

Genetic diversity of segregating *Carica papaya* genotypes using the Ward-MLM strategy

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ABSTRACT. Progenies from crosses between distinct accessions are potentially exploitable sources of genetic variability, through either evaluation or selection of segregants to obtain promising genotypes. Successful selection can be achieved based on a large number of descriptors that are simultaneously evaluated. Multivariate methods are used initially that fulfill two prerequisites: the estimation of similarity (or dissimilarity) measures between the parents and the use of a clustering technique for group formation. The multivariate method using Ward - Modified Location Model (MLM) allows the determination of the number of groups and their means with high precision, favoring the use of all information on the genotypes. We quantified the genetic diversity in a segregating F_2 population of papaya consisting of 92 plants based on morphoagronomic descriptors, using the Ward-MLM strategy. This method clustered the 92 plants into three groups, with the most promising genotypes for selection of plant architecture clustered into group I; the descriptors that contributed the most to the genetic diversity were fruit length, petiole length, first fruit insertion height and fruit mass.

Key words: BLUP/REML; Genetic value; Multivariate method

INTRODUCTION

World papaya production reached 1.4 million tons in 2016, and the main papaya producing countries were India, Brazil, Indonesia, Nigeria, and Mexico. The crop has *gained* prominence in Brazil because it adapts perfectly to the local edaphoclimatic conditions. In 2016, papaya cultivation comprised an area of approximately 30,372 hectares, mainly in the states of Bahia, Espírito Santo, Rio Grande do Norte and Minas Gerais (FAO, 2016; IBGE, 2016).

In 2014, Brazilian papaya export grew 17.9% in tons when compared to the previous year, and Espírito Santo was the largest exporter and the second largest producer (MDIC, 2015). One of the main obstacles to the growth of the crop is the small number of cultivars currently cultivated, which generates uniformity in the plantations and, consequently, low genetic variability. Within the "Solo" group, the cultivars 'Golden' and 'Golden THB' are currently grown for export and 'Sunrise Solo' for the domestic market (Serrano and Catanneo, 2010; Ruggiero et al., 2011).

To increase variability in papaya, the genetic diversity of germplasm banks such as Caliman Agrícola has been studied (Barbosa et al., 2011, Silva et al., 2017). Information on diversity facilitates the selection and development of segregating progenies (Jesus et al., 2013; Pinto et al., 2013) and hence of new cultivars and/or hybrids for farmers (Silva et al., 2008; Ramos et al., 2011).

In a study with papaya, great variability has been found from generations F_2 to F_4 , maximizing potentials for selection (Karunakaran et al., 2010). Progenies from crosses between distinct accessions are potentially exploitable sources of genetic variability, through either evaluation or selection of segregants to obtain promising genotypes. Successful selection can be achieved based on a large number of variables that are simultaneously evaluated. Multivariate methods for grouping elements are selected by researchers initially fulfilling two prerequisites: the estimation of similarity (or dissimilarity) measures between the parents and the use of a grouping technique for group formation (Loarce et al., 1996; Cruz et al., 2014).

The multivariate method using the Ward - Modified Location Model (MLM), which was proposed by Franco et al. (1998), is a strategy to measure variability, so that quantitative and qualitative descriptors can be used simultaneously. This strategy allows the determination of the number of groups and their means with high precision, favoring the use of all the information on the genotypes (Crossa and Franco, 2004; Amaral Júnior et al., 2010).

Various studies have used MLM strategy on some crops such as coffee (Rodrigues et al., 2016), corn (Ortiz et al., 2008), tomato (Gonçalves et al., 2009), guava (Campos et al., 2013), and castor bean (Oliveira et al., 2013). Studies using the MLM strategy in papaya are still unknown.

We quantified the genetic diversity in a segregating F_2 population of papaya consisting of 92 plants based on morphoagronomic descriptors, using the Ward-MLM strategy.

MATERIAL AND METHODS

The F_2 population evaluated in this study was obtained from the self-fertilization of F_1 plants derived from the controlled crossing of the contrasting lines 'Baixinho de Santa Amália' (BSA) and 'Golden Pecíolo Curto' (short petiole) (GPC), belonging to the 'Solo' group. These are genotypes derived from potentially endogamous species, from the Caliman Agrícola S/A germplasm bank, maintained in more than eight consecutive self-fertilizations. BSA was selected from germinal mutation, in a commercial plantation of cultivar 'Improved Sunrise Solo'

in the State of Espírito Santo, in 1980, and its main characteristic is low height (Cattaneo, 2001). GPC plants are tall, with small leaves and a short leaf petiole (Silva et al., 2017).

The experiment was carried out at Santa Teresinha Farm belonging to Caliman Agrícola S/A, in the municipality of Linhares, Espírito Santo (19° 11' 49" S; 40° 05' 52" W; 30 m altitude), between February 2012 and December 2014 (Figures 1 and 2). The climate of the region is type AWi (tropical humid), with a rainy summer and a dry winter.

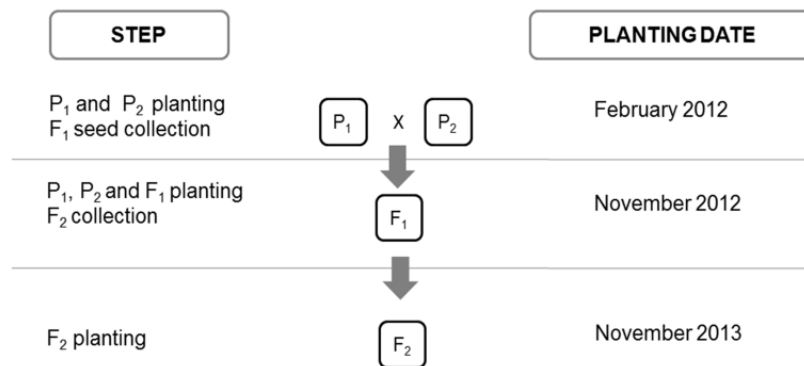


Figure 1. Planting schedule and seed collection of the study generations from the cross “Baixinho de Santa Amália x Golden Pecíolo Curto”

The first planting with the parents was carried out in February 2012. After eight months, GPC was used as a pollen donor for BSA to generate the F₁ population. F₁ seeds were sown in November 2012 together with the parents to generate the F₂ population and replenish F₁, P₁ and P₂ seeds. F₂ was obtained by self-fertilization of F₁ plants and taken to field in November 2013, where all 92 hermaphrodite plants were evaluated.

F₁ hermaphrodite plants performed natural self-pollination. The flowers were selected, marked and bagged (paper bags) before anthesis. The population was obtained in the field, taking every care to guarantee safety and reliability of the work.

The experiment was carried out with one hermaphrodite plant per hole planted at between-row spacing of 3.6 m and in-row spacing of 1.5 m, following cultural practices recommended for the crop (Costa and Costa, 2013).

Phenotypic evaluations on all hermaphrodite plants of the F₂ generation were taken at 10 months after planting., and the following descriptors were measured: stem diameter (SD) at 20 cm above the ground level, using a pachymeter; plant height (PH) from the ground level (from the root collar) to the insertion point of the newest leaf, using a measuring tape; first fruit insertion height (FFIH) from the ground level to the peduncle of the first fruit, using a measuring tape; petiole length (PL) of three fully developed leaves, at the middle height of the panel of each plant, using a measuring tape; leaf length (LL) from the base of the midrib to its tip, in the median lobe, using a measuring tape; maximum leaf width (MLW), measuring the largest width on the same leaves used to measure PL, using a measuring tape; total number of fruits (TNF), counting of all fruits on the plant; fruit mass (FM) of three fruits per plant at maturation stage I (Fuggate et al., 2010), using a precision scale (expressed as grams); fruit length (FL) and fruit diameter at the midpoint of the fruit (FD), both using a pachymeter; greatest thickness of fruit pulp (GTP) and smallest thickness of fruit pulp (STP) taken after cutting the fruit horizontally in the equatorial region, using a ruler; soluble solids (SS) by direct reading in a digital refractometer and expressed as °Brixin; internal fruit firmness (FIRM) measured by cutting the

fruit horizontally in two halves and measuring the resistance of the pulp at three equidistant points using a penetrometer (Instrutherm, model PTR-100) with a 7.9 mm diameter tip and expressed as kg cm^{-2} ; estimated production of first year (PROD) in kilogram per plant estimated for the first year based on number of fruits and mass per fruit. The morphological descriptors SD, PH, FFIH, PL, LL, MLW, FL, FD, GTP, and STP were expressed as centimeters.

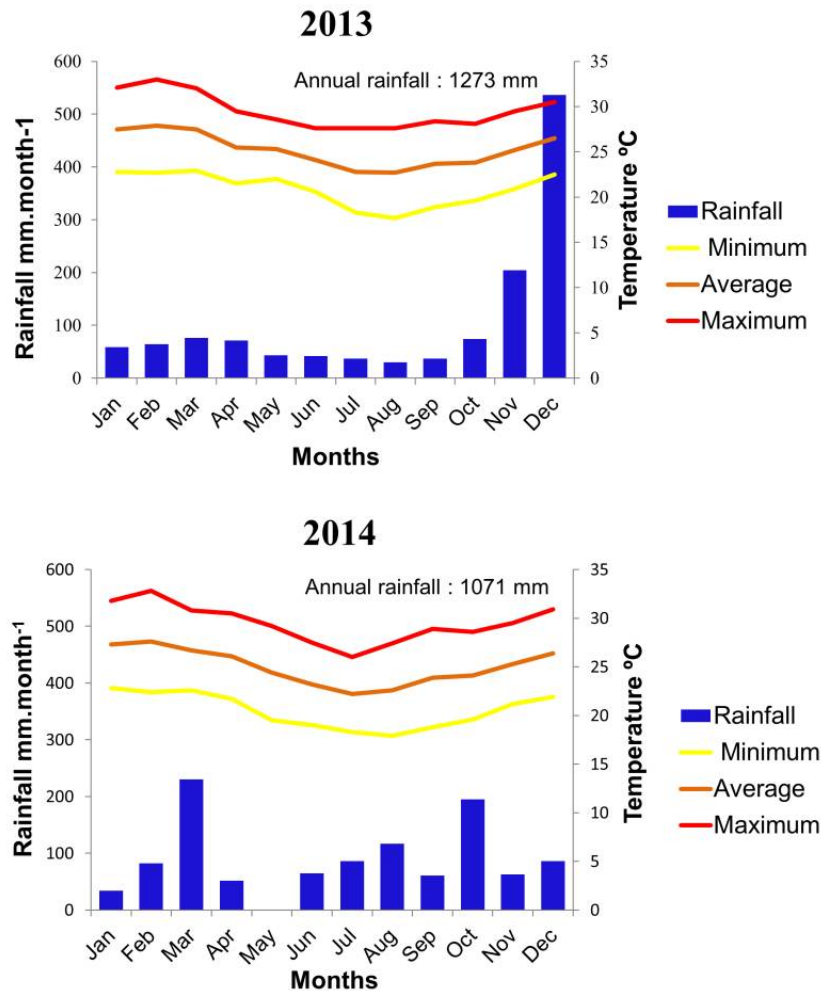


Figure 2. Meteorological data of the experimental area for 2013 and 2014. Source: Caliman Agrícola S/A (2016).

Statistical significance was examined by the F-test, and the descriptors were evaluated simultaneously by the Ward-MLM strategy (Franco et al., 1998). The clusters were obtained using the distance matrix and the pseudo-F and pseudo- t^2 criteria, defining the optimal number of groups based on the set of descriptors studied. With the optimum number of groups defined, the hierarchical classification was performed, providing the parameters to implement the final step of the MLM model (Cossa and Franco, 2004). The difference between groups and the correlation between the variables and the canonical variable were assessed graphically using the Candisc procedure in SAS version 9.1.3 (SAS, 2003).

RESULTS

In this study, the quantification of the genetic diversity in the F₂ population (Figure 3) using the Ward - MLM strategy for the 15 quantitative descriptors evaluated in the 92 genotypes allowed the formation of three groups, which was optimal for the genotypes studied, showing an increment of 38.94 (Table 1).

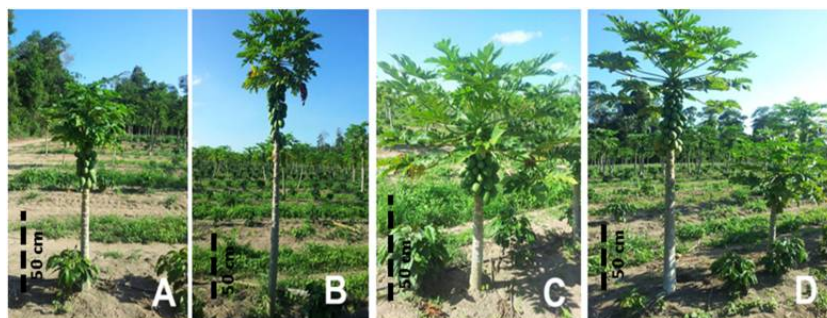


Figure 3. Variability in the F₂ papaya population from the cross of “Baixinho de Santa Amália” and “Golden Pecíolo Curto”, at 300 days after planting.

- A – Intermediate plant height and short petiole;
 B – Tall plant height with long petiole;
 C – Short plant height with long petiole;
 D – Short and tall plant heights with long petiole.

Table 1. Mean and standard deviation of the descriptors for each of the three groups formed by the Ward-MLM strategy of 92 genotypes in the F₂ papaya population.

Descriptors	Groups			Mean and standard deviation
	I (47)	II (27)	III (18)	
SD	9.83±1.10	9.83±1.51	11.78±0.94	10.21±1.42
PH	170.30±47.60	186.19±23.81	216.17±25.47	183.93±41.61
FFIH	61.90±17.97	90.00±16.80	85.28±13.59	74.72±21.32
PL	39.45±8.18	54.54±7.93	66.22±3.95	49.11±13.02
LL	26.11±3.47	29.52±3.23	33.46±1.48	28.55±4.20
MLW	38.53±5.44	43.67±4.62	51.53±3.01	42.58±6.88
TNF	56.02±14.36	36.70±11.70	66.39±11.70	52.38±16.96
FM	305.86±60.81	380.14±45.74	428.33±88.32	351.62±80.07
FL	11.15±0.71	12.67±0.54	12.50±0.84	11.86±1.00
FD	7.55±0.59	8.00±0.44	8.42±0.65	7.85±0.65
GTP	2.24±0.22	2.42±0.13	2.53±0.17	2.35±0.22
STP	1.54±0.16	1.68±0.13	1.73±0.19	1.62±0.17
SS	10.88±0.93	11.87±0.74	11.99±0.88	11.39±1.00
FIRM	11.47±0.70	11.58±0.63	11.58±0.55	11.52±0.65
PROD	17.12±5.33	13.89±4.47	28.16±6.87	18.33±7.39

SD - stem diameter (cm); PH - plant height (cm); FFIH - first fruit insertion height (cm); PL - petiole length (cm); LL - leaf length (cm); MLW - maximum leaf width (cm); TNF - total number of fruits (unit); FM - fruit mass (g); FL - fruit length (cm); FD - fruit diameter at the midpoint of the fruit (cm); GTP - greatest thickness of fruit pulp (cm); STP - smallest thickness of fruit pulp (cm); SS - total soluble solids (°Brix); FIRM - internal fruit firmness (kg cm⁻²); PROD - estimated production of first year (kg.plant⁻¹).

The Ward-MLM strategy defines the optimal group number through the logarithmic function of Log-Likelihood probability following the criteria of pseudo-t², pseudo-F, and

the logarithmic function of verisimilitude (SAS, 2000). Risk profile graphs have been used to verify the number of groups, and the point of maximum growth has been defined for the optimal number of groups that may vary with the species, number of accessions, or number and types of descriptors evaluated (Gonçalves et al., 2009).

Group III consists of 18 genotypes with the highest values for most of the descriptors in the study: SD, PH, PL, LL, MLW, TNF, FM, FL, GTP, STP, SS and PROD. Fraife Filho et al. (2001) and Silva et al. (2007) reported that plants with the greatest stem diameters are more productive, and the selection of tall plants, with larger fruits and more productive, is possible. Thus, crosses of the best genotypes of group III and those of group I and II can be performed for better exploitation of heterosis.

Group I is formed by 47 genotypes with the lowest means for the descriptors PH, FFIH, PL, LL, MLW, FM, FL, FD, GTP and STP. This group can be exploited through the selection of genotypes with the lowest means for PH, FFIH, PL, LL and MLW, which are descriptors determining plant architecture. These descriptors favor the selection of potential genotypes for protected environments because their smaller architecture phenotype facilitates harvesting, and with lower values of PL, LL and MLW, the planting density is a strategy to be exploited. It is possible to select plants in group I with higher values of PH and lower PL, LL and MLW that can be effective to obtain cultivars to increase conventional plant density.

Group I showed low FFIH with mean of 61.90 cm, a considerable value when compared with the other groups. Therefore, it is possible for the genotypes of group I to compensate for the lower values found for PROD, since the reduction in the insertion height of the first fruit favors a longer harvest period resulting in a longer production cycle (Dantas and Lima, 2001; Lim and Hawa, 2007).

The 27 genotypes of group II have intermediate values for almost all the descriptors studied, except for TNF and PROD, with the lowest values. However, selection in group II can be efficient for plant architecture by selecting intermediate genotypes for PH, PL, MLW and LL, or even exploitation of heterosis by crossing its genotypes with group III, aiming to increase productivity in response to plant architecture.

The fruit descriptors FM, FL, FD, GTP, STP, SS and FIRM in the three groups classify the 92 genotypes as belonging to the "Solo" group, with fruits weighing 300 to 650 grams. Considering these results, efficient selections for fruit weight within the "Solo" group can be performed, as well as crosses aiming at heterosis exploitation, with group III formed by genotypes with the greatest fruit mass, followed by group II, and group I with the lowest means.

The pulp descriptors GTP and STP, particularly GTP, had values greater than 2.0 cm, which is important because it is directly related to pulp yield and commercial value. According to Yamanishi et al. (2006), mean thickness greater than 2.0 cm favors the trade market.

SS is one of the main descriptors of organoleptic properties in papaya, and means varied from 10.88 °Brix for group I to 11.99 °Brix for group III. These results are corroborated by Dias et al. (2011), who reported variation from 7.25 to 11.52 °Brix in a study with 27 genotypes and Marin et al. (2006), who found 7.85 to 12.65 °Brix in a study with hybrids.

FIRM means were around 12.0 kg.cm⁻² for the three groups, and these values characterize good pulp firmness, while lower results have been reported in commercial

cultivars of papaya (Fontes et al., 2008; Viana et al., 2015). Good pulp firmness in association with the results found for GTP and STP directly influences fruit resistance against mechanical damages in transport and fruit postharvest life.

The descriptors that contributed most to genetic diversity based on the first canonical variable were FL, PL, FM and FFIH, which relate to production and plant architecture, with weight coefficients of 80.8, 77.7, 60.4 and 69.2 (Table 2).

Table 2. Canonical variables for the 15 quantitative descriptors in genotypes of the F₂ population of papaya.

Descriptors	Canonical variable (VC)	
	VC1	VC2
SD	0.212	0.609
PH	0.322	0.361
FFIH	0.692	0.037
PL	0.777	0.520
LL	0.158	0.298
MLW	0.587	0.586
TNF	-0.357	0.663
FM	0.604	0.364
FL	0.808	0.079
FD	0.475	0.360
GTP	0.528	0.301
STP	0.471	0.212
SS	0.560	0.157
FIRM	0.088	0.019
PROD	0.058	0.816

Efficient selection should meet some prerequisites, in particular the heritability, which must be significant to be maintained in the next generations. According to Silva et al. (2008) and Karunakaran et al. (2010), in studies carried out with papaya, the descriptors plant height, first fruit insertion height, stem diameter, fruit mass, fruit length, pulp thickness, and total soluble solids have high heritability, which favors selection.

The dissimilarity between the groups based on the Mahalanobis distance by the Ward-MLM strategy, shows that groups II and III are the closest (19.95), while groups I and III were the most distant (21.09) (Table 3). Crosses between groups I and III can be performed to exploit heterosis by producing transgressive individuals for the descriptors of plant architecture and production.

Table 3. Distance between groups formed by the Ward-MLM strategy based on 15 quantitative descriptors in genotypes of the F₂ population of papaya.

Groups	II (27)	III (18)
I (47)	20.59	21.09
II (27)	-	19.95

The first two canonical variables obtained by the Ward-MLM strategy explained 100% of the total variation, allowing the visualization of the existing genetic variability by graphic dispersion (Figure 4). The high value indicates that the graphic representation based

on the two canonical variables is suitable to delineate the groups and the genotypes within them.

The F_2 population of papaya has promising genotypes for selection aiming at the descriptors of interest to be included in a papaya breeding program, and selection is a tool to exploit the genetic diversity, performance of parents, and allelic complementarity. In a study exploiting the genetic variability in the F_2 population of the hybrids Tainung and Calimosa, Oliveira et al. (2012) achieved satisfactory results by selecting 18.30 and 24.61% of the genotypes considered as promising for selection of lineages.

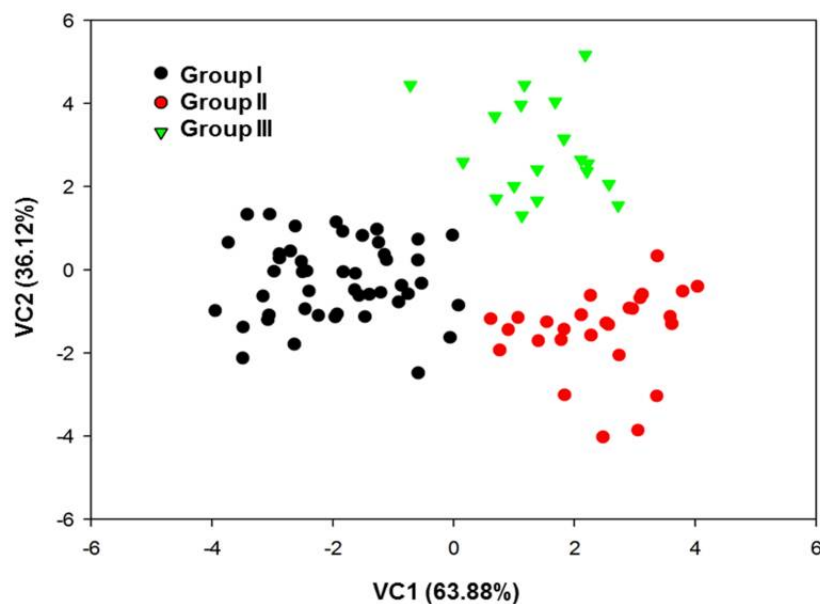


Figure 4. First two canonical variables (VC1 and VC2) for the three groups formed by the Ward-MLM strategy, based on 15 quantitative descriptions in genotypes of the F_2 population of papaya.

CONCLUSIONS

The Ward-MLM strategy allowed the identification of variability within the segregating F_2 population of papaya and enabled a consistent formation of three groups. The most promising genotypes for selection aiming at plant architecture were clustered in group I. The descriptors that contribute most to the genetic diversity are fruit length, petiole length, first fruit insertion height and fruit mass.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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