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Genetic divergence in Brazilian varietal pineapple hybrids

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ABSTRACT. Knowledge about the genetic variability of pineapple makes it possible to select characteristics of interest and plan crosses to obtain a promising cultivar for the market. This study aimed at the characterization and genetic divergence of pineapple clones from the crosses: IAC Fantástico x Jupi, BRS Imperial x Pérola, BRS Imperial x Smooth Cayenne and BRS Vitória x Smooth Cayenne. The study was carried out at the State University of Mato Grosso, Tangará da Serra campus crop fields. The municipality (Tangará da Serra) markets the Pérola and Jupi cultivars as fresh fruit. The other cultivars were used to obtain traits of interest. A randomized block design with three replications and five plants per plot was used; 42 characteristics (qualitative and quantitative) were analyzed. Vegetative and inflorescence characteristics were measured at inflorescence initiation. Fruit data was collected after harvesting. Variables were analyzed using the Ward-MLM procedure in the SAS program. Three distinct groups were found among the cross progenies. Individuals in group III had the highest averages for fruit mass with and without crown (1600 and 1486 g respectively), length (19.41 cm) and average fruit diameter (12.06 cm). This is an advantage for the producer, as larger fruits have smaller crowns. It also included individuals without thorns, with fruits of cylindrical shape and yellowish pulp, soluble solids and acidity levels within the recommended range, being the most suitable for fresh consumption. Groups I and II had lower means for the characteristics and were similar to each other. The greatest distance was observed between groups II and III; therefore, it is recommended to cross the best individuals of these groups to explore the heterotic effect and expand the existing genetic variability.

Key words: *Ananas comosus*; Genetic variability; Genetic enhancement; Cultivar crosses; Joint analysis

INTRODUCTION

Pineapple (*Ananas comosus* var. *comosus*) is a fruit that stands out among the most cultivated tropical fruit species and is of great economic importance worldwide (Lira Júnior et al. 2021). Distributed in 83 countries, the world pineapple production was estimated at 30,455,692 tons, harvested in a wide area of 1,177,546 hectares in 2020. The biggest producers were the Philippines, China, Costa Rica and Brazil with a production of 2.70, 2.64, 2.62 and 2.45 million tons respectively (FAO 2020).

In Brazil, almost all production that supplies the national market is intended for fresh fruit consumption. Exports included fruit, prepared and processed pineapple, as well as pineapple juice, which corresponded to about 29% 2 and 69% of the exports, respectively (CONAB, 2020). The Pérola cultivar is the most planted in the country, occupying 85% of the crop area. This cultivar has soft pulp, ranging from white to pale yellow, is juicy, with a good aroma and total soluble solids of 13 to 16º Brix, characteristics that please consumers. However, it has thorns on its leaves, making it difficult for the producer to handle and cultivate it and susceptibility to fusariosis, a fungal disease (*Fusarium guttiforme*) that severely affects Brazilian production (Matos 2018; Sanewski et al., 2018).

For the development of genetic improvement programs, hybridization is a fundamental strategy to generate variability. Pineapple is self-incompatible and highly heterozygous. In this way, the crossing of two contrasting cultivars will generate a segregating population thus promoting greater diversity for characteristics related to the plant and fruit, favoring the selection process (Sanewski 2011; Zhao and Qin 2018). Knowledge about diversity estimates based on phenotypic traits of agronomic interest can provide useful information to guide breeding programs. In general, the analysis of genetic diversity aims to identify more divergent individuals for important agronomic traits. Its importance is highlighted because the more genetically distant individuals are more likely to have distinct alleles and when these are associated with high means, they produce a high heterotic effect (Wang et al. 2017; Ismail et al, 2020).

Thus, the phenotyping performed using qualitative and/or quantitative descriptors makes it possible to identify promising genotypes for the traits of interest, which is essential to guide the next stages of the program. In this context, the use of multivariate algorithms that aim to quantify the dissimilarity between individuals is considered an essential tool for plant improvement (Gonçalves et al. 2008). Among the multivariate methods, the Ward - Modified procedure Location Model (MLM), proposed by Franco et al. (1998), consists of an excellent strategy for quantifying variability, as it uses quantitative and qualitative variables simultaneously.

This strategy allows defining the optimal number of groups and calculating the average of the groups with high precision, using all available information about the genotypes (Crossa and Franco 2004). With this, it is possible to separate an original group of observations into several subgroups, to obtain homogeneity within and heterogeneity between the subgroups, according to the criterion of similarity or dissimilarity adopted (Cruz and Carneiro 2006). Makaranga et al. (2018) evaluated pineapple genetic diversity in Tanzania based on microsatellite markers and detected four different groups, with low genetic diversity among accessions. In Brazil, Machado et al. (2011) evaluated five qualitative and 11 quantitative variables and Melon et al. (2015) evaluated 31 qualitative descriptors and 21 quantitative descriptors in pineapple genotypes based on the algorithm described by Gower. Both authors observed the formation of several groups, indicating that the method was efficient to represent the genetic diversity among the evaluated genotypes. Therefore, considering the importance of this information, assuming that crosses between current cultivars can generate a segregating population with high variability favoring the identification of promising clones, this study aimed to phenotype the pineapple population and estimate the divergence between individuals based on the joint analysis of qualitative and quantitative variables using the Ward-MLM procedure.

MATERIAL AND METHODS

Obtaining the varieties and cultivation conditions

In 2012, an active germplasm bank was implemented in the experimental area of the State University of Mato Grosso (Unemat), followed by agronomic characterization and analysis of genetic divergence between accessions by Melão et al. (2015) and the evaluation of genetic resistance of accessions by Garcia et al. (2015). After these evaluations, the following crosses were performed: IAC Fantástico x Jupi, BRS Imperial x Pérola, BRS Imperial x Smooth Cayenne and BRS Vitória x Smooth Cayenne, which originated the population evaluated in this work (Table 1).

Table 1. Number of pineapple genotypes obtained from four crosses planted in Tangará da Serra-MT, Brazil, in 2021.

Genitors		Number of genotypes
Male	Female	
Smooth Cayenne	BRS Imperial	24
Smooth Cayenne	BRS Vitória	8
Jupi	IAC Fantástico	25
Peróla	BRS Imperial	34
Total		91

The crosses were carried out during the month of May 2014, from 06:30 to 09:00 according to Spironello et al. (1994). It was not necessary to emasculate the flowers of the female parents, because the plants have high incompatibility. For cross-pollination, it was necessary to remove the flowers of the male parents with the help of tweezers and place them in Petri dishes, later, the anthers were detached to pollinate the flowers of the female parents. The tweezers were always sterilized with 70% alcohol when the male and female parent was changed, to avoid contamination.

At 120 days after the crosses, the fruits were harvested when they were ripe and the seeds were removed. These were disinfected with 70% alcohol and sodium hypochlorite for 15 minutes, then placed to dry on paper towels. For seed germination, sand and 250 mL transparent gerbox plastic box (11x11x3.5 cm) were sterilized in an autoclave. Subsequently, the sand was placed in the boxes, seeded and sent to a growth chamber at 24°C, with a photoperiod of 12 hours, being wetted three times a day with sterile distilled water.

In December 2014, the seedlings were grown in a screen-covered greenhouse with 70% shading, and transplanted into 128-cell Styrofoam trays. The substrate used was according to Lima et al. (1994) and foliar fertilization was carried out weekly with Platon© at a dosage of $1mL^{-1}$. The first 30 days irrigation was intermittent, 1 minute every hour, eight times a day, then it was adjusted to three times a day. In April 2015, the seedlings were transplanted to the bed located in a greenhouse, covered by a screen with 50% shading. The construction site was built with the aid of a micro tractor, where the enchanter was coupled, with a width of 1 meter and a height of 20 cm. The flowerbed was covered with black TNT fabric. For planting the seedlings, openings were made in the fabric with a spacing of 20x20cm. The liming and fertilization of planting and coverage of pineapple seedlings were carried out according to soil analysis, following the recommendations of Cunha et al. (2005). Irrigation was performed by micro-sprinkler twice a day. Leaf fertilization was carried out weekly with Platon© at a dosage of $2mL^{-1}$. Each genotype was multiplied in the field via conventional seedlings, for two generations, aiming to increase the number of seedlings for carrying out the experiment. The experiment was installed in the Unemat experimental area, located in the municipality of Tangará da Serra-MT, Brazil (14º39' south latitude and 57º25' west longitude and altitude of 321 m). The region's climate is characterized as humid tropical, with a dry winter and rainy summer, with an average annual rainfall of 1,830 mm (Martins et al. 2010; Alvares et al. 2013).

The planting of the experiment in the field was carried out in September 2018, in a randomized block design, with three replications and five plants per plot. The planting spacing was 1.20 x 0.40 x 0.40 m (double rows). Puppet-type seedlings of sizes between 35 and 45 cm were used. Irrigation was performed by sprinkling three times a week, when necessary. The liming and fertilization of planting and coverage of pineapple seedlings were carried out according to the soil analysis, following the recommendations of Cunha et al. (2005). In September 2019, artificial floral induction was performed using 50 mL of syrup per plant. The syrup was prepared by adding 1 mL of Ethrel 720, 20 g of urea and 0.35 g of quicklime or hydrated lime for each 1 liter of water. The application was carried out in the afternoon, with the aid of a 20L costal pump. Weed control was initially carried out with manual weeding. Subsequently, chemical weeding with the herbicide Krovar (diuron + bromacil) at a dosage of 4 kg ha^{-1} was used, which allowed efficient weed control.

Phenotyping of morphoagronomic characteristics

Forty-two traits were evaluated, 16 qualitative (three vegetative and 13 fruit characters) and 26 quantitative traits (four vegetative and 21 fruit characters) as shown in

Table 2. This morphoagronomic characterization followed the pineapple germplasm characterization and evaluation catalog proposed by Queiroz et al. (2003). The qualitative characteristics referring to the vegetative and inflorescence were evaluated during the beginning of the development of the inflorescence and for the characteristics referring to the fruits, they were evaluated after their harvest.

For the quantitative characteristics, the vegetative and inflorescence data were evaluated in the initial flowering stage of the plant. For the characteristics referring to the fruits, they were collected at the point of harvest stage, with physiological maturation (open mesh, that is, the fruits had spaces between the fruits extending and acquiring a light green color) (MAPA 2002).

Table 2. Morphoagronomic characteristics evaluated in the 91 pineapple clones.

Qualitative characteristics	
Plant habit	The position of the leaves of the plant was observed according to their angle of insertion and pendency. Being classified as open, when the leaves have an angle of approximately 90º starting from the stem of the plant. Erect, when the leaves are arranged at an angle of less than 90º and decumbent, when all the leaves are in the same direction.
Thorns	The presence and characteristics of the thorns on the leaves were observed. The leaf may be all prickly, with the thorns evenly distributed, possibly only at the apex of the leaves, or the leaf may not contain thorns.
Presence of anthocyanin	The presence of the purple color in the leaves and its intensity were examined. Anthocyanin is present at high or medium levels, or absent in the leaves.
External color of the fruit	The external color of the fruit was observed, and the ratings were assigned as white - cream, green, yellowish green, yellow, golden yellow, orange, light purple, dark purple, brown, or black.
Homogeneity in the external color of the fruit	It was observed whether the external coloring was regular, gradually regular, or irregular (with colored spots).
Fruit shape	The fruit was analyzed and classified according to its shape: inverted trapezoidal (narrower base), cylindrical, ovoid, conical, trapezoidal (wider base) or globose.
External aroma of the fruit	It was noted whether or not there was aroma in the fruits.
Eye profile	The profile of the eyes was evaluating in the middle part of the fruit. Classification is prominent, slightly prominent, flat, or concave.
Cracks or fissures in the eyes	It was noted whether or not there were cracks or fissures in the middle part of the fruit.
Size of eye bracts	The size of the eye bract was measured using a caliper in the middle part of the fruit and the size of the bract compared with the size of the eye of the fruit. It can be ¼ of the eye, ½ of the eye, ¾ of the eye, equal to the eye or larger than the eye.
Pulp color	The pulp color was evaluated in the middle part of the fruit, being classified as creamy white, pale yellow, yellow, or intense yellow.
Pulp texture	Pulp was evaluated between the heart and the locule of the slice of the middle part of the fruit and characterized as smooth, juicy, or fibrous.
Pulp aroma	Through smell, the aroma of the fruit pulp was classified, ranging from little, medium, and strong.
Depth of the eyes in relation I'm the central axis	Eye depth was observed and classified as shallow, intermediate, or deep.
Insertion of the crown into the fruit	The insertion of the crown into the fruit was observed and divided into light collar, wide collar, or no collar.
Crown habit	The position of the crown leaves was analyzed according to their insertion angle, being erect when the leaves were facing upwards, with an angle less than 90º, open with an angle of approximately 90º and decumbent when the leaves were facing only one side of the crown.
Quantitative characteristics	
Plant height	With a measuring tape, the height of the plant was measured from the ground to the highest leaf in the natural position of the plant in centimeters.
D sheet length	Leaf D was measured in centimeters from its insertion on the stem the tip of the leaf, using a measuring tape.
Sheet width D	On the same sheet previously measured and with the tape, the widest part of the sheet was measured.
Number of active sheets	The number of green and / or active leaves of the plant was counted.
Number of seedlings	The number of pups (plantlets) per plant was counted.

Fruit paste with crown	The fruits were weighed with crowns.
Fruit mass without crown	The fruits were weighed without crowns.
Fruit length	This was measured longitudinally, from the base of the fruit to the apex, without the crown.
Top fruit diameter	Next to the crown this was measured transversally..
Average fruit diameter	This was measured in the middle part of the fruit, transversely.
Bottom diameter of the fruit	The fruit was measured close to the peduncle.
Horizontal eye diameter	Evaluated in the middle part of the fruit with the aid of a caliper.
Vertical eye diameter	Evaluated in the middle part of the fruit with the aid of a caliper.
Diameter of the central shaft	The fruit was cut transversally in the middle part and the maximum diameter was measured, with a caliper.
Number of crowns	The number of crowns on the harvested fruits was counted.
Crown weight	the crowns were weighed on a precision scale, in grams.
Crown length	This was measured from the base of the crown to the longest leaf in its natural form.
Total soluble solids	With the extraction of the juice, two drops were dripped into a digital refractometer and the values were given in % in °Brix.
Total titratable acidity	This was determined by titration. 10 mL of pineapple juice were added to beakers, then 50 mL of distilled water and 2 drops of phenolphthalein solution were added, and then the solution was titred with 0.1 M sodium hydroxide (NaOH) under constant stirring, until a pink color persisted for 30 seconds. With the amount of NaOH used, the percentage of citrus acid in the juice was calculated.
Ratio (SST/ATT)	Determined by dividing the values found for soluble solids and titratable acidity.

Analysis of genetic variability

Quantitative and qualitative variables were analyzed simultaneously, using the Ward-MLM procedure to compose the genotype groups, using the Cluster procedure and interactive matrix programming (IML) of the SAS program (SAS Institute 2009). For use of the Ward clustering method, the distance matrix was obtained by the Gower algorithm (Gower 1971).

The definition of the number of groups was through the logarithmic probability function (Log - Likelihood) according to the criteria of pseudo - F and pseudo - t² combined with the likelihood profile, associated with the likelihood ratio test (Franco et al. al. 1998). Based on the definition of the ideal number of groups, the hierarchical classification was obtained using the Ward method, which provided initial values of the parameters needed to implement the final stage of the MLM model (Franco et al. 1998).

The Ward-MLM strategy consists of two phases, in which, in the first phase, the groups are defined by the Ward clustering method (Ward Junior 1963), using the Gower dissimilarity matrix (Gower 1971), and in the second phase, the mean vector of the quantitative variable for each subpopulation, regardless of the values of the qualitative variable, being estimated by the MLM procedure.

Gower dissimilarity index was used because the set of variables under study formed a mixed group. Next, Gower 's algorithm was employed (Gower 1971) to obtain the distance matrix for using Ward's clustering method. The Gower index uses qualitative and quantitative data to generate a single dissimilarity index, ranging from 0 to 1. Dissimilarity was given by:

$$S_{ij}= \frac{\sum_{k=1}^{p} W_{ijk} .S_{ijk}}{\sum_{K=1}^{p} W_{ijk}} \qquad \text{(Eq. 1)}$$

where k is the number of variables (k = 1, 2, …, p= total number of evaluated characteristics); i and j, any two individuals; W-$_{ijk}$ is a weight given to the ijk comparison, assigning value 1 for valid comparisons and value 0 for invalid comparisons (when the value of the variable is absent in one or both individuals); S-$_{ijk}$ is the contribution of the variable k to the similarity between individuals i and j, having values between 0 and 1. On the other hand, when the variable is quantitative:

$$S_{ij}= \frac{|Y_{ik} - Y_{jk}|}{R_k} \quad \text{(Eq. 2)}$$

where the amplitude R_{-k} = the amplitude of variation of the variable k, having values between 0 and 1. The value of W_{ijk} was used to define the contributions of individuals S-ijk . Thus, when the value of the variable k is absent in one or both individuals, $W_{ijk} = 0$ or, otherwise, it is equal to 1.

The distances between accessions were observed in the file named *distifile* generated by SAS (2003). Then, the logarithm graph of the likelihood function was obtained, maximized, according to the MLM method, where the best number of groups for the analysis was defined. Finally, the complete MLM analysis was performed for the number of groups (g) defined, describing the results of the classification, with a table describing the groups formed and the canonical analysis for the quantitative variables, using, for the latter, the *canfile*, obtained by SAS (2003), containing the canonical coordinates for the observations.

RESULTS AND DISCUSSION

Based on the log-likelihood function, the ideal number of groups was established at three. Group I consisted of 34 clones, group II of 25 and group III of 32 (Table 3). There was polymorphism for few analyzed variables, however, for the most interesting ones from the market point of view, such as fruit mass with crown and fruit length, the groups were, which reveals the existence of morphoagronomic variability between clones for these and other features. For plant-related variables (plant height, D-leaf length and number of seedlings), the three groups had similar values (Table 3). The average height of the Smooth Cayenne cultivar, the most cultivated in the world, is 100 cm (Chan et al., 2003), a value close to that observed in the three groups. However, these attributes of plant development vary according to genotype, cultural practices and edaphoclimatic conditions.

The length of the leaf D is used as an indicator of the ideal period for artificial floral induction and for the prediction of fruit mass at harvest (Guarçoni and Ventura 2011). In this study, the clones showed values below 1 m in the three groups, which, together with the height of the plant, show their vigor. It is noteworthy that the values of length and width of the D leaf are strongly influenced by both the genotype and the environment (Küster et al. 2018).

The clones in group III had a larger number of active leaves (36.92) and also a greater width of the D leaf (5.52), consequently greater leaf area (Table 3), increasing the photosynthetic rates and transpiration of the plants. This reflects on the plant's ability to intercept radiation and effect gas exchange with the environment, thus constituting an important indicator of plant productivity (Favarin et al. 2002). The number of leaves of all clones was similar to the Pérola cultivar, which, at the time of floral induction, had 25 to 30 leaves. The reduced number is attributed to the greater spacing between leaves, although this is offset by their greater length (Siebeneichler et al. 2008). However, these characteristics are also related to the previously mentioned developmental attributes and the wide genetic variability of the cultivars used in crosses.

Table 3. Means of the quantitative variables of the three groups formed by the Ward-MLM strategy and the canonical variables for the pineapple clones.

Characteristics	Groups			Canonical variables	
	G1 (34)	G2 (25)	G3 (32)	VC1	VC2
Plant height (cm)	93.54	94.64	96.58	0.05	-0.10
Leaf length D (cm)	87.70	83.56	87.91	0.18	0.08
Leaf width D (cm)	4.98	5.09	5.52	0.19	-0.26
Number of active leaves (cm)	29.30	30.59	36.92	0.28	-0.34
Number of seedlings (un)	5.69	6.05	5.52	-0.10	-0.01
Fruit mass with crown (kg)	1.26	1.21	1.60	0.47	-0.32
Fruit mass without crown (kg)	1.14	0.96	1.49	0.65	-0.26
Fruit length (cm)	15.87	13.79	19.41	0.59	-0.21
Upper diameter of the fruit (cm)	8.88	9.5	9.42	-0.04	-0.21
Mean fruit diameter (cm)	10.68	10.6	12.06	0.41	-0.34
Lower diameter of the fruit (cm)	9.26	9.08	10.00	0.36	-0.23
Horizontal eye diameter (cm)	2.16	1.99	2.15	0.35	0.20
Vertical eye diameter (cm)	2.39	2.27	2.55	0.39	-0.09
Diameter of the central axis (cm)	1.99	2.09	2.09	0.01	-0.15
Number of crowns (un)	1.13	3.26	1.33	-0.23	-0.14
Crown weight (g)	122.58	247.16	113.72	-0.49	-0.20
Length of crown (cm)	16.37	19.41	14.75	-0.43	-0.01
Total soluble solids (°Brix)	13.43	14.02	13.15	-0.26	-0.02
Average total soluble solids (°Brix)	14.59	14.97	14.74	-0.07	-0.08
Total titratable acidity (%AC)	0.90	0.91	1.19	0.20	-0.19
Average total titratable acidity (%AC)	0.80	0.87	0.92	0.10	-0.35
Higher Ratio	16.56	17.66	13.72	-0.41	0.20
Mean Ratio	20.26	18.72	17.15	-0.14	0.37
Lower Ratio	24.45	21.67	20.42	-0.08	0.46

Clones from group III had the highest averages for fruit mass with and without crown (1.60 and 1.49kg respectively), length (19.41 cm) and average fruit diameter (12.06 cm). This is an advantage for the producer as larger and heavier fruits have smaller and lighter crowns and this combination ends up attracting consumer preference (Table 3). On the other hand, groups I and II had the lowest means for these characteristics and were more similar to each other (Table 2). The MD-2 cultivar used worldwide as a cultivar for fresh fruit, produces a fruit ranging from 1.3 to 2.5 kg. Smooth Cayenne, on the other hand, produces fruits weighing 1.5 to 2.5 kg, used in processing industries, and the cultivar Pérola, used in Brazil in natura, produces fruits weighing 0.9 to 1.6 kg (Sanewski et al., 2018). The values observed in this study for these variables are close to those found for the most used varieties; therefore, the clones present in group III are the most indicated for the continuation of the genetic improvement program aiming at the consumption of fruits in natura.

Fruit mass within a breeding program, as well as other traits, varied between clones because the parents were genetically different. In the case of pineapple that is vegetatively propagated, if a promising clone is identified in the segregating population, it can be cloned and distributed, as the genotype is fully inherited.

As for the number of crowns, it was observed that the fruits belonging to group II had more than three crowns, consequently, greater weight (Table 3). This increase has a negative effect on the plant, and may cause fasciation of the fruit, making its commercialization unfeasible. According to Rohrbach and Johnson (2003), several crowns (two or more) develop when young fruits are exposed to high temperatures at the beginning of the development stage.

Other hypotheses were formulated to explain the emergence of more than one crown on the fruit. Bartholomew and Sanewski (2018) report that multiple crowns may have a genetic or environmental origin, and may be associated with high soil fertility and rapid plant growth after a period of prolonged drought, if such conditions occur at the time of inflorescence onset (Collins 1960 ; Py et al. 1987). They may also be related to calcium deficiency (Swete Kelly and Bartholomew 1993). This problem deserves attention, since the Brazilian trade is mainly based on the sale of the fruit in natura with the presence of the crown. Therefore, when it comes to productivity, the clones belonging to group II are not promising because, in addition to having a greater number of crowns, they also have a lower fruit mass. The other quantitative variables related to the fruit did not vary between groups, such as the upper and lower diameter of the fruit (cm), diameter of the horizontal and vertical eyes of the fruit (cm) and diameter of the central axis (cm). The same was observed for chemical characteristics such as total soluble solids (13.15 to 16.22 ºBrix), titratable acidity (0.74 to 1.19) and ratio (13.72 to 24.45) for the upper and lower portions of the fruit respectively, being considered of lesser relative importance for genetic diversity in this study (Table 3). The total soluble solids content is the parameter most correlated with food quality and is always used as a quality criterion to select fruits suitable for the fresh market (Smith 1988). A minimum total soluble solids requirement of 12º Brix is used in Brazil, Hawaii and Australia (MAPA 2008; Lobo and Yahia 2017). In India, the preference is from 13.8 to 17% and the ratio varies from 20.83 to 27.24 (Morton, 1987). These values were similar to those observed in this work. It is noteworthy that the ratio is a quality index that is related to the sweetness of the fruit, therefore fruits with a higher ratio have a more pronounced sweetness and, consequently, greater acceptance by the consumer (Viana et al. 2013). It is noteworthy that the acid level is higher at the top of the fruit, while the total soluble solids are higher at the bottom due to maturity differences within the fruit (Paull et al., 2017).

Based on the first canonical variable, the quantitative characteristics that most contributed to the existing genetic diversity among the clones were crownless fruit mass (0.65) and fruit length (0.59) (Table 3). The fruit mass is the characteristic of greatest interest to the producer, combined with the absence of thorns on the leaves and resistance to fusariosis. The characteristics mass of the uncrowned fruit and fruit length provided greater variability among clones and are positively correlated, since the greater the length of the fruit, the greater its mass.

Analyzing the qualitative characteristics, heterogeneity was observed between groups for all variables, except for plant habit, in which the open type predominated (Table 4). Although the other variables were heterogeneous both within and between groups, some characteristics predominated, such as the presence of medium anthocyanin in the leaves, external color of the green fruit, cylindrical fruit shape, absent external aroma of the fruit, prominent profile of the eyes, presence of slits. or fissures in the fruit, size of the eye bracts of ¾ of the eye, texture of the succulent pulp, shallow depth of the eyes in relation to the central axis and insertion of the crown to the fruit with a wide collar.

Fruit shape is an important quality characteristic. For the export market *in natura*, the conical fruit is rejected from the moment of packaging, preferring fruits with a cylindrical shape. The demand for the industrial market is also for cylindrical fruits, as they have a higher pulp yield (Py et al. 1984). In this sense, more than 40% of the clones evaluated in each group had a cylindrical fruit shape, being interesting for later selection.

Table 4. Variables and number of genotypes per group for qualitative characteristics in each of the three groups (GI, GII and GIII) formed by the Ward MLM strategy for the pineapple clones.

Characteristics	Classification	Groups		
		GI (34)	GII (25)	GIII (32)
Plant Habit	Open	33	25	31
	Erect	1	0	1
Thorns	With	20	13	18
	Without	14	12	14
Presence of anthocyanin	Absent	6	7	9
	Mean	19	11	17
	A lot	9	7	6
Fruit external color	Green	21	13	16
	Yellowish green	9	4	7
	Light purple	1	5	4
	Dark purple	3	3	5
Homogeneity in the external color of the fruit	Gradually regular	12	11	13
	Regular	21	14	19
Fruit shape	Cylindrical	16	10	15
	Ovoid	2	2	1
	Conical	5	7	11
	Trapezoidal	4	2	4
	Globose	7	4	1
External fruit aroma	Present	3	3	3
	Absent	31	22	29
Eye profile	Prominent	24	20	16
	Little prominent	9	4	15
	Flat	1	1	1
Cracks or fissures in the eyes	Absent	0	4	1
	Present	34	21	31
Size of the eye bracts	¼ of the eye	1	2	0
	½ of the eye	8	7	9
	¾ of the eye	21	14	20
	equal to eye	4	2	3
Pulp color	White-cream	16	11	14
	Pale yellow	12	10	16
	Yellow	6	3	2
	Intense yellow	0	1	0
Pulp texture	Mild	11	6	14
	Succulent	21	18	18
	Fibrous	2	1	0
Pulp aroma	Little	12	15	14
	Medium	15	5	10
	A lot	7	5	8
Depth of the eyes in relation to the central axis	Shallow	29	24	28
	Intermediate	5	1	4
Insertion of the crown in the fruit	With light collar	14	10	12
	With wide collar	5	2	2
	Without collar	15	13	18
Habit of the crown	Erect	16	13	10
	Open	17	10	22
	Decumbent	1	2	0

The texture of the pulp in the clones was predominantly juicy in all groups and the depth of the eyes in relation to the central axis was shallow, with more than 85.3% between groups. These characteristics are also important for a clone, as it has a low fiber content and a higher amount of pulp. These characteristics can be added to clones that have a cylindrical fruit shape, making the clone more promising, with a higher number of agronomic characteristics of interest to the producer and consumer.

The population showed variability for the characteristic spinescence. Of the evaluated clones, 44.3% did not have spines. This is advantageous, showing that it is possible to select genotypes without thorns, adding other important characteristics mentioned above. Regarding the crown habit, 31% of the clones had the erect type, it is important to highlight that the three groups had a small crown, ranging from 14.7 to 19.4cm (Table 3). Small, upright crown facilitates packing and transporting the fruit.

Quality attributes have varied importance according to the interests of each segment of the market chain. Growers prioritize appearance, that is, absence of defects, high profitability in production, as well as ease of harvesting and transportation, as well as resistance to disease. Retailers and wholesalers opt for the appearance of the fruit, which is considered the most important attribute, emphasizing firmness and good storage capacity of the fruit (Chitarra and Chitarra 2005). Therefore, it is possible to select clones within groups that facilitate harvesting and transport.

For the variables pulp color and aroma, there was a predominance of white-cream and pale yellow with an average of 44.9 and 41.7% respectively. Regarding aroma, the highest percentages were observed between little, 46.3% and medium, 31.8% in all groups, showing great variability among clones. The characteristics of greatest interest to the producer and, consequently, to the genetic improvement of the pineapple plant for *fresh consumption,* are the yellow peel and pulp (ripe fruit) and the cylindrical shape (Melão et al. 2015). Groups I and III had the highest number of clones with these characteristics.

The three groups presented promising clones in relation to desirable qualitative characteristics such as absence of thorns, cylindrical fruit, and yellow pulp (Table 4). The crossing between these makes it possible to aggregate these advantageous attributes in a single clone. Commercially, in addition to the absence of thorns and the yellow to intense yellow pulp color, the cylindrical fruit shape is also one of the most sought-after characteristics in other breeding programs, as it can be used for export, since conical fruits are not well accepted. abroad. The most commercially accepted cultivar abroad for *in natura* consumption is MD-2. It has become the market standard for the fresh produce trade, as it produces cylindrical fruit, with square shoulders and a yellow flesh color (Sanewski et al., 2018). These characteristics of fruit color, fruit shape and pulp color are the most evaluated by consumers (Sampaio et al. 2011).

Relating the quantitative (Table 3) and qualitative (Table 4) variables, it can be noted that group III allocated clones of commercial interest with higher means than the other groups, for fruit mass with and without crown and fruit length. It also presented interesting individuals with no thorns, conical fruit, and yellowish pulp color. Within the groups, specific clones that present these characteristics can be selected for later crosses. For *in natura* consumption, group I presented 12 clones with yellowish pulp color, group II, 10 and group III, 16 clones. This pulp color is the objective of several breeding programs, however, it must be linked to other attributes, such as greater fruit mass and high total soluble solids content. Therefore, group III clones that meet all these requirements are more promising for fresh consumption.

Through the graphical analysis of the canonical variables, the greatest distance was observed between groups II and III (21.70) (Table 5). This indicates that they present greater genetic diversity for the characters studied. Despite being the largest distance observed between the groups, this distance is still considered small, which can be explained by the fact that the segregating population has common parents. The cultivars used as pollen receivers (I AC Fantástico, BRS Imperial and BRS Vitória) have the Smooth Cayenne cultivar as a common parent, hence the proximity between the clones (Sanewski et al., 2018).

Table 5. Distance between groups formed by the Ward-MLM method for quantitative and qualitative variables of pineapple clones.

Groups	II	III
I	15.06	10.96
II	--	21.70

In turn, the smallest distance was observed between groups I and III, with an estimated value of 10.96, showing high similarity between the clones that make up these groups (Table 5). To avoid reducing genetic variability, as well as restricting possible gains obtained with selection, crosses between clones from groups I and III are not recommended. This is because genetically related parents tend to have many common alleles and therefore offer little advantage (Cruz et al. 2014).

By graphical analysis of the canonical variables (CAN), the first two variables obtained by the Ward-MLM method explained 100% of the total variation of the clones (Figure 1). According to Cruz and Carneiro (2006), if the first two canonical variables allow estimates above 80% of the total variation, a satisfactory interpretation of the variability between accessions can be obtained, making the two-dimensional graphic representation possible, as occurred in this study. In Figure 1, it can be noted how the clones belonging to group II are more distant from each other, with greater variability within when compared to the other groups. This variability within the group can be explored as long as they present characteristics of interest to the breeder.

In genetic improvement, genetic diversity studies are of fundamental importance for the choice of genotypes to be used as parents, since the genetic distance between the parents is indicative of the heterotic expression in the progenies (Cruz et al. 1994), which increases the possibility of obtaining superior individuals in segregating populations. These authors also state that, in addition to the divergence between parents, it is essential to consider individual merit for the characteristics that one is interested in improving. This study, based on the genetic divergence of segregating pineapple populations, enabled the identification of superior clones, mainly with regard to the agronomic characteristics of the fruits, which will provide more advantageous gains due to their selection.

As most of the current breeding programs are focused on the development of cultivars for the *in natura* market, such as Unemat, the most desired traits in the main breeding programs according to Py et al. (1984) and Ramalho et al. (2009) are plants without thorns, cylindrical fruit shape, yellow pulp color and high content of total soluble solids. Therefore, crosses between group III clones that had higher agronomic performance and absence of thorns, with group II clones that have cylindrical fruits, pulp ranging from pale yellow to yellow and juicy texture can be recommended, aiming at complementing favorable alleles and greater exploration of the heterotic effect for the evaluated characteristics.

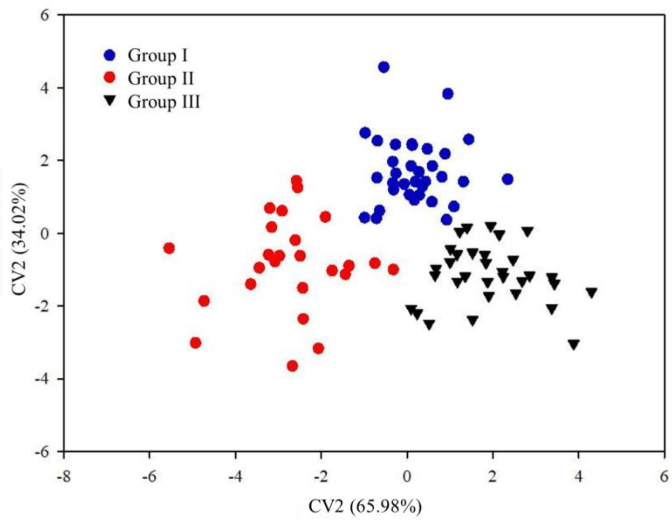

Figure 1. Dispersion of the first two canonical variables (CV1 and CV2) for the three pineapple clone groups formed by the Ward-MLM procedure.

CONCLUSIONS

We found genetic variability among the pineapple genotypes for the main quantitative and qualitative morpho-agronomic variables, such as mass of the fruit without crown, length of the fruit, plants without thorns, fruit with a cylindrical shape and with a whitish to yellow pulp color.

The Ward MLM grouping method provided a graphic visualization of the genetic divergence between the pineapple clones, in which the formed groups presented very different characteristics. To explore the existing variability, crosses between clones with desirable morphoagronomic characteristics would be recommended between individuals from groups II and III.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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