

Variation among pineapple half-sibs and selecting genitors based on potential genetic divergence

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Abstract

Knowledge about variation and relative importance of agronomic traits for genetic divergence studies can reveal useful information to guide the breeding programs. The objective of this study was to evaluate the phenotypic variation, and select of pineapple half-sib seedlings based on genetic divergence of fruit and plant traits. 'Pérola' cultivar (female genitor) received a pollen mix as from cultivars 'MD-2', 'BRS Imperial' and 'BRS Vitória' (male genitors). Four hundred twenty-nine F₁ individuals propagated from seeds were evaluated under field conditions. Descriptive statistics, Singh's (1981) relative contribution, and Tocher's cluster optimization methods based on the distances matrix were estimated. Coefficients of variation ranged from 9.89 % to 63.79 %. Regarding total variance, fruit traits grouped 52.69%, while plant traits accumulated 47.30%. These results demonstrated that evaluated traits contribute for half-sib progeny relative discrimination and that none of them should be discarded for studies of diversity. Among the 12 heterotic clusters formed, group VII, IX and VIII are recommended to compose hybridization blocks and evaluation cycles of phenotypic stability for use *per se*. These groups have a broad heterotic potential, and desirable agronomic traits, mainly regarding to high means for fruit mass without crown (FMWC) upper than 4,000g and soluble solids content (SSC) around 20-21°Brix, which can be used by the IPA's pineapple breeding program.

Keywords: *Ananas comosus*; Plant breeding; Variance; Dissimilarity; Clustering

1 Introduction

Pineapple (*Ananas comosus* var. *comosus*) is a fruit that standing out among the most cultivated tropical fruit species and high economic importance around the world. Distributed about 80 countries, the world pineapple production was estimated at 28,179,348 tons, harvested in a wide area of 1,125,307 ha, in 2019 [1]. The largest producing of 3,328,100 tons was the Costa Rica, follow of 2,747,856 tons (Philippines) and 2,426,526 tons (Brazil).

The main pineapple cultivar planted in Brazil, known as 'Pérola' or 'Branco de Pernambuco' [2], it has vigorous growth, with long leaves and dark green color. Its fruit has a conical shape, mainly directed to the domestic fresh fruit market. Average fruit weight is 1.6kg; the pulp has a cream-white color with abundant juice and intense aroma, and total soluble solids are between 13-16 °Brix. This cultivar is highly susceptible to fusariosis, a fungal disease (*Fusarium guttiforme*) that severely affects Brazilian production.

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In Brazil, the development of new pineapple cultivars aims to combine genes that control resistance to fusariosis and leaf margins without spines in a single plant, as well as genes of dozens of other traits related to improvement of fruit quality and greater yield stability [3,4,5].

Routinely, pineapple breeding programs generates and processes data bases with thousands of observations referring to dozens of traits related to the productivity and fruit quality, aiming to identify unexplored patterns or behaviors in this broad data set [3,6,7]. Knowledge about phenotypic variation of agronomic traits and diversity among genotypes based on dissimilarity coefficients can reveal useful information to guide breeding programs.

In general, analysis of the predictive genetic diversity aims identify more divergent individuals with high averages for important agronomic traits. Its importance is highlighted because more divergent individuals are the most suitable to produce a high heterotic effect. The degree of genetic diversity expresses some measure or coefficient of dissimilarity based on morphological, physiological or molecular differences between individuals [8,9,10].

The expectation of heterosis manifested among descendants is a function of the gene dominance effects to the interest trait and square of the genetic frequencies difference of their parents, in addition to epistatic effects, which are generally neglected [11,12].

These studies are applicable to identify promising F_1 individuals with wide divergence and higher mean for those traits related to the fruit quality and plant productivity. After this step these selected individuals can be used for cultivation *per se* and/or like a potential genitor in hybridization plans [6,7,13].

The Brazilian pineapple breeding programs have focused on recombining alleles of qualitative traits that control spineless leaf margin and resistance to fusariosis (*Fusarium guttiforme*) [3,4,5]. However, other many quantitative traits should be also considered for the selection strategies, associated to fruit quality and higher production stability.

The objective of this study was to evaluate the phenotypic variation of fruit and plant traits among pineapple half-sibs, and select individuals based on potential genetic divergence for genitors in hybridization plans of the breeding program of the Agronomic Institute of Pernambuco – IPA, Brazil.

2 Material and methods

The field trial was carried out in the Experimental Station of Itambé, Pernambuco State, Brazil (lat 7° 24' 16.80" S, long 35° 10' 54.00" W, alt 190 m asl) belonging to the Agronomic Institute of Pernambuco (IPA). The municipality of Itambé is located in North region of the 'Zona da Mata'. The climate of this region is hot and humid As (Köppen) with 25°C of mean annual temperature, and 1.200 mm.year⁻¹ of mean rainfall.

2.1 Plant materials

Plant material was represented by 429 half-sib individuals of pineapple (*Ananas comosus* var. *comosus*). These individuals (seedlings) propagated from seed were developed by the IPA's Pineapple Genetic Breeding Program. Seedlings were obtained via pollination using 'Pérola' as female genitor, that it received a pollen mix from 'MD-2', 'BRS Imperial' and 'BRS Vitória' as male genitors.

Table 1 Agronomic information about the cultivars used as genitors

Traits	Cultivars			
	'Pérola'	'MD-2'	'BRS Imperial'	'BRS Vitória'
Fusariosis	Susceptible	Susceptible	resistant	Resistant
Fruit mass(kg)	0.9 - 1.6	1.3 - 2.5	0.9 - 1.3	1.5
Soluble solids (°Brix)	13 – 16	15 – 17	14 – 18	15.8
Pulp color	White	Yellow	yellow	White
Fruit shape	Conical	cylindrical	cylindrical	Cylindrical
Leaf margin	Spiny	spiny tip or base	piping	Piping

Source: Sanewski et al. [14], Junghans [15].

These cultivars were chosen due to their contrasting and complementary agronomic traits (Table 1). Regarding the origin these cultivars, 'Pérola' is a pre-Columbian plant, cultivated by indigenous peoples before Europeans arrived in America.

Regarding the origin these cultivars; 'Pérola' is a pre-Columbian plant, cultivated by indigenous peoples before Europeans arrived in American continent; 'MD-2' is across between 'PRI 58-1184' and 'PRI 59-443', the parentage includes 'Smooth Cayenne' and others several cultivars. It was first marketed by Del Monte Inc. in 1996; 'BRS Imperial' is a cross between 'Perolera' and 'Smooth Cayenne' performed by EMBRAPA and released 2003; 'BRS Vitória' is a cross between 'Primavera' and 'Smooth Cayenne' performed by EMBRAPA and released in 2006 [14,15].

2.1.1 Cultivation of genitor plants

In 2014 May, genitors were planted in plastic bucket of volume for 10 L, containing soil (2/3) and cattle manure (1/3) as substrate, and 10 g of simple superphosphate per 10 L of the mixture was added, that were kept in greenhouse with micro drip irrigation (Figure 1). Twenty slips to each cultivar-genitor was used, being one slip per plastic bucket.



Figure 1 Genitor plants cultivated in plastic bucket for cross pollination. IPA's Pineapple Genetic Breeding Program.
Photo Lira Júnior, J. S.

2.1.2 Floral induction treatment

When genitors completed 11 months old, floral induction treatment was performed in Abril 2015. Calcium carbide via liquid form as floral inductor was used. The appearance of inflorescences occurred between 2015 June and 2015 July.

2.1.3 Pollination

Pollination in the morning (from 6 am to 9 am), daily, until closure all flowers was performed (Figure 2). Both male and female genitor inflorescences were protected with kraft paper bag to avoid pollen contamination of unknown origin. Anthers were collected in flowers from male genitors ('MD-2', 'BRS Imperial' and 'BRS Vitória') and rubbed lightly over stigma of the female genitor flowers. After pollination, inflorescences were protected again.



Figure 2 Pollination of Pérola's emasculated flowers (Female genitor). IPA's Pineapple Genetic Breeding Program. Photo Lira Júnior, J. S

2.1.4 Germination and acclimatization

In December 2015, seeds extracted in the over-ripe 'Perola' fruits (Figure 3A). These seeds were washed in a sieve under running water and dried in kiln at 37°C for 48 hours.

In January 2016, seeds in plastic trays with blotting paper as a substrate and moistened with distilled water were sowed. Trays were sealed with PVC film and kept inside a seed germination chamber with 25 °C and under continuous light. Germination for up to 75 days after sowing was verified. Germinated seeds that emitted radicles when visible to naked eye were considered (Figure 3B).



Figure 3 Seeds extracted in the over-ripe fruit (A) and germinated in plastic trays with blotting paper as a substrate (B). IPA's Pineapple Genetic Breeding Program. Photos Lira Júnior, J. S.

In April 2016, at 90 days after sowing, acclimatization process was started, when this seedlings were transplanted into styrofoam trays containing commercial substrate Plantmax HT, and maintained under a shedding screen (50%) and micro-spray irrigation (Figure 4A).

At 100 days, after transplanting, these seedlings were individualized into black polyethylene bags (15cm x 10cm) containing 2/3 of soil + 1/3 of tanned bovine manure, and maintained under full sun with micro-spray irrigation (Figure

4B). This acclimatization period lasted for about 13 months, until May 2017, when seedlings reached approximately height of 30cm suitable for field planting.

2.2 Planting seedlings

Field trial lasted for about 23 months (from 2017 May to 2019 April). This period started with planting of individuals in the simple spacing of 1.2m x 0.6m, and cultivated according to rainfed system (Figure 5). Fertilization were performed following results of the soil fertility analysis, as recommendations reported by [16]: P = 5mg/dm³; pH = 5,7 (H₂O); Ca = 3,7cmolc.dm⁻³; Mg = 1,00cmolc.dm⁻³; Na = 0,03cmolc.dm⁻³; K = 0,27cmolc.dm⁻³; Al = 0,00cmolc.dm⁻³; H = 5,36cmolc.dm⁻³; S = 5,00cmolc.dm⁻³; CTC = 10,40cmolc.dm⁻³. The rainfall data for cultivation period on the field are presented in the Table 2. Plants do not received treatment for floral induction, whose inflorescences naturally were emitted.

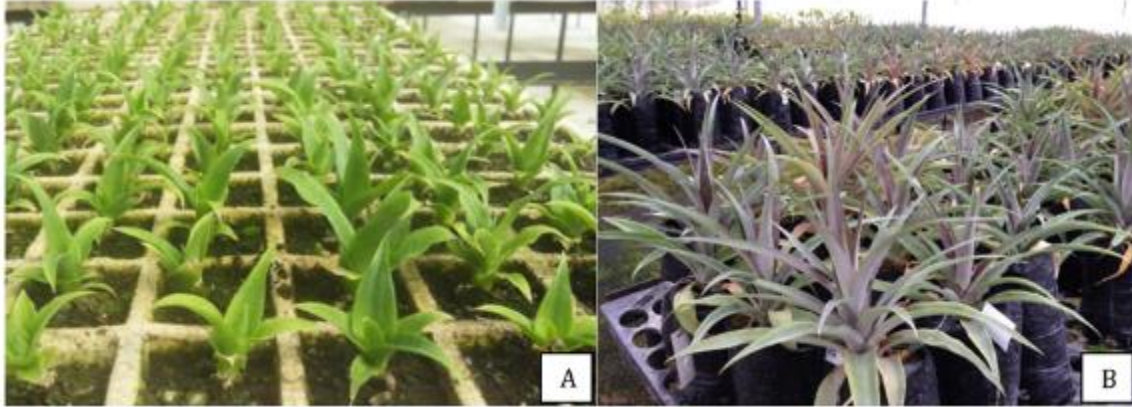


Figure 4 Transplanting (A) and acclimatization (B) of seedlings. IPA's Pineapple Genetic Breeding Program. Photos Lira Júnior, J. S.



Figure 5 Cultivation of half-sib progeny propagated from seed (sexual cycle), and developed by the IPA's Pineapple Genetic Breeding Program. Experimental Station of Itambé, PE, Brazil. Photo Lira Júnior, J. S.

Table 2 Monthly distribution of rainfall during the field cultivation period and historical monthly mean of 40 years. Agronomic Institute of Pernambuco State (IPA), Experimental Station of Itambé, Pernambuco State, Brazil.

Period	Month												Totals
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
2017/2018	147	191	380	83	63	40	5	8	201	217	105	235	1,675
2018/2019	175	136	124	2	4.5	3	4.7	48	82	155	193	144	1,072
Mean*	154	216	191	93	58	28	21	27	65	68	124	154	1,204

Source: Agência Pernambucana de Águas e Clima – APAC (<http://www.apac.pe.gov.br/>); * Mean of 40 years

2.3 Measured traits

Individual plants at the time of the ripe fruit harvest were measured. Fruit maturation for harvest was determined visually, and considering at least 50% change on coloring of the shell from green to yellowish or orange.

The follow 17 traits were evaluated, which nine are related to the fruit: 1) FMWC = fruit mass without crown (g), 2) CM = crown mass (g), 3) FL = fruit length (cm), 4) CFB = circumference of the fruit base (cm), 5) CFMT = circumference of the fruit middle third (cm), 6) CFA = circumference of the fruit apex (cm), 7) CL = crown length (cm), 8) DCA = diameter of the central axis (mm), and 9) SSC = soluble solids contents (°Brix); and eight are related to the plant: 10) PHFB = plant height to the fruit base (cm), 11) PH = plant height (cm), 12) PL = peduncle length (cm), 13) PD = peduncle diameter (mm), 14) LL = leaf length D (cm), 15) LW = leaf width (cm), 16) DSBL = diameter of the stem base with leaves (mm), 17) NAL = number of active leaves.

2.4 Statistical analysis

2.4.1 Descriptive statistics and relative contribution

The sampled data were used for calculate descriptive statistics for assessed fruit and plant traits: Mean (\bar{X}); minimum value (min. V.); maximum value (max. v.); standard deviation ($\hat{\sigma}$); coefficient of variation (CV%); lower limit (LL) and upper limit (UL), at the confidence interval with probability of 95%.

The methodology of Singh [17] relative contribution (S_j) for define which traits are most important for divergence among half-sib individuals was used, according to the following expression:

$$\text{Equation (I)} \sum_{j=1} S_j = \sum_{i < i'} \sum_{i'} d_{ii'}^2,$$

where: $d_{ii'}^2$ is the Square of the Standardized Euclidean Distance among half-sib individuals was estimated according to the following expression:

$$\text{Equation (II)} d_{ii'}^2 = \frac{1}{v} \sum_j (Y_{ij} - Y_{i'j})^2,$$

where: Y_{ij} is the value of the i -th individual, regarding to j -th trait, and v is the number of evaluated traits; Original data were standardized due to the different units of measurement, resulting in a mean = 0 and variance = 1, according to the following expression;

$$\text{Equation (III)} y_{ij} = \frac{Y_{ij}}{\hat{\sigma}_{y_j}},$$

where: $\hat{\sigma}_{y_j}$ is the standard deviation of the trait Y .

2.4.2 Tocher's cluster optimization method

In order to identify genitors to compose hybridization blocks and evaluation cycles of phenotypic stability for use per se, genetic diversity by estimating measures of dissimilarity was studied among individuals based on the 17 evaluated traits. Firstly, It was considered data set formed by those individuals with values \geq than overall means for fruit mass

without crown (FMWC) and soluble solids content (SSC). As reported by Cabral et al. [3,18] to many importance traits for the genetic improvement of pineapples in Brazil, this selection was designed from these values for fruit mass without crown (FMWC) and soluble solids contents (SSC).

In sequence, measures of dissimilarity were obtained by estimating the Square of the Mean Euclidean Distance (d_{ii}^2 , - Equation II) from the standardized data (y_{ij} - Equation III). The generated matrix was used in the cluster analysis by the Tocher optimization method, adopting as a criterion that the mean intragroup distance must be lower than the mean intergroup distance [12]. All statistical analyzes were performed using the software Genes [19].

3 Results and discussion

3.1 Descriptive statistics and relative contribution

Table 3 Descriptive statistics and Singh's relative contribution of plant and fruit traits measured on the 429 pineapple half-sib individuals, propagated by seeds (sexual cycle), under rainfed cultivation system. IPA-Experimental Station of Itambé, Pernambuco State, Brazil

Traits	CV (%)	Value		Mean	Standard deviation	CI (95%)		S_j (%)
		Min.	Max.			LL	UL	
1. Fruit	-	-	-	-	-	-	-	52.69
FMWC(g)	30.43	382.00	5,393.00	1,992.10	606.35	1,930.62	2,050.65	3.76
CM(g)	63.79	10.00	360.00	90.96	58.03	85.08	96.57	7.06
FL (cm)	24.31	10.80	48.20	23.73	5.77	23.14	24.28	6.12
CFB (cm)	10.41	24.00	56.20	39.96	4.16	39.53	40.36	4.30
CFMT (cm)	9.89	25.80	51.20	38.21	3.78	37.82	38.57	5.70
CFA (cm)	18.52	15.80	46.20	29.36	5.44	28.81	29.88	8.24
CL (cm)	38.04	3.20	36.70	13.43	5.11	12.91	13.92	5.98
DCA (mm)	22.56	10.00	42.00	20.47	4.62	20.00	20.91	5.35
SSC (°Brix)	16.38	9.60	26.40	15.93	2.61	15.66	16.18	6.18
2. Plant	-	-	-	-	-	-	-	47.31
PHFB (cm)	15.45	18.20	68.40	46.12	7.13	45.39	46.80	5.18
PH (cm)	18.07	30.30	114.10	79.55	14.38	78.09	80.93	7.56
PL (cm)	16.14	9.7	34.7	19.29	3.11	18.97	19.59	3.99
PD (mm)	18.10	16	61	31.63	5.72	31.04	32.17	4.16
LL (cm)	13.53	54.6	118.4	85.76	11.60	84.58	86.88	8.50
LW (mm)	13.68	39	88	60.33	8.25	59.49	61.12	7.29
DSBL (mm)	14.99	36	152	83.89	12.57	82.61	85.09	3.02
NAL	22.02	11	58	36.74	8.09	35.92	37.52	7.61

CV = coefficient of variation; lower limit (LL) and upper limit (UL), at the confidence interval (CI) with probability of 95 %; S_j = relative contribution (%); FMWC = fruit mass without crown; CM = crown mass; FL = fruit length; CFB = circumference of the fruit base; CFMT = circumference of the fruit middle third; CFA = circumference of the fruit apex; CL = crown length; DCA = diameter of the central axis; SSC = soluble solids contents; PHFB = plant height to the fruit base; PH = plant height; PL = peduncle length; PD = peduncle diameter; LL = leaf length D; LW = leaf width; DSBL = diameter of the stem base with leaves; NAL = number of active leaves.

Descriptive statistics based on phenotypic data revealed a high variation for fruit and plant traits (Table 3). This result is important because it indicates a high potential for selecting descendants with recombinant traits for fruit quality and agronomic attributes that were inherited from their parents ('Pérola', 'MD-2', 'BRS Imperial', and 'BRS Vitória').

Those traits that are related to the fruit reached coefficients of variation (CV's) between 9.89% and 63.79%, which resulted amplitude of 53.90% and, consequently, revealed a wide dispersion of the data around the respective means (Table 3). Plant traits presented CV's from 13.53% to 22.02%, which resulted an amplitude of 8.49% and, consequently, a lowest data dispersion, when it is compared to the group of fruit traits (Table 3).

For example, a portion of this variation can be observed in the Figure 6 for fruit and plant traits among some individuals that were developed by IPA's Pineapple Breeding Program.



Figure 6 Sample of the phenotypic variation (coloring, shape, size, and growth habit) for fruit (A) and plant (B) traits among some half-sib individuals. IPA's Pineapple Genetic Breeding Program. Experimental Station of Itambé, PE, Brazil. Photos Lira Júnior, J. S.

Considering similar traits, these results are comparable to the CV's of 9.01% and 46.11%, reported by Cabral et al. [18] for selected F₁ genotypes that by hybridization among different cultivars were obtained. These authors commented that high CV's are expected because they are hybrid progenies with a great variability among descendants. According to Chan [6], pineapple cultivars are heterozygous and hybridization among them often leads to development of fertile seeds that contains a wide spectrum of genotypic combinations. From this segregating F₁ population it is possible to select excellent individuals with trait recombinants within a progeny composed by numerous descendants.

Among the fruit traits that reached the highest CV values: Crown mass (CM) varied from 10g to 360g with a standard deviation of 58.03g around the mean of 90.96g; crown length (CL) varied from 3.2cm to 36.7cm, which resulted in a standard deviation of 5.11cm, around the mean of 13.43cm; fruit mass without crown (FMWC) reached from 382g to 5,393g that resulted in a standard deviation of 606.35g around the mean of 1,992.10g, and 95% confidence interval of 1,930.62g to 2,050.65g (Table 3).

Regarding the traits that achieved intermediate CV values: Fruit length (FL) varied from 10.8cm to 48.2cm, whose standard deviation was 5.77cm around the mean of 23.73cm; soluble solids contents (SSC) varied from 9.6°Brix to 26.40°Brix, with standard deviation of 2.61°Brix around the mean of 15.93°Brix and 95% confidence interval of 15.66°Brix to 16.18°Brix; circumference of the fruit apex (CFA) reached from 15.80 to 46.20 cm with standard deviation of 5.44cm, around the mean of 29.36cm, and lower limit of 28.81cm and upper limit of 29.88cm, at the confidence interval with probability of 95%; diameter of the central axis (DCA) varied from 10mm to 42mm, with standard deviation of 4.62mm and around mean of 20.47mm.

Lowest CV values were achieved for circumferences of the fruit middle third (CFMT) and of the fruit base (CFB). Consequently, these traits stood out among those that exhibited the smaller variations: While CFMT varied from 25.80cm to 51.20cm, with standard deviation of 3.78cm around the mean of 38.21cm; CFB achieved mean of 39.96cm, ranging from 24.00cm to 56.20cm and standard deviation 4.16cm.

Amid plant traits that achieved the highest CV value: Number of active leaves (NAL) varied from 11 to 58, with standard deviation of 8.08 around mean of 36.74.

For those plant traits that reached the intermediate CV values: Peduncle diameter (PD) with mean of 31.63mm and standard deviation of 5,72mm, achieved values between 16mm and 61mm; Plant height (PH) presented standard deviation of 14.38cm and mean of 79.55cm, with variation between 30.30cm and 114.10cm; Peduncle length (PL) varied from 9.7cm to 34.7cm, with standard deviation of 3.11cm, and around the mean of 19.29cm; Plant height to the fruit base (PHFB) presented variation from 18.20cm to 68.40cm, standard deviation of 7.13cm, and around the mean 46.12cm.

Regarding to plant traits which achieved the smallest CV values: diameter of the stem base with leaves (DSBL) with mean of 83.89mm and standard deviation of 12.57mm, varied from 36mm to 152 mm; Leaf width (LW) varied from 39mm to 88mm, with standard deviation of 8.25mm, and around the mean of 60.33mm; Leaf length (LL) varied from 54.6cm to 118.4cm with standard deviation of 11.60cm, and around mean of 85,86cm.

These results are within of the wide range from 85.38g to 176.45g for the mean crown weight and from 1,102.38g to 1,857.27g for fruit weight [18], regarding to selected F₁ genotypes in progenies from different crosses. According to Chan and Lee [20], fruit weight of F₁ pineapple hybrids selected for early fruiting varied from 1.36kg to 1.73kg. According Nhat Hang et al. [13], from several F₁ hybrids three were better in terms of fruit cylinder shape, fruit weight (1,500g-1,760g), and edible portion (56.3%-75.4%). Lira Júnior et al. [21] reported genotypic values (BLUP) of pineapple hybrids between 1.60kg and 2.72kg for fruit mass without crown.

Regarding to plant traits, Cabral et al. [18] reported means between 29.63cm and 61.18cm for plant height and 16.37cm a 27.14cm for peduncle length. According to these authors, an elite pineapple cultivar must exhibits a smallest peduncle length than 20cm to avoid sunburn and breakdown peduncle.

For soluble solids contents (SSC) these results (Table 3) exceed the mean values of 13.21°Brix at 14.99°Brix [18]. Lira Júnior et al. [21] reported genotypic values (BLUP) of F₁ hybrids between 15.90°Brix and 21.86°Brix for soluble solids contents. According to Nhat Hang et al. [13], total soluble solids varied from 18.4% to 19.4% in a segregating progeny. Sriporaya et al. [7] evaluated a total of 886 F₁ individuals and three of them ranged from 18.0°Brix to 22.2°Brix for soluble solids contents.

Respecting to the relative contribution analysis, while fruit traits grouped 52.69% of the total variance, plant traits accumulated 47.31% (Table 3). The higher individual contributions were expressed by LL, CFA, NAL, PH, LW, CM, SSC, and FL traits, with values ranging from 6.00% to 8.50%. In an intermediate group, CL, CFMT, DCA, PHFB, CFB, and DP traits contributed with values between 4% and 6%. Despite these lower values of contributions, between 3% and 4%, PL, FMWC, and SD traits are important for relative importance study in the plant genetic breeding.

These results indicate that evaluated traits are essentials and contribute, relatively, for discrimination of the evaluated F₁ individuals, being not recommended discard them for genetic divergence studies.

3.1.1 Tocher's cluster optimization method

Among the 429 individuals evaluated, 144 were used for genetic diversity analysis with high means for fruit mass without crown (FMWC) \geq 1,992.10g and soluble solids content (SSC) \geq 15.93°Brix. Tocher's optimization grouping analysis resulted in the formation of 12 heterotic groups (Table 4).

The largest number of 95 individuals (65.97%) was gathered by group I, which contains individuals 04-62 and 04-23 with smallest distance measurement ($D^2 = 0.0895$) and, therefore, presented the least dissimilarity (Table 4). The maximum distance ($D^2 = 5.1371$) was estimated between individuals 125-274 and 125-18, respectively, clustered in groups VI and VII, with 5 and 4 individuals each group.

Group VII (125-363; 125-85; 04-60; 125-274) stood out for the highest averages for FMWC (4,244g), DCA (28.75mm), PD (46.75mm), and DSBL (107.50mm) (Table 5). In addition to other attributes, groups IX and VIII, which stood out for the highest averages for SSC of 21.10°Brix and 20.80°Brix, respectively, clustered individuals 125-40; 125-292; 125-218; 125-95; and 125-372. In addition to other attributes, groups IX and VIII, which stood out for the highest averages for SSC of 21.10°Brix and 20.80°Brix, respectively, clustered individuals 125-40; 125-292; 125-218; 125-95; and 125-372. The performance *per se* of these individuals should be evaluated with a view to recommending those with best performances and greatest phenotypic stability.

As reported by Melão et al. [8], the existence of genetic variability is essential to start a breeding program. From the analysis of genetic divergence, these authors suggested groups of pineapple accessions for hybridizations based on morphological and agronomic characteristics among 19 accessions of pineapple.

From the conception of a diverse genetic base population with high means, and satisfactory agronomic performance, it is possible to potentialize the heterosis effects, increasing the chances of selecting transgressive individuals [11,22].

Table 4 Clustering of 144 individuals of pineapple half-sibs by Tocher's optimization method using the Square of the Standardized Euclidean Distance based on 17 fruit and plant traits. IPA-Experimental Station of Itambé, Pernambuco State, Brazil

Groups	Number of individuals	Identification
I	95	04-62; 04-23; 04-08; 125-333; 125-354; 125-254; 125-235; 125-93; 125-144; 125-220; 125-276; 125-348; 125-211; 125-252; 125-26; 125-173; 04-85; 125-133; 125-172; 125-76; 125-129; 125-347; 125-111; 125-192; 125-142; 125-158; 125-66; 125-191; 125-50; 125-217; 125-62; 125-364; 125-130; 125-97; 125-11; 125-65; 125-175; 125-281; 125-224; 04-102; 125-303; 125-236; 125-168; 125-156; 125-43; 125-323; 04-14; 125-222; 125-05; 125-341; 125-150; 04-99; 125-148; 04-84; 04-55; 04-95; 04-50; 03-26; 125-151; 04-64; 04-19; 125-337; 125-124; 04-63; 125-202; 125-38; 125-247; 125-289; 125-371; 125-10; 125-277; 04-106; 125-313; 04-32; 125-108; 04-54; 125-265; 04-72; 125-315; 125-241; 125-08; 04-11; 125-71; 125-57; 125-244; 125-338; 125-45; 125-286; 125-163; 125-128; 125-106; 125-51; 04-81; 04-92; 125-132
II	14	125-199; 125-358; 125-114; 03-28; 04-39; 125-189; 04-103; 125-310; 125-369; 125-178; 125-362; 125-304; 125-41; 125-70
III	5	125-339; 125-20; 125-109; 125-343; 04-94
IV	5	125-250; 03-03; 125-283; 125-346; 125-257
V	4	125-110; 04-86; 125-330; 04-52
VI	5	125-210; 125-18; 04-57; 125-278; 125-314
VII	4	125-363; 125-85; 04-60; 125-274
VIII	3	125-218; 125-95; 125-372
IX	2	125-40; 125-292
X	5	125-42; 04-25; 125-256; 03-34; 125-320
XI	1	03-21
XII	1	03-23
Maximum distance = 5.1371		125-274 and 125-18
Minimum distance = 0.0895		04-62 and 04-23

n = number of individuals per group.

For example, in Figure 7 stands out individual 125-363 that is member of the heterotic group VII, at the time of harvest for analysis. It is observed that the plant is vigorous with spreading growth habit, and fruit has the potential for high pulp productivity, as the fruit mass without crown reached 3,905g and soluble solids contents 16°Brix. According to Lira Júnior [21], this same individual was selected and presented genotypic values (REML/BLUP) of 2.54kg for fruit mass without crown and 15.98°Brix for total soluble solids (TSS), conical fruit shape, pulp's golden yellow coloration and spiny leaf margin spinescence.

These results indicate a high heterotic potential of the group VII with a very high mean for FMWC in future hybridization programs with groups IX and VIII, both with highlights for the high means of SSC. The high divergence between these analyzed groups, probably, is due to their genetic origin and the degree of parentage among evaluated individuals. The high level of genitor's heterozygosis with contrasting and complementary traits contributed possibly to this result, reproducing a high rate of recombination among half-sib descendents.

Table 5 Means of fruit and plant traits for 12 groups of pineapple half-sibs formed by Tocher’s optimization method using the Square of the Standardized Euclidean Distance. IPA-Experimental Station of Itambé, Pernambuco State, Brazil

Traits	Means/Group											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
FMWC(g)	2,271	2,331	3,029	2,218	3,228	2,192	4,244	2,142	2,111	2,589	3,095	2,410
CM(g)	69.46	127.43	80.20	31.60	69.25	152.40	70.75	130.00	158.50	60.80	205.00	81.00
FL (cm)	26.72	21.44	36.06	28.90	27.10	23.44	38.28	28.33	20.70	27.50	22.70	23.00
CFB (cm)	41.62	43.91	44.12	40.16	48.60	42.00	49.13	38.89	37.00	43.42	50.40	45.00
CFMT (cm)	38.74	43.68	39.98	36.88	46.58	41.20	46.00	35.63	41.20	40.82	49.20	42.80
CFA (cm)	28.21	37.41	22.34	24.56	35.90	35.84	22.90	24.80	37.00	25.02	38.00	30.40
CL (cm)	11.96	17.69	14.08	7.62	11.40	22.32	10.53	12.90	17.40	11.58	11.30	8.40
DCA (mm)	20.52	24.93	22.20	18.40	20.00	26.40	28.75	22.33	21.00	23.20	18.00	16.00
SSC (°Brix)	16.79	17.59	15.64	18.36	15.50	15.92	16.45	20.80	21.10	18.16	16.20	17.00
PHFB (cm)	47.53	46.74	49.74	55.04	44.33	46.68	50.63	55.90	52.75	41.28	46.40	58.40
PH (cm)	81.77	81.64	93.54	83.84	84.15	90.24	85.58	95.00	97.20	78.74	88.60	108.20
PL (cm)	19.21	19.30	19.60	18.24	16.48	20.14	16.08	22.87	19.35	15.40	16.40	24.60
PD (mm)	33.37	33.64	32.60	35.80	30.50	33.40	46.75	37.67	37.00	34.60	30.00	31.00
LL (cm)	88.99	84.71	107.54	80.96	83.35	100.36	101.18	90.97	79.80	90.78	87.30	101.20
LW (mm)	61.60	64.93	66.00	53.80	57.50	65.40	71.75	66.00	52.00	70.40	54.00	74.00
DSBL (mm)	88.09	88.71	92.60	86.60	93.50	90.80	107.50	95.67	89.00	89.60	85.00	82.00
NAL	38.24	43.86	39.00	47.60	39.25	35.20	49.75	45.00	52.00	46.80	54.00	32.00

FMWC, fruit mass without crown; CM, crown mass; FL, fruit length; CFB, circumference of the fruit base; CFMT, circumference of the fruit middle third; CFA, circumference of the fruit apex; CL, crown length; DCA, diameter of the central axis; SSC, soluble solids contents; PHFB, plant height to the fruit base; PH, plant height; PL, peduncle length; PD, peduncle diameter; LL, leaf length D; LW, leaf width; DSBL = diameter of the stem base with leaves; NAL = number of active leaves.



Figure 7 Fruit maturation of the hybrid IPA 125-363 from the base to the top. Experimental Station of Itambé, PE, Brazil. Photo Lira Júnior, J. S.

4 Conclusion

Concerning to phenotypic variation, crown mass (CM), crown length (CL) and fruit mass without crown (FMWC) reach the largest data dispersions, while circumference of the fruit middle third (CFMT) and circumference of the fruit base (CFB) achieve the smallest;

Respecting to the relative importance for the total variance, both groups of traits gather almost the same proportions. While fruit traits cluster 52.69% of the total variance, plant traits accumulate 47.31%. Individually, none of them should be discarded for the genetic divergence studies among pineapple half-sibs;

Group VII (125-363, 125-85, 04-60, and 125-274), group IX (125-40 and 125-292) and group VIII (125-218, 125-95, and 125-372) are recommended to compose hybridization blocks and evaluation cycles of phenotypic stability for use *per se*. These groups have a broad heterotic potential, and desirable agronomic traits, mainly regarding to high means for fruit mass without crown (FMWC) upper than 4,000g and soluble solids content (SSC) around 20-21°Brix, which can be used by the IPA's pineapple breeding program.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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