidium guajava* L.), with 15,2 thousand hectares of planted area and average yield of 22.7 t ha⁻¹. The annual production of guavas in the country is approximately 345.000 tons with production value of 331.9 million reais (IBGE 2014). Guava is grown in commercial orchards throughout the national territory, from South to Northern Brazil (Natale et al. 2009). Currently, some of the main producing centers are developing countries, which lack the resources for adequate scientific research programs aimed at the effective technological advance for the culture of guava, namely: Brazil, Mexico, India, China, Pakistan and South Africa (Pereira and Kavati 2011).

The genus *Psidium* has about 150 species, including the main species *P. guajava* L. (guava, 2n = 22), *P. cattleianum* Sabine (sweet araçá, araçá de praia, araçá-de-coroa) and *P. guineense* Swartz or *P. araçá* Raddali (true araçá or acid araçá) (Pereira 1995; Pommer et al. 2006). Brazil concentrates much of the diversity of this genus, 61 species, 45 of which are endemic, distributed throughout the territory,

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mainly in the central and south-eastern regions. Some species are also distributed in northern South America, the West Indies, North America, the Andes and Southern Brazil (Pommer et al. 2006, Sobral et al. 2015).

In Brazil, “guava decline” decimated many orchards. In this complex disease, parasitism due to the phytonematode *M. enterolobii* predisposes guava plants immune to *Fusarium solani* to the extensive root rot caused by this fungus. This leads to nutritional deficiencies, chlorosis, tip burn and falling leaves, sharp fall in productivity and plant death. This is an irreversible process that lasts only a few months (Gomes et al. 2008; Gomes et al. 2014).

The synergistic effect of this disease is corroborated by the results of experiments in vases indicating that *M. enterolobii* is not highly aggressive to guava when it attacks alone (Gomes et al. 2008, 2011; Almeida et al. 2011; Gomes et al. 2014). Bioassays with *F. solani* isolates from different regions of Brazil conducted in growth chamber confirmed that “guava decline” is the causal agent of the destruction of about 5000 ha of commercial guava. This results in huge economic impact for producers, since the losses were estimated in more than US \$ 70 million (Pereira et al. 2009; Gomes et al. 2014).

Several strategies for the control or management of this disease have been investigated, but no short-term solution has been achieved yet (Freitas et al. 2014; Freitas et al. 2016a).

One alternative to fight the disease can be the use of species of *Psidium sp.* araçá trees for the introgression of resistance genes in susceptible commercial cultivars. Araçás are sources of resistance to the nematode and can be successfully crossed with guava. They are a more advantageous alternative to avoid guava decline in commercial orchards (Carneiro et al. 2007; Almeida et al. 2009; Miranda et al. 2012; Martins et al. 2013).

It is therefore essential to study the *Psidium spp.* inherited resistance to *M. enterolobii*, mainly due to: i) the relevance of scientific research on resistance against this disease, compared with other diseases of annual and perennial crops involving nematodes, ii) the significance of scientific and practical studies involving *M. enterolobii* per se, since this nematode is considered a threat to agriculture throughout the world, because of its wide range of hosts and virulence multiple resistance genes effective against other species of *Meloidogyne sp.*, and iii) the understanding of the mechanisms involved in nematode resistance may allow breeders and nematologists to find new strategies for the control or management of guava decline.

Interspecific crosses are generally used in genetic breeding programs, because they generate segregating populations with high genetic variability, which increases selection efficiency in these generations. Selection success depends not only on the variability of the experimental material, but also on the accuracy of the methods used. Thus, accurate analytical methods must be employed to estimate the variance components and allow the prediction of the individual genetic values of future selection candidates (Santos et al. 2015; Borges et al. 2010).

Therefore, the mixed models methodologies can be used as an optimal selection procedure for improving the accuracy of the selection process. This methodology is used to estimate the components of variance by the Restricted Maximum Likelihood - REML and predict genotypic values by the Best Linear Unbiased Prediction - BLUP (Resende 2002; Alves and Resende 2008).

The REML / BLUP methodology has been increasingly adopted in plant breeding, especially in perennials, such as rubber (Kalil et al. 2000), eucalyptus (Rocha et al. 2006), coffee (Petek et al. 2008), acerola (Paiva et al. 2002), cupuaçu (Alves and Resende 2008), papaya (Oliveira et al. 2012), açaí (Teixeira et al. 2012) and passion fruit (Santos et al. 2015).

Thus, this work aimed to: i) obtain and assess interspecific hybrids of *Psidium spp.* for resistance to the nematode *M. enterolobii*; ii) carry out a study on disease resistance heritage; iii) estimate genetic parameters and obtain the genetic value for the traits evaluated using the REML / BLUP mixed models methodology; and iv) carry out selection at plant level within progeny, seeking to identify superior genotypes obtained from interspecific cross between *P. guajava* and *Psidium sp.* Thus, superior individuals selected for resistance to *M. enterolobii* can be backcrossed with guava in future stages of the Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF guava breeding program in order to obtain cultivars resistant to the disease and make them available to new farmers.

Material and methods

Genetic material

Nine hundred and seventeen interspecific hybrids, obtained from crosses using *P. guajava*, *P. cattleyanum*, and *P. guinensis* from the UENF genetic breeding program, were assessed. These parents were chosen for

their known resistance and/or susceptibility to *M. enterolobii* (Miranda et al. 2012). The araçá *P. cattleyanum*, parent resistant to *M. enterolobii*, and the araçá *P. guineensis*, parent susceptible, was used to generate segregating populations for conducting the mapping in further steps of the program. The genotypes of *P. guajava* were also susceptible. The hybridizations were performed in greenhouse using: *P. guineense* (P36) x *P. cattleyanum* (P11); *P. guajava* (13.2II) x *P. cattleyanum* (CV4); *P. guajava* (13.4II) x *P. cattleyanum* (P33); *P. guajava* (13.4II) x *P. cattleyanum* (P53); *P. cattleyanum* (CV8) x *P. guineense* (CV11); and *P. cattleyanum* (CV1) x *P. guineense* (CV11).

P. guineense (P36), *P. guajava* (13.2II), *P. guajava* (13.4II), *P. cattleyanum* (CV8), and *P. cattleyanum* (CV1) were used as female parents (Table 1).

Interspecific hybridization

The plants of all accessions were pruned for synchronized floral induction in order to enable the performance of manual crosses. The flower buds of the female parents were emasculated in the pre-anthesis stage. Emasculation is the removal of petals, sepals and anthers. The flowers of the male parents (araçá trees) were always collected on the day of pollination and were placed in a petri dish.

Emasculation was held on the day pollination occurred. Donor flowers were macerated by hand on a Petri dish, and the pollen of the male parent was deposited and distributed across the surface of the stigma. After pollination, the buttons were labeled with the IDs of the parents and protected with TNT bags to prevent contamination, either by wind or pollinators. The developing fruits were monitored and then

Table 1 Genotypes *P. guineense*, *P. cattleyanum* and *P. guajava* were used in interspecific crosses to obtain segregating populations of *Psidium spp*

| Code | Progenies |
|--|---|
| P36 ¹ x P11 ² | (<i>P. guineense</i> x <i>P. cattleyanum</i>) |
| 13.2II ³ x CV4 ² | (<i>P. guajava</i> x <i>P. cattleyanum</i>) |
| 13.4II ³ x P33 ² | (<i>P. guajava</i> x <i>P. cattleyanum</i>) |
| 13.4II ³ x P53 ² | (<i>P. guajava</i> x <i>P. cattleyanum</i>) |
| CV8 ² x CV11 ¹ | (<i>P. cattleyanum</i> x <i>P. guineense</i>) |
| CV1 ² x CV11 ¹ | (<i>P. cattleyanum</i> x <i>P. guineense</i>) |

1 and 3 = Susceptible genotypes to *M. enterolobii* (Miranda et al. 2012); 2 = Resistant genotypes *M. enterolobii* (Miranda et al. 2012)

harvested, when physiologically mature. The seeds were counted and placed in a paper bag properly identified for storage in a freezer.

Sowing and conductance of the experiment

Sowing was performed in 128-cell germination trays containing artificial substrate (Plantimax®). Then, the trays were kept in a nebulization chamber until seed germination, which occurred between 22 and 30 days.

To characterize the resistance heritage, the H1 generation seedlings were conducted under greenhouse conditions, transplanted to 5-L plastic containers that received one seedling per unit, with properly fertilized substrate, and maintained in a greenhouse.

Inoculum

A pure isolate of *M. enterolobii* was maintained in tomato plants. To prepare the inoculum, the method proposed by Cotter et al. (2003) was modified: parasitized roots were put into 1-L flasks filled with 500 mL of water. The flasks were stirred in mixer (Tecnal® TE240 model) for 4 min. The suspension was sieved in 100 and 500 mesh sieves and the nematode eggs were retained in the latter.

The seedlings were inoculated at the stage of four to six pairs of leaves. Each seedling received 10 mL of suspension with 1000 eggs distributed in four holes around the stem. The guava tree cv Paluma, known to be susceptible to *M. enterolobii* (Burla et al. 2010), was used as a reference in all lots to ensure the viability of the inoculum and inoculation method. One hundred and thirty-five days after inoculation, the assessments were performed, as proposed by Miranda et al. (2009): for the extraction of eggs and second stage juveniles (J₂), the plants had half their root systems extracted and processed as described above. The only modification was shaking the roots in sanitary water aqueous solution (sodium hypochlorite 2%) 6%, instead of pure water. The remaining half of the roots of the plants resistant to nematodes was replanted in pots maintained in greenhouse for their preservation.

The suspension of eggs and J₂ obtained from each plant was homogenized and three 1 ml aliquots were used for counting in Peter's laminas. The counts were multiplied by two – since only half the root system was processed - and expressed as final nematode population (Pf). The final classification of plants for resistance to nematodes was based on the reproduction factor

(RF = Pf/1000) sensu Oostenbrink (1966): FR = 0 = immune, FR <1 = resistant and FR > 1 = susceptible.

Resistance inheritance analysis

The study on inheritance was based on the evaluation of the reproduction factor, which resulted in three classes (K): immune, resistant and susceptible. We used the chi-square test (χ^2), 5% probability to adjust the proportions observed to those expected based on the hypothesis of monogenic inheritance with incomplete dominance (1:2:1) for resistance control. The calculated chi-square (χ^2_c) was estimated using the Genes software system (Cruz 2013), by the following statistics:

$$\chi^2_f = \frac{(O_i - E_i)^2}{E_i}$$

Where:

- O_i number of individuals observed in the i -th phenotypic class;
- E_i expected number of individuals in the i -th phenotypic class; and
- K number of phenotypic classes.

Mixed model for evaluation and selection of plants and estimation of genetic parameters

The analysis was performed using the Selegen-REML / BLUP software system (Resende 2002), based on the following statistical model $y = Xr + Zg + Wp + e$ where y is the vector of data; r is vector of repetition effects (here assumed as fixed) added to the overall average; g is the vector of individual genetic effect (here assumed as random); p is the vector of the plot effects (random); and e is the vector of error (random). Upper case letters refer to incidence matrices for the effects mentioned above. The following variance components were estimated (individual REML):

- σ^2_g Individual genotypic variance;
- σ^2_f Individual phenotypic variance;
- h^2_a Individual narrow-sense heritability;
- h^2_{mp} Heritability based on the average progeny;
- Acprog Accuracy in the selection of progeny.

Results

Achievement of segregating population

Three hundred eighty-six *Psidium spp.* interspecific crosses were performed, with average fruit set rate of 13.44% (Table 2). The CV8 x CV11 cross presented the highest fruit set (40%). For the crosses P36 x P11, 13.2II x CV4 and 13.4II x P33, only one fruit was obtained for each cross, while the cross 13.4II X P53 provided only two. CV1 X CV11 presented 24.13% of fruit set in the crosses performed. However, despite such low fruit set rate, a considerable amount of seeds was obtained (1835), with average percentage germination of 71.9%. In the cross between 13.4II and P33, for example, only one fruit was obtained, but it contained 335 seeds, out of which 257 germinated, totaling 76.7% germination.

The crosses P36 X P11 and CV8 X CV11, both hybridizations between *P. cattleianum* and *P. guineense*, showed the lowest and highest fruit set rate, 0.5 and 40%, respectively.

Low fruit set rate was observed for the crossing of greater interest (guava x araçá), 5, 5, and 6% for 13.2II X CV4; 13.4II X P33; and 13.4II X P53, respectively. However, the germination percentage of the seeds derived from these crosses was high, 86.1, 76.7 and 87.6%, for the same crosses, respectively.

Resistance inheritance

The hypothesis of monogenic inheritance with incomplete dominance (three classes: immune, resistant and susceptible) for resistance to *M. enterolobii* was not confirmed by the segregation observed in the evaluated crosses. The number of resistant and susceptible individuals obtained from the crosses was significantly different from the number expected ($X^2 > 5.91$; G.L. 2; $p > 0.05$) (Table 3).

Genetic parameter estimation via mixed models

In the crosses assessed, the phenotypic variance values were higher than those of the respective genotypic variances. The phenotypic variance values ranged from 1.47 to 3900.96 (Table 4). The genotypic variances, in turn, ranged from 0.15 to 390.09.

The narrow sense individual heritability estimate for the trait RF was 20% in all interspecific crosses performed. In this study, the accuracy values ranged from 75 to 81%.

Table 2 Number of crosses conducted, rate of fruit set (%), number of fruits, number of seeds, and percentage of germination of the *Psidium* spp. interspecific crosses

| Parents | Number of crosses conducted | Fruit set rate (%) | Number of fruits | Number of seeds | Germination (%) |
|--------------|-----------------------------|--------------------|------------------|-----------------|-----------------|
| P36 X P11 | 200 | 0.50 | 1 | 297 | 66.7 |
| 13.2II X CV4 | 20 | 5.00 | 1 | 101 | 86.1 |
| 13.4II X P33 | 20 | 5.00 | 1 | 335 | 76.7 |
| 13.4II X P53 | 34 | 6.00 | 2 | 394 | 87.6 |
| CV8 X CV11 | 25 | 40.00 | 10 | 298 | 67.1 |
| CV1 X CV11 | 87 | 24.13 | 21 | 410 | 47.6 |
| Average | | 13.44 | | | 71.9 |
| Total | 386 | | 36 | 1835 | |

Genotype selection and genetic gain estimation via BLUP

The thirty best genotypes for each cross were selected for smaller factor of reproduction of *M. enterolobii*. Thus, for the selected individuals, the nematode reproduction factor ranged from 0 to 88 in the crosses. In the interspecific guava x araçá (*P. guajava* x *P. cattleyanum*) crosses, the reproduction factor of *M. enterolobii* ranged from 0 to 36.80 for 13.2II x CV4; from 0 to 3.60, for 13.4II x P33; and from 0 to 88 for 13.4II x P53 (Table 5). Considering the interspecific araçá x araçá (*P. cattleyanum* x *P. guineense*) crosses, the reproduction factor ranged from 0 to 0.60 for P36 x P11; from 0 to 0.08 for CV1 x CV11; and from 0 to 2.40 for CV8 x CV11. In general, the CV1 x CV11 cross presented the lowest values for the reproduction factor and the 13.4II x P53 cross, the highest (Table 6).

The gains obtained in the guava (*P. guajava*) x araçá (*P. cattleyanum*) cross ranged from 0 to 52.73% for the 13.2II x CV4 cross; from 0 to 8.07% for 13.4II x P33; and from 0 to 1.18% for 13.4II x P53 (Table 5). The best genotypes among those selected for the crosses 13.2II x CV4; 13.4II x P33 and 13.4II x P53 were, respectively, 25, 62 and 121, and both presented zero for gain and reproduction factor.

For the araçá (*P. cattleyanum*) x araçá (*P. guineense*) crosses, in the P36 x P11 cross, the gain ranged from 0 to 1.83%; for CV1 x CV11, from 0 to 2.88%; for CV8 x CV11, from 0 to 11.15% (Table 6). In progenies of the araçá x araçá cross, those from the cross between P36 and P11 stood out, with maximum gain of 1.83%. Genotypes 253 and 91 stood out among those selected for P36 x P11 and CV8 x CV11, respectively, with gain equal to zero. As for CV1 x CV11 cross, the genotypes

86, 82, 53, 51, 48, 36, 33 and 29 obtained gain equal to zero.

Discussion

Resistance to Meloidogyne

The low fruit set rate of the crosses is probably due to incompatibility caused by the pre and/or post-fertilization barrier. The pre-fertilization incompatibility barriers result from the delayed or inhibited growth of the pollen tubes and lack of pollen grain germination. After fertilization, the main barriers are the embryo death due to the degeneration of the endosperm and total or partial sterility of hybrid plants (Van Creijl et al. 1997). In studies on resistance to *F. solani* in the segregating populations obtained by interspecific crosses between *Passiflora edulis* and *Passiflora mucronata*, Freitas et al. (2016b) obtained a variable number of crosses, with hybrids showing different fruit set rates. According to the authors, the greater number of fruits and seeds obtained in the cross in which *P. mucronata* was used as female parent was due to differences in the size and shape of the reproductive system structure, since the pollen grains of *P. edulis* are larger and in great amount, when compared to those of *P. mucronata*, which favors this crossing via. The same was not observed in this study.

Freitas et al. (2015) studied the resistance to *Cowpea aphid-borne mosaic virus* (CABMV) in segregating populations obtained by crossing *P. edulis* and the hybrid H5–14 (*P. edulis* x *P. setacea*). For such populations obtained in two different ways (crosses and reciprocals), it was observed 1.35% of fruit set, and 77% of fruit set for reciprocals. The authors report that the hybrid used at crosses may have presented evidence of male sterility

Table 3 Chi-square between expected and observed number of resistant, immune and susceptible classes in *Psidium spp.* progenies, assuming monogenic inheritance with incomplete dominance ($GL = 2$)

| Cross | Phenotypic Class | Fe | Fo | χ^2_{calc} |
|--------------|------------------|--------|-----|------------------------|
| P36 X P11 | Immune | 63.25 | 49 | 3.2104 |
| | Resistant | 126.50 | 54 | 41.5513 |
| | Susceptible | 63.25 | 150 | 118.9812 |
| | | | | 163.7430 |
| 13.2II X CV4 | Immune | 7.50 | 7 | 0.0333 |
| | Resistant | 15.0 | 7 | 4.2666 |
| | Susceptible | 7.50 | 16 | 9.6333 |
| | | | | 13.9333 |
| 13.4II X P33 | Immune | 20.0 | 9 | 6.0500 |
| | Resistant | 40.0 | 34 | 0.9000 |
| | Susceptible | 20.0 | 37 | 14.4500 |
| | | | | 21.4000 |
| 13.4II X P53 | Immune | 71.75 | 9 | 54.8789 |
| | Resistant | 143.50 | 12 | 120.5034 |
| | Susceptible | 71.75 | 266 | 525.8963 |
| | | | | 701.2787 |
| CV8 X CV11 | Immune | 22.75 | 17 | 1.4532 |
| | Resistant | 45.5 | 70 | 13.1923 |
| | Susceptible | 22.75 | 4 | 15.4532 |
| | | | | 30.0989 |
| CV1 X CV11 | Immune | 44.0 | 33 | 2.7500 |
| | Resistant | 88.0 | 63 | 7.1022 |
| | Susceptible | 44.0 | 80 | 29.4545 |
| | | | | 39.3068 |

$$\chi^2_{\text{tab}} = 5.991$$

(Freitas et al. 2015). Due to the lack of research on *Psidium spp.*, further studies are needed to clarify the mechanisms that affect the fruit set rate of interspecific crosses between guava and araçá. Pre or post-fertilization barriers may be affecting the performance of these crossings.

No evidence of monogenic inheritance was observed in all populations obtained in the present study. All values obtained establish that multiple alleles may affect the control of resistance to *Meloidogyne enterolobii*. Similar results can be found in other perennial plants (Junghans et al. 2003; Dumsday et al. 2003; Acosta-Leal and Xiong 2008; Bastiaanse et al. 2015; Naresh et al. 2016). Different results of this study were reported by Costa et al. (2012). The authors assessed resistance to *M. enterolobii* in hybrid guava and araçá and that resistance to the nematode is simply inherited with alleles

Table 4 Estimates of the components of the genotypic variance among progenies of full siblings (σ_g^2), individual phenotypic variance (σ_f^2), narrow sense individual heritability (h_a^2), progeny selection accuracy (Acprog) obtained by the REML procedure, for the reproduction factor (RF) in crosses of species of *Psidium spp.* Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, 2014/2015

| Cross | σ_g^2 | σ_f^2 | h_a^2 | Acprog |
|--------------|--------------|--------------|--------------|--------|
| P36 X P11 | 390.09 | 3900.96 | 0.20 + -0.11 | 0.81 |
| 13.2II X CV4 | 14.19 | 141.87 | 0.20 + -0.33 | 0.75 |
| 13.4II X P33 | 12.13 | 121.35 | 0.20 + -0.20 | 0.79 |
| 13.4II X P53 | 241.43 | 2414.36 | 0.20 + -0.10 | 0.81 |
| CV8 X CV11 | 230.11 | 2301.14 | 0.20 + -0.19 | 0.79 |
| CV1 X CV11 | 0.15 | 1.47 | 0.20 + -0.13 | 0.80 |

σ_g^2 = genotypic variance; σ_f^2 = phenotypic variance; h_a^2 = narrow sense heritability; Acprog = accuracy in progeny selection

displaying a dominance effect. However, inconsistent data and chi-square could not be obtained because of the small number of hybrid plants assessed (10 plants). The suggested establishment of a strategy to obtain segregating populations, aimed at resistance to various types of diseases, focused on obtaining populations with high effective number, can increase the chances to obtain individuals with resistance alleles.

Estimates of genetic parameters are essential for breeding programs, since they help plant breeders making decisions about the breeding method, conductance and selection (Amaral et al. 2009). High values for phenotypic variance are expected in segregating populations, whose quantitative inheritance traits present continuous phenotype distribution. The trait evaluated, reproduction factor (RF), is a polygenic trait greatly affected by environmental change, which contributes to the high levels of the phenotypic variance obtained. A similar result was found by Santos et al. (2015) in the study on the segregating population from the interspecific cross of *Passiflora spp.* whose phenotypic variance values were high. The authors attributed these results to a strong environmental effect on the traits evaluated.

The narrow sense heritability refers to the fraction of the genotypic differences between the parents, which are expected to be recovered among the descendants (Gonçalves et al. 2007). Thus, the narrow-sense heritability was used in this study to calculate the selection gains, since broad-sense heritability takes the total genetic variation into account, which is only partly transmitted to offspring.

For the trait RF, the estimate of the narrow sense individual heritability was ranked as medium magnitude, according to Farias Neto et al. (2013).

Table 5 Genetic gain and new predicted averages for reproduction factor estimated by REML/BLUP in *P. guajava* x *P. cattleyanum* crosses. Universidade Estadual do Norte Fluminense Darcy Ribeiro. Campos dos Goytacazes. RJ. 2014/2015

| Order | 13.2II x CV4 | | | | 13.4II x P33 | | | | 13.4III x P53 | | | |
|---------|--------------|-------|----------|-------------|--------------|------|----------|-------------|---------------|-------|----------|-------------|
| | Genotype | FR | Gain (%) | New Average | Genotype | FR | Gain (%) | New Average | Genotype | FR | Gain (%) | New Average |
| 1 | 25 | 0.00 | 0.00 | 6.59 | 62 | 0 | 0.00 | 5.70 | 121 | 0 | 0.00 | 112.16 |
| 2 | 24 | 0.00 | 0.61 | 6.64 | 59 | 0 | 0.18 | 5.72 | 115 | 0 | 0.04 | 112.20 |
| 3 | 20 | 0.00 | 1.21 | 6.68 | 57 | 0 | 0.35 | 5.73 | 111 | 0 | 0.07 | 112.24 |
| 4 | 16 | 0.50 | 1.82 | 6.72 | 53 | 0 | 0.70 | 5.74 | 98 | 0 | 0.12 | 112.28 |
| 5 | 26 | 0.60 | 2.58 | 6.77 | 51 | 0 | 0.88 | 5.75 | 91 | 0 | 0.15 | 112.33 |
| 6 | 21 | 0.80 | 3.33 | 6.82 | 56 | 0.04 | 1.05 | 5.77 | 84 | 0 | 0.19 | 112.37 |
| 7 | 17 | 1.30 | 4.09 | 6.87 | 48 | 0.04 | 1.40 | 5.78 | 83 | 0 | 0.23 | 112.42 |
| 8 | 22 | 1.40 | 4.85 | 6.92 | 43 | 0.08 | 1.58 | 5.80 | 82 | 0 | 0.27 | 112.46 |
| 9 | 30 | 1.80 | 5.76 | 6.98 | 49 | 0.16 | 1.93 | 5.81 | 81 | 0 | 0.31 | 112.50 |
| 10 | 29 | 2.40 | 6.67 | 7.04 | 52 | 0.2 | 2.11 | 5.82 | 80 | 0 | 0.35 | 112.55 |
| 11 | 12 | 0.00 | 7.58 | 7.10 | 42 | 0.2 | 2.46 | 5.84 | 79 | 0 | 0.39 | 112.59 |
| 12 | 7 | 0.00 | 8.48 | 7.16 | 41 | 0.24 | 2.63 | 5.86 | 61 | 0 | 0.43 | 112.64 |
| 13 | 3 | 0.00 | 9.39 | 7.22 | 46 | 0.44 | 2.98 | 5.87 | 55 | 0 | 0.47 | 112.68 |
| 14 | 1 | 0.00 | 10.45 | 7.29 | 60 | 0.48 | 3.16 | 5.89 | 63 | 0.16 | 0.51 | 112.73 |
| 15 | 13 | 0.20 | 11.52 | 7.36 | 54 | 0.48 | 3.51 | 5.90 | 64 | 0.2 | 0.55 | 112.78 |
| 16 | 9 | 0.40 | 12.88 | 7.44 | 58 | 0.96 | 3.86 | 5.92 | 60 | 0.32 | 0.60 | 112.82 |
| 17 | 10 | 0.60 | 14.24 | 7.54 | 75 | 1 | 4.04 | 5.94 | 89 | 0.4 | 0.63 | 112.87 |
| 18 | 5 | 0.80 | 15.91 | 7.65 | 73 | 1.04 | 4.39 | 5.95 | 62 | 0.4 | 0.68 | 112.92 |
| 19 | 6 | 1.00 | 17.73 | 7.77 | 44 | 1.08 | 4.74 | 5.97 | 57 | 0.48 | 0.72 | 112.97 |
| 20 | 2 | 1.25 | 20.00 | 7.91 | 61 | 1.32 | 4.91 | 5.99 | 65 | 0.6 | 0.77 | 113.01 |
| 21 | 11 | 1.70 | 22.42 | 8.08 | 67 | 1.36 | 5.26 | 6.01 | 48 | 0.6 | 0.80 | 113.06 |
| 22 | 8 | 2.80 | 25.61 | 8.28 | 63 | 1.96 | 5.61 | 6.03 | 53 | 0.75 | 0.85 | 113.11 |
| 23 | 18 | 10.20 | 29.09 | 8.52 | 65 | 2.12 | 5.96 | 6.04 | 76 | 0.8 | 0.89 | 113.16 |
| 24 | 14 | 13.60 | 32.88 | 8.77 | 66 | 2.36 | 6.32 | 6.06 | 114 | 1.00 | 0.94 | 113.21 |
| 25 | 23 | 21.00 | 35.45 | 8.94 | 72 | 2.56 | 6.67 | 6.08 | 85 | 1.00 | 0.98 | 113.26 |
| 26 | 4 | 16.80 | 37.88 | 9.10 | 64 | 3.6 | 6.84 | 6.09 | 67 | 2.00 | 1.03 | 113.31 |
| 27 | 28 | 27.80 | 41.52 | 9.34 | 38 | 0 | 7.19 | 6.12 | 54 | 2.2 | 1.07 | 113.36 |
| 28 | 15 | 24.20 | 43.48 | 9.47 | 33 | 0 | 7.54 | 6.13 | 87 | 4.00 | 1.11 | 113.41 |
| 29 | 27 | 30.00 | 46.21 | 9.65 | 29 | 0 | 7.72 | 6.15 | 227 | 88.00 | 1.15 | 113.46 |
| 30 | 19 | 36.80 | 52.73 | 10.08 | 24 | 0 | 8.07 | 6.17 | 152 | 88.00 | 1.18 | 113.48 |
| Average | 6.59 | | | | 5.70 | | | | 112.16 | | | |

Fehr (1987) states that the effectiveness of breeding is greater when the magnitude of the coefficient of heritability for the trait under study is known, since it assist defining the selection strategies and predicting genetic gain. As observed in this study (Table 4), other authors also report that most quantitative traits of economic importance present around 20% narrow sense individual heritability (Farias Neto and Resende 2001; Soh et al.

2003; Farias Neto et al. 2008; Lopes et al. 2012; Farias Neto et al. 2013). Thus, the magnitude of heritability for the trait under study is within the expected range.

The accuracy values in the present study were considered high (Table 4). The genotypic assessment quality should be primarily inferred according to its accuracy. Accuracy values higher than 70% are good enough to indicate precise inference about the genetic value of

Table 6 Genetic gain and new predicted averages for reproduction factor estimated by REML/BLUP in *P. cattleyanum* x *P. guineense* crosses. Universidade Estadual do Norte Fluminense Darcy Ribeiro. Campos dos Goytacazes. RJ. 2014/2015

| Order | P36 x P11 | | | | CV1 x CV11 | | | | CV8 x CV11 | | | |
|---------|-----------|------|----------|-------------|------------|------|----------|-------------|------------|------|----------|-------------|
| | Genotype | FR | Gain (%) | New Average | Genotype | FR | Gain (%) | New Average | Genotype | FR | Gain (%) | New Average |
| 1 | 253 | 0.00 | 0.00 | 20.75 | 86 | 0.00 | 0.00 | 1.04 | 91 | 0.00 | 0.00 | 10.85 |
| 2 | 239 | 0.00 | 0.05 | 20.76 | 82 | 0.00 | 0.00 | 1.04 | 89 | 0.00 | 0.28 | 10.88 |
| 3 | 238 | 0.00 | 0.10 | 20.77 | 53 | 0.00 | 0.00 | 1.04 | 86 | 0.00 | 0.55 | 10.91 |
| 4 | 237 | 0.00 | 0.14 | 20.78 | 51 | 0.00 | 0.00 | 1.04 | 82 | 0.00 | 0.83 | 10.94 |
| 5 | 236 | 0.00 | 0.24 | 20.79 | 48 | 0.00 | 0.00 | 1.04 | 81 | 0.00 | 1.11 | 10.97 |
| 6 | 235 | 0.00 | 0.29 | 20.81 | 36 | 0.00 | 0.00 | 1.04 | 73 | 0.00 | 1.38 | 11.00 |
| 7 | 234 | 0.00 | 0.34 | 20.82 | 33 | 0.00 | 0.00 | 1.04 | 61 | 0.00 | 1.75 | 11.04 |
| 8 | 233 | 0.00 | 0.39 | 20.83 | 29 | 0.00 | 0.00 | 1.05 | 59 | 0.00 | 2.03 | 11.07 |
| 9 | 232 | 0.00 | 0.43 | 20.84 | 26 | 0.00 | 0.96 | 1.05 | 58 | 0.00 | 2.30 | 11.11 |
| 10 | 231 | 0.00 | 0.53 | 20.86 | 22 | 0.00 | 0.96 | 1.05 | 56 | 0.00 | 2.67 | 11.14 |
| 11 | 230 | 0.00 | 0.58 | 20.87 | 20 | 0.00 | 0.96 | 1.05 | 55 | 0.00 | 3.04 | 11.18 |
| 12 | 229 | 0.00 | 0.63 | 20.88 | 15 | 0.00 | 0.96 | 1.05 | 54 | 0.00 | 3.32 | 11.22 |
| 13 | 228 | 0.00 | 0.67 | 20.89 | 4 | 0.00 | 0.96 | 1.05 | 50 | 0.00 | 3.69 | 11.25 |
| 14 | 227 | 0.00 | 0.77 | 20.91 | 71 | 0.01 | 0.96 | 1.05 | 88 | 0.06 | 4.06 | 11.29 |
| 15 | 209 | 0.00 | 0.82 | 20.92 | 35 | 0.02 | 0.96 | 1.05 | 57 | 0.12 | 4.42 | 11.33 |
| 16 | 249 | 0.06 | 0.87 | 20.93 | 30 | 0.04 | 0.96 | 1.05 | 90 | 0.18 | 4.79 | 11.37 |
| 17 | 245 | 0.06 | 0.96 | 20.94 | 27 | 0.04 | 0.96 | 1.05 | 52 | 0.18 | 5.16 | 11.42 |
| 18 | 251 | 0.12 | 1.01 | 20.96 | 28 | 0.06 | 0.96 | 1.05 | 51 | 0.24 | 5.62 | 11.46 |
| 19 | 250 | 0.12 | 1.06 | 20.97 | 37 | 0.08 | 1.92 | 1.06 | 76 | 0.42 | 5.99 | 11.50 |
| 20 | 246 | 0.12 | 1.16 | 20.98 | 24 | 0.08 | 1.92 | 1.06 | 48 | 0.42 | 6.36 | 11.55 |
| 21 | 252 | 0.18 | 1.20 | 21.00 | 12 | 0.08 | 1.92 | 1.06 | 84 | 0.60 | 6.82 | 11.60 |
| 22 | 244 | 0.18 | 1.25 | 21.01 | 176 | 0.00 | 1.92 | 1.06 | 83 | 0.66 | 7.28 | 11.64 |
| 23 | 243 | 0.18 | 1.35 | 21.02 | 175 | 0.00 | 1.92 | 1.06 | 53 | 0.66 | 7.74 | 11.69 |
| 24 | 240 | 0.24 | 1.40 | 21.04 | 174 | 0.00 | 1.92 | 1.06 | 49 | 0.96 | 8.20 | 11.74 |
| 25 | 241 | 0.30 | 1.45 | 21.05 | 171 | 0.00 | 1.92 | 1.06 | 87 | 1.14 | 8.66 | 11.79 |
| 26 | 242 | 0.36 | 1.54 | 21.07 | 170 | 0.00 | 1.92 | 1.06 | 60 | 1.20 | 9.12 | 11.84 |
| 27 | 224 | 0.36 | 1.59 | 21.08 | 165 | 0.00 | 1.92 | 1.06 | 85 | 1.92 | 9.68 | 11.90 |
| 28 | 247 | 0.42 | 1.69 | 21.09 | 162 | 0.00 | 1.92 | 1.06 | 47 | 1.98 | 10.14 | 11.95 |
| 29 | 144 | 0.54 | 1.73 | 21.11 | 156 | 0.00 | 1.92 | 1.07 | 62 | 2.10 | 10.69 | 12.01 |
| 30 | 248 | 0.60 | 1.83 | 21.12 | 154 | 0.00 | 2.88 | 1.07 | 63 | 2.40 | 11.15 | 12.07 |
| Average | 20.75 | | | | 1.04 | | | | 10.85 | | | |

the progenies. Accuracy is associated with selection precision and represent the main element in genetic breeding that is influenced by a breeder in order to maximize genetic gain (Resende 2002).

Genetic gains for resistance

The 30 best genotypes from the six populations studied were selected for the variable reproduction factor. The

genetic gains were predicted and the new averages estimated were close to the overall average for all variables under analysis (Tables 5 and 6), since the target is a reduced reproduction factor. Thus, the genotypes with lower values were selected.

The genetic values predicted by BLUP consider the values observed without the environmental effects. Therefore, contrary to what happens with the species of vegetative propagation, wherein all genotypic value is

capitalized, in the case of allogamous species or those from cross-pollination, which undertake progeny tests, only additive effects are transmitted to offspring, which should be used as parents in the next generation (Alves and Resende 2008). The genetic gain estimated by BLUP is equivalent to the average predicted breeding values for the selected genotypes, and the new average refers to the overall average added to the gain. This leads to improved average population for the traits assessed (Santos et al. 2015).

The prediction of the genetic values of superior genotypes is one of the major problems in the breeding of any species, since it requires the true values of variance components. The use of more sophisticated methods, such as the REML / BLUP provides better estimates for these parameters. This procedure takes into account the true values of the variance components, which are estimated by the restricted maximum likelihood procedure (REML). These components in turn interact in the BLUP mixed model equations and provide the genetic values. Thus, the REML / BLUP strategy implies a genotypic rather than phenotypic selection because it considers the treatment effects (genotype) as random (Resende and Duarte 2007).

Thus, the (REML/BLUP) strategy efficiently identified the genotypes most resistant to *M. enterolobii* to be used in backcrossing with guava for continuing the UENF guava breeding program. Individuals 121, 115, 111, 98, 91, 84, 83, 82, 81, 80, 79, 61 and 55, from the cross between guava 13.4II (susceptible) and araçá P53 (resistant), showed the smallest gains for the reproduction factor. Further backcrosses of these individuals selected with the guava recurrent parent may generate a new variety resistant or immune to *M. enterolobii*.

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