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# Cross preferences and genetic diversity of Psidium interspecific hybrids through morphoagronomic traits and resistance to *Meloidogyne enterolobii*

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**Abstract:** The introgression of *M. enterolobii* resistance-related genes in guava breeding programs can be compromised by incompatibility among *Psidium* species. This study aimed to evaluate the female parent preference and genetic diversity of Psidium interspecific hybrids using morphoagronomic traits and resistance to *M. enterolobii*. There were evaluated cross successes and germination from crosses between accesses of *P. cattleyanum*, *P. guineense* and *P. guajava* and the genetic diversity by Ward-MLM method of hybrids according to descriptors developed for the genus. Crosses were more successful when *P. cattleyanum* was the female parent. Germination was more successful in crosses involving *P. cattleyanum* and *P. guajava*. Four groups were formed. The group IV clustered the most resistant genotypes, composed by genotypes of *P. cattleyanum* x *P. guineense*, while the group II was the most susceptible. The groups I and III grouped some genotypes of *P. cattleyanum* x *P. guajava* with low levels of susceptibility. There are preferences of female parent species among crosses. Some individuals of groups I and III can be used as source of resistance genes for the breeding program, due the presence of favorable alleles inherited from guava parent. The high susceptibility leads to reduction in root development.

**Key words:** Genetic diversity, guava decline, interspecific hybridization, root-knot nematode, ward-MLM.

## INTRODUCTION

The guava (*Psidium guajava* L.) is an important crop for Brazilian pomology, highlighting the states of São Paulo and Pernambuco (IBGE 2016). The Brazilian guava production count with a wide diversity of varieties, being some of them dedicated to *in natura* fruit consume (Ogawa, Pedro Sato, Kumagai and Sassaoka varieties) and to industry process fruits (Paluma, Rica and Século XXI) varieties (Pereira & Kavati 2011).

However, some concerns come up as the disease that becoming widespread worldwide, the guava decline. It is a synergic association between the nematode *Meloidogyne enterolobii* Yang and Eisenbank, 1983 (syn. *Meloidogyne mayanguensis* Rammah & Hirschmann 1988), known as root-knot nematode, and the fungus *Fusarium solani*, promoting symptoms as galls on plant root, tanning and yellowing on leaves, defoliation of whole aerial part and, consequently, the plant death (Gomes et al. 2014, Sousa et al. 2017). The impact promoted by guava decline in Brazil has been threatening all production chain of guava in many poles, estimating a loss of million dollars (Gomes et al. 2011). In India, Jain et al. (2007) estimated that crop losses due plants parasitic nematodes were about of $3.5 billion. Searching for efficient methods of control against this pathogen, several strategies were employed (Almeida et al. 2011, 2012, Robaina et al. 2015), however, the introduction of resistant plants of guava in orchards is the most efficient control against the nematode.

Different sources of resistance genes can promote a durable resistance in cultivars against any pathogen and some species of the same genus Psidium, commonly known as "araçá", carry these *M. enterolobii* resistance-related genes (Miranda et al. 2010). The species *P. guineense* Swarts and *P. cattleyanum* Sabine, although have less economic importance than guava, have genotypes that could potentially be used in guava breeding programs as source of resistance genes through interspecific hybridization for introgression on target population (Costa et al. 2017; Gomes et al. 2017). Occasionally, species of the same genus can present cross-incompatibility in several crop species (Lu et al. 2014, Martins et al. 2015, Maune et al. 2018). This phenom limits the number of samples of a segregant population, which can lead to poor estimation of genetic divergence among individuals or groups of individuals. In *Psidium* spp., genetic diversity study was employed by Santos et al. (2011) to determinate an effective discrimination between "araçá" and guava accessions evaluated for some morpho-agronomic traits. In breeding programs aiming the obtention of inbred lines, determinate the contrasting genotypes is useful to characterize heterotic groups and to explore the maximum of heterosis (Yuwono et al. 2017).

Therefore, multivariate methods are more efficient in quantifying dissimilarity between individuals, because it allows to consider many traits at the same time, even from different natures (Gonçalves et al. 2008, Campos et al. 2013). In this way, the Ward - Modified Location Model (MLM) procedure is a great strategy to quantify variability using both quantitative and qualitative variables (Franco et al. 1998). Some diversity studies have been developed through the Ward-MLM method. In cultivated species, divergence in lim'e bean accesses originated from different domestication centers was identified (Oliveira-Silva et al. 2017), while in peanut populations, groups with moderate variability were observed (Nunes da Luz et al. 2014). In cases of fruit trees, genetic divergence was verified in passion fruit progenies using quantitative and qualitative variables (Silva et al. 2014) and in different species of the genus *Passiflora*, the method allowed a clear differentiation of 11 species (Paiva et al. 2014).

Thus, the objective of this study was to determinate the cross preference among three *Psidium* species, as well as to characterize and estimate the genetic diversity of first generation of interspecific hybrids ($H_1$) of *Psidium* based on quantitative and qualitative traits related to morphoagronomic and response to inoculation with *M. enterolobii*. There were used seedlings to characterize genotypes due to the easiness of quantifying nematodes in the roots. This is the first study in which the Ward-MLM method is applied to discriminate Psidium genotypes according to *M. enterolobii* resistance and other morpho-agronomic traits in early stages.

## MATERIALS AND METHODS

### Experiment structure

The experiment was installed in a greenhouse at *Universidade Estadual do Norte Fluminense Darcy Ribeiro* - UENF in April of 2015, using interspecific hybrids between resistant genes donator specie (*P. cattleyanum*) and resistant genes receptor species (*P. guajava* and *P. guineense*) (Miranda et al. 2012) to compound a segregate population.

The crosses were carried out stepwise, according to the floral synchronism between parents, therefore all following procedures of evaluation were carried out stepwise. The accesses used as parents were obtained from germplasm bank of UENF (Table I) and crossed randomly, according to recommendation of Alves and Freitas (2007). A total of 412 crosses between *P. cattleyanum* vs. *P. guineense* and 284 crosses between *P. cattleyanum* vs. *P. guajava* were carried out at late 2014. There were evaluated, at the total, 885 genotypes of the five families for diversity study.

Therefore, the seeds collected from crosses were planted in trays and kept in a nebulization chamber until germination, which occurred between 22 and 30 days. The $H_1$ generation seedlings were conducted under greenhouse conditions, transplanted to 5 L plastic pots that received one seedling each, with properly fertilized substrate, and maintained in greenhouse.

## Inoculation procedure

There were used tomato roots as inoculum source, planted on sterile soil being inoculated with *M. enterolobii* isolates previously collected from guava orchards in São João da Barra, RJ. After the development of the sixth pair of Psidium plant leaves, each pot received a 5 mL nematode egg concentration inoculated around the plant. The nematode egg concentration inoculated had an initial population (Pi) with 1,000 eggs of *M. enterolobii*. The inoculation procedure and the period of 135 to 180 days, consisting of the inoculation until evaluation, follows the methodology proposed by Burla et al. (2010).

**Table I. Families of interspecific hybrids of *Psidium* discriminating the female and male species, and data about the cross performance.**

| Families* | Parents | NC** | CS (%) | NF | NS | G (%) |
|---|---|---|---|---|---|---|
| CV1 X CV11 | *P. cattleyanum* x *P. guineense* | 87 | 24.13 | 21 | 410 | 47.60 |
| CV11 X CV8 | *P. guineense* x *P. cattleyanum* | 100 | 1.00 | 1 | 21 | 4.70 |
| CV11 X UENF-20181 | *P. guineense* x *P. cattleyanum* | 200 | 0.50 | 1 | 297 | 66.70 |
| CV6 X 13.10 I | *P. cattleyanum* x *P. guajava* | 30 | 10.00 | 3 | 16 | 43.80 |
| CV6 X 9.7 I | *P. cattleyanum* x *P. guajava* | 20 | 5.00 | 1 | 77 | 3.90 |
| CV8 X CV11 | *P. cattleyanum* x *P. guineense* | 25 | 40.00 | 10 | 298 | 67.10 |
| UENF-20181 X 9.7 I | *P. cattleyanum* x *P. guajava* | 20 | 10.00 | 2 | 8 | 0.00 |
| UENF-20181 X 9.9 I | *P. cattleyanum* x *P. guajava* | 25 | 4.00 | 1 | 8 | 12.50 |
| UENF-20175 X 9.7 I | *P. cattleyanum* x *P. guajava* | 40 | 5.00 | 2 | 5 | 80.00 |
| 9.9 I X P52 | *P. guajava* x *P. cattleyanum* | 50 | 2.00 | 1 | 117 | 76.90 |
| 13.4 II X P51 | *P. guajava* x *P. cattleyanum* | 34 | 6.00 | 2 | 394 | 87.60 |
| 13.4 II X UENF-20176 | *P. guajava* x *P. cattleyanum* | 20 | 5.00 | 1 | 335 | 76.70 |
| 9.5 II X UENF-20181 | *P. guajava* x *P. cattleyanum* | 25 | 4.00 | 1 | 68 | 92.60 |
| 13.2 II X CV4 | *P. guajava* x *P. cattleyanum* | 20 | 5.00 | 1 | 101 | 86.10 |
| Total | | 696 | 6.60 | 46 | 1,858 | 67.40 |

***The prefix CV means that patents are accesses of *Psidium* sp. placed in greenhouse, the prefix UENF means that parents are accesses of *Psidium* sp. placed at field and the other parents are genotypes of guava under field experimentation. **Number of crosses (NC); Crosses success rate (CS); Number of fruits (NF); Number of seeds (NS); Germination rate (G).**

## Qualitative variables

We evaluated the plants according fourteen qualitative morphological descriptors from each plant, being four binary and ten multicategory, based on International Union for the Protection of New Varieties of Plants (UPOV) document as described at the Table II. These characteristics were all evaluated when the progenies showed the sixth pair of leaves, just before the inoculation procedure.

## Quantitative variables

Six quantitative variables measured from each plant were evaluated in segregate population. Plant height was obtained using a measuring tape since soil to the tip of plant. Number of leaves was obtained by counting leaves. Fresh mass of aerial part and root was obtained using an electronic balance model Shimadzu BLZ3200H. Fresh volume of root was measured using cylinder graduated by the difference between column water after root submersion. The variables associated to roots were processed according to the method proposed by Miranda et al. (2010) for screening guava plants to *M. enterolobii* resistance, where the root plant was halved allowing the plants to continue their development, while the other part of root was used as sample for analysis. These sample were used to estimate the reproduction factor (RF) following the methodology proposed by Oostenbrink (1966), where $RF = P_i/P_f$, being $P_i$ the initial population and $P_f$ final population, and RF > 1 means that genotype is susceptible for nematode infection, RF < 1 means that genotype is resistant for nematode infection and RF = 0 means that genotype is immune for nematode infection.

**Table II.** Descriptors used to evaluate morphologic traits from interspecific hybrids of *Psidium*.

| Descriptor | Characteristic | Code |
|---|---|---|
| Pubescence on lower side of young leaf | Absent or very sparse | 1 |
| | Sparse | 3 |
| | Medium | 5 |
| | Dense | 7 |
| | Very Dense | 9 |
| Twisting | Absent | 1 |
| | Present | 9 |
| Pubescence on lower side of fully developed leaf | Absent | 1 |
| | Present | 9 |
| Shape of leaf | Round | 1 |
| | Ovate | 2 |
| | Obovate | 3 |
| | Trullate | 4 |
| | Obtrullate | 5 |
| | Oblong | 6 |
| Shape of base | Obtuse | 1 |
| | Rounded | 2 |
| | Cordate | 3 |
| Shape of tip | Attenuate | 1 |
| | Apiculate | 2 |
| | Acute | 3 |
| | Obtuse | 4 |
| | Rounded | 5 |
| Relief of surface of upper side | Smooth | 3 |
| | Medium | 5 |
| | Wrinkled | 7 |
| Curvature in cross section | Weak | 3 |
| | Medium | 5 |
| | Strong | 7 |
| Curvature of midrib | Absent | 1 |
| | Present | 9 |
| Degree of curvature of midrib | Weak | 3 |
| | Medium | 5 |
| | Strong | 7 |
| Undulation of margin | Absent | 1 |
| | Present | 9 |
| Degree of undulation of margin | Weak | 3 |
| | Medium | 5 |
| | Strong | 7 |
| Green color | Grey green | 1 |
| | Yellow green | 2 |
| | Green | 3 |
| | Dark green | 4 |
| Color of midrib on lower side | Cream | 1 |
| | Yellow | 2 |
| | Reddish | 3 |

## Genetic diversity analysis

The clustering method approached to estimate the genetic divergence was the Ward-MLM technique proposed by Franco et al. (1998), where the Gower's algorithm (Gower 1971) was used to estimate the distance between access contemplating both quantitative and qualitative variables. This method was processed by software SAS, through the commands CLUSTER and IML (SAS institute 2011). The optimal number of groups was defined based on pseudo-f and pseudo-t² criteria, associated to likelihood profile and likelihood test ratio. The MLM procedure estimated the mean vector of quantitative traits for each subpopulation. The distance between groups and the correlation of variables with canonical variables was obtained by CANDISC procedure. The distance, proposed by Matusita (1956), adapted by Krzanowski (1983) and Franco et al. (1998) for distribution of variables, defined the dissimilarity of groups based on generalized Mahalanobis distance, where each plant of the group was considered as replication.

## RESULTS

### Interspecific crosses

The results obtained from the crosses (Table I) demonstrate that the highest cross success rates between *P. cattleyanum* x *P. guineense* were observed in crosses CV8 x CV11 and CV1 x CV11 (40% and 24.13%, respectively). For crosses between *P. cattleyanum* x *P. guajava*, the progenies with highest cross success rates were CV6 x 13.10I and UENF-20181 x 9.9I (10% each).

There were remained 1,858 seeds from the crosses, however, the germination rate above 70% were only observed for the crosses *P. guajava* x *P. cattleyanum*, highlighting the crosses 9.5II x UENF-20181, 13.4II x P51, 13.2II x CV4, 9.9I x P52 and13.4II x UENF-20176, with germination rate of 92.6%, 87.6%, 86.1%, 76.9% and 76.7%, respectively, as well as the cross *P. cattleyanum* (UENF-20175) x *P. guajava* (9.7I) which presented germination rate of 80%. Among 70% and 40% of germination rate, the crosses *P. cattleyanum* (CV8) x *P. guineense* (CV11), *P. guineense* (CV11) x *P. cattleyanum* (UENF-20181), *P. cattleyanum* (CV1) x *P. guineense* (CV11) and *P. cattleyanum* (CV6) x *P. guajava* (13.10I) were observed in this group, showing the rates 67.1%, 66.7%, 47.6% and 43.8%, respectively.

To follow up the study, only 885 seeds had emerging success and vigor enough to reach at the six pairs of leaves admitted here for the characterization and diversity analysis.

### General classification of population

The 885 genotypes were mostly classified as absent or very sparse pubescence in lower side of young leaves (40.11%), followed by medium, sparse, dense and very dense (27.23%, 24.75%, 6.33% and 1.58%, respectively) (Figure 1).

The descriptor twisting of leaves showed most of genotypes with absence (96.95%), as well observed for the mostly genotypes when evaluated pubescence in downside of mature leaves (98.87%). For developed leaves the genotypes were classified mostly as oblong shape (68.81%), followed by oblanceolate (30.85%), ovulate (0.23%) and obovate (0.11%). For shape of base all genotypes were classified as obtuse (100%) and for tip of leaves the genotypes were classified as obtuse (69.83%) and as acute (30.17%).

The curvature in cross section has the most of genotypes classified as medium (50.40%) followed by weak (29.60%) and strong (20.00%). The descriptor degree of curvature of midrib, that was observed in 69.49% of genotypes, had weak (49.27%), medium (40.81%) and strong (9.92%), while the other 30.51% did not presented this characteristic. In turn, the degree of undulation of margin was observed in only 69.83% and the most of these genotypes was classified as weak (58.74%), followed by medium (33.66%) and strong (7.60%).

The descriptor green color classified the genotypes mostly as dark green leaves (58.54%), followed by green (41.46%) and the color of midrib on lower side were yellow for the most genotypes (74.69%), followed by cream (13.01%) and red (12.09%).

### Clustering

According to pseudo-F and pseudo-t$^2$ criteria, the 885 genotypes were clustered in three and two groups, respectively. The logarithmic function of probability based at highest increment on log-likelihood's value determined the formation of four groups as optimal number of groups, as illustrate the figure 2.

### Clusters characterization

The group I was composed by 44.3% of genotypes of progeny CV11 x UENF-20181, 26.0% of progeny CV8 x CV11, 15.9% of progeny CV1 x CV11, 7.8% of progeny 13.4II x UENF-20176 and 6% of progeny 13.4II x P51, a total of three hundred thirty-four genotypes, being this the only one group that allocated genotypes of all crosses. The group II was composed by 92.2% of genotypes of progeny 13.4II x P51, 6.4% of progeny CV11 x UENF-20181 and 1.4% of progeny CV8 x CV11, a total of 281 genotypes. The group III was composed by 59.2% of genotypes of progeny CV11 x UENF-20181, 35.4% of progeny 13.4II x UENF-20176 and 5.4% of progeny 13.4II x P51, a total of 147 genotypes. All group IV was composed by genotypes of progeny CV1 x CV11, a total of 123 genotypes.

In group I, the genotypes were represented mostly with absence of pubescence on lower side of young leaves (52.99%), absence of twisting (96.71%), absence of pubescence on lower side of developed leaves (99.40%), oblong shape of leaves (55.99%), obtuse shape of base (100.00%), obtuse shape of tip (58.08%), smooth surface of upper side (39.22%), medium curvature in cross section (45.51%), presence of curvature of midrib (57.49%), weak degree of curvature of midrib (59.90%) when observed, presence of undulation of margin (58.08%), weak degree of undulation of margin (68.04%) when observed, dark green color of leaves (62.28%) and yellow color of midrib on lower side (68.26%) (Figure 3).

The second group had the most of genotypes with medium pubescence on lower side of young leaves (39.86%), absence of twisting (98.93%), absence of pubescence on lower side of developed leaves (97.51%), oblong shape (98.58%), obtuse shape of base (100.00%), obtuse shape of tip (98.58%), wrinkled surface of upper side (71.53%), medium curvature in cross section (51.96%), presence of curvature of midrib (98.58%), weak degree of curvature of midrib (54.51%) when observed, presence of undulation of margin (98.58%), weak degree of undulation of margin (60.65%) when observed, dark green color of leaves (61.21%) and yellow color of midrib on lower side (80.43%).

The third group was represented by genotypes with medium pubescence on lower side of young leaves (39.46%), absence of twisting (91.16%), absence of pubescence on lower side of developed leaves (100.00%), oblong shape (98.64%), obtuse shape of base (100.00%), obtuse shape of tip (100.00%), wrinkled surface of upper side (56.46%), medium curvature in cross section (50.43%), presence of curvature of midrib (99.32%), medium degree of curvature of midrib (54.11%) when observed, presence of undulation of margin (99.32%), medium degree of undulation of margin (47.26%) when observed, dark green color of leaves (61.22%) and yellow color of midrib on lower side (68.71%).

The fourth group clustered genotypes with characteristics of absence of pubescence on lower side of young leaves (96.75%), absence of twisting (100.00%), absence of pubescence on lower side of developed leaves (99.19%), obtrullate shape (100.00%), obtuse shape of base (100.00%), acute shape of tip (100.00%), smooth surface of upper side (90.24%), medium curvature in cross section (60.16%), absence of curvature of midrib (100.00%), absence of undulation of margin (99.19%), weak degree of undulation of margin (100.00%) when observed, dark green color of leaves (58.54%) and yellow color of midrib on lower side (86.18%).

## Descriptive statistical

The group II present the highest mean of reproduction factor, 130.00 and 42.73 standard deviation, while the group IV present the lowest mean 1.11 and standard deviation 1.08, the group I and III present mean 4.28 and 6.13, respectively, and standard deviation 9.07 and 9.80, respectively (Figure 4).

For number of leaves, the highest mean belongs to group I with 42.38 and standard deviation 19.20, followed by group IV with mean 40.27 and standard deviation 11.65. The groups II and III presented mean 36.34 and 28.39, respectively, and standard deviation 11.61 and 11.21, respectively.

The height of plant presents the highest mean for group III, followed by groups IV, II and I, with values 43.71, 37.31, 36.68 and 29.48 cm, respectively, and the standard deviation of these groups was 9.85, 7.14, 10.92 and 7.76 cm, respectively.

For fresh mass of aerial part, the highest mean was registered by group IV with mean 27.44 g and standard deviation 9.48 g, the second highest mean belongs to groups I and III, both present mean 17.99 and standard deviation 9.10 and 9.44 g, respectively. The lowest mean was registered by group II with 17.90 g of mean and 8.55 g of standard deviation.

The highest mean of fresh mass of root belongs to group IV, with 33.85 g, presenting 9.95 g of standard deviation. The groups I, III and II presenting mean 7.49, 6.29 and 5.28 g, respectively, with standard deviation 4.86, 2.72 and 2.38 g, respectively.

Similar rank was observed for fresh volume of root, where the group IV presented mean of 27.60 mL and standard deviation 10.95 mL, followed by group I with 6.27 mL of mean and 4.75 mL of standard deviation, group III with 5.16 mL of mean and 2.68 mL of standard deviation, and group II with 4.09 mL of mean and 2.36 mL of standard deviation.

## Canonical correlation

The procedure CANDISC (Table III) estimated a positive and strong correlation of RF with canonical variable 1 (0.841), while root fresh mass and root fresh volume showed negative and strong correlation with the same canonical variable (-0.755 and -0.717, respectively). For the correlation of the canonical variable 2 and reproduction factor, root fresh mass and root fresh volume, the results were all positives (0.538, 0.601 and 0.548, respectively) and were not so strong as canonical variable 1. Therefore, the second canonical variable do not represent the correlation between these characteristics as well as the first canonical variable. The characteristics number of leaves, plant height and fresh mass of aerial part showed low values

**Table III. Two first canonical variables correlated with six quantitative variables measured in interspecific hybrids of *Psidium*.**

| **Variables*** | **Canonical Variables** | |
|---|---|---|
| | **Can1** | **Can2** |
| RF | 0.841 | 0.538 |
| NL | -0.099 | 0.000 |
| PH | 0.045 | 0.172 |
| MA | -0.275 | 0.255 |
| RM | -0.755 | 0.601 |
| RV | -0.717 | 0.548 |

***RF (Reproduction Factor), NL (Number of leaves), PH (Plant Height), MA (Mass of Aerial Part), RM (Root Mass) and RV (Root Volume).**

of coefficients of correlation with both canonical variables, suggesting no correlation between these characteristics with the others.

### Distance between groups

According to Ward-MLM method, the estimation of distance between groups (Table IV), based on generalized distance of Mahalanobis, showed that the group II presented highest distance with group IV (70.95), while the most similar groups were I and III (5.77). The groups I and II presented values of distance 26.83, while the groups I and IV presented value of distance 40.68, otherwise, the groups II and III presented value of distance 26.26, and the groups III and IV presented value of distance 45.06 (Figure 5).

## DISCUSSION

### Interspecific crosses of *Psidium* species

The results elucidated that interspecific crosses among *Psidium* species were not homogenous and some crosses have advantages when carried out in one direction. This was noted when analyzed the cross-success rates, as observed when the *P. cattleyanum* was the female parent, where rates were under than 10%. Furthermore, the highest cross success rates were observed in crosses among *P. cattleyanum* and *P. guineense*. Studies carried out for interspecific hybridization in *Capsicum* showed difficulties caused by pre-fertilization barriers, such as possibility of crosses only in one direction, as example of crosses between *C. baccatum* and *C. frutescens*, showing non-germination of pollen grains or stopping the pollen tube growing (Martins et al. 2015). Many seeds obtained by crosses suffered with lack of vigor, or even germinated. These phenomena are usually common in interspecific hybridization (Parton et al. 2001, Martins et al. 2015, Okamoto and Ureshino 2015). The reduction in the number of progenies analyzed in the study (from 1,858 seeds to 885) could be affected by a post-fertilization barrier in interspecific hybridization among *Psidium* species, which can be confirmed by cytological studies.

**Table IV.** **Mahalanobis distance among four groups formed by Ward-MLM method from interspecific hybrids of *Psidium*.**

| Group | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | - | 26.83 | 5.77 | 40.68 |
| 2 | | - | 26.26 | 70.95 |
| 3 | | | - | 45.06 |
| 4 | | | | - |

Some genotypes suffered from lack of vigor, causing losses of genotypes mostly registered in progenies of *P. cattleyanum* x *P. guajava*. The same lack of vigor was reported by Costa et al. (2012), that had unsuccess in interspecific hybridization among guava and *P. cattleyanum*, however, these authors had success in cross among *P. guajava* and *P. guineense*. The high number of different parents used, as well as crosses made in this study, was the differential to acquisition of satisfactory number of viable seeds among *P. cattleyanum* and *P. guajava*.

Besides that, the basic chromosome number of *Psidium* species is x = 11, however, *P. guajava* is the only diploid specie in this genus (2n = 2x = 22), while *P. guineense* and *P. cattleyanum* are admitted as polypoid (2n = 4x = 44) (Costa et al. 2008, Souza et al. 2015). The difficult to cross *P. guajava* and *P. cattleyanum* presented here could be an effect of the ploidy level differences, reducing the viability of crosses due the called triploid block (Marks 1966). It's expected that progenies of cross *P. cattleyanum* (2n = 44) x *P. guajava* (2n = 22) might present karyotype with 2n = 3x = 33 chromosomes, generating rarely gametes with up to 22 chromosomes, or usually sterile, due to unbalanced chromosome numbers caused by a failure to separate odd-numbered chromosome sets during meiosis (Köhler et al. 2010). The next generation ($H_2$)

should have some genotypes with chromosomal complement tetraploid. However, if considering backcross with *P. guajava*, triploid hybrids should be expected in the first generation of backcross.

## Cluster characterization

The number of groups formed using the Pseudo-F and Pseudo-t² criteria, associated to likelihood function, grouped the evaluated genotypes in the best way possible. These cluster number optimization criteria are preferable for non-accept a subjective clustering, however, the different number of accesses, descriptors and traits evaluated, can result in different number of groups (Gonçalves et al. 2009). Campos et al. (2013), evaluating the diversity of 138 guava access based on morphological, agronomical and physic-chemical descriptors, used this methodology and obtained eight groups in their analysis.

Considering the study of Santos et al. (2008) and comparing the data, the standard of segregation of genotypes in group I showed average similarities to guava in characteristics of curvature in shape of leaves, cross section and curvature of midrib. In group II, the similarities of genotypes with guava were observed in curvature in cross section, curvature of midrib and undulation of margin. The group III showed similarities in curvature of midrib, undulation of margin and degree of undulation of margin. For group IV, all genotypes showed standard segregation like *P. cattleyanum*. The group IV obtained in this study also clustered the genotypes with the lowest RF, followed by group I and III. Although Souza et al. (2014) clustered mostly genotypes resistant to *M. enterolobii* in one group, being these genotypes a *P. cattleyanum* access, the present study is the first one to estimate the diversity among interspecific hybrids of Psidium. Miranda et al. (2012) suggested that identifying resistant genotypes for *M. enterolobii* in accesses of *Psidium spp*. could open a novel path for strategies in guava breeding programs.

## Canonical Variables

The estimative of eigenvalues of the first two canonical variables obtained in this study represented 95.21% of total variation. It’s a value above the suggested by Gonçalves et al. (2009), which recommend that estimative above 80% is satisfactory for interpretation of variability among access. The correlation estimated about the six quantitative variables, showed that the RF of nematode have a high negative correlation with root variables on the first canonical variable. According Gomes et al. (2014) one of primary symptoms caused by *M. enterolobii* is the necrosis in all kind of root tissues, which, consequently, leads to losses of fine roots of absortion, by this way, infected genotypes have low mass and volume of roots compared to resistant genotypes, suggesting that the low mass and volume of roots are associated with the damage caused by *M. enterolobii*. However, carry out indirect selection through fresh mass and volume of roots might not provide an effective accuracy for screening resistant genotypes to *M. enterolobii*.

## Distance between groups

The distance of groups I and III, composed by *P. cattleyanum* x *P. guajava* progenies, from the group IV, composed by *P. cattleyanum* x *P. guineense* progenies, where genotypes showed low mean of RF, was relatively high and it can be exploited for the ongoing guava breeding program. However, the genotypes obtained in group I and III could be preferable for backcross because they have more probabilities to drag agronomic favorable genes in linkage to resistant genes rather than genotypes from group IV, that have no or few related genes to *P. guajava*. The backcross method is efficient for introgression of desired genes in populations already improved, such as commercial varieties and hybrids (Frisch & Melchinger 2005). Directing crosses through divergent clusters may avoid the narrowing of genetic base of a breeding program by expanding the genetic variability, providing options for the development of new varieties and hybrids (Govindaraj et al. 2015). Silva et al. (2014) had well used the Ward-MLM as strategy to define heterotic groups, and then advance the generations of passion fruit in a program of recurrent selection.

## CONCLUSIONS

There are some preferences among crosses of *Psidium* sp. The crosses aiming the introgression of resistant genes had great results in *P. cattleyanum* x *P. guineense*, in comparison to *P. cattleyanum* x *P. guajava* families, however the germination rate was higher in *P. gujava* x *P. cattleyanum* families. Therefore, only with high number of cross-pollinations between *P. guajava* and *P. cattleyanum* there will be possible to reach a satisfactory number of interspecific hybrid seeds.

The Ward-MLM method was efficient to cluster genotypes from similar crosses. The group I and III could cluster several resistant genotypes, being some of them originated from crosses between *P. cattleyanum* and *P. guajava*. The potential of these clusters for use in the guava breeding program on going is based on the presence of *P. guajava* as parent where some agronomic traits are already present.

Among quantitative traits evaluated, the first two canonical variables could demonstrate that the susceptibility to *M. enterolobii* is associated to the loss of fresh mass of roots and fresh volume of roots. However, it is still interesting select genotypes through reproduction factor of *M. enterolobii* rather than roots-related variables measured in this study, due the high information promoted by reproduction factor according to the resistance of the genotype.

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Odimar Almeida performed the measurements and data analysis. Alexandre Pio Viana conceived the idea. Vicente Gomes carried out the crosses and planned the experiments. Ricardo de Souza provided the lab facilities to process samples. Paulo Santos, Antônio Amaral Júnior and Daniele Rodrigues supported the analysis concepts.