

## Phenotyping of *Passiflora edulis*, *P. setacea*, and their hybrids by a multivariate approach

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**ABSTRACT.** Morphological characterization is the most accessible and used method to quantify the genetic diversity of the available germplasm. The multivariate statistical method is highly important for this purpose. This study aimed to characterize parents and hybrids of *Passiflora* according to morphoagronomic descriptors and estimate the genetic divergence between them based on the joint analysis of qualitative and quantitative variables using the Ward-modified location model (MLM) procedure. One hundred and thirty-eight individuals were assessed (10 *P. edulis*, 10 *P. setacea*, and 118 interspecific hybrids) using 23 quantitative and 12 qualitative descriptors. The values for the quantitative descriptors were measured and subjected to multivariate statistics using the Ward-MLM strategy. Large genetic variability was detected by the morphoagronomic data in the 138 genotypes that were evaluated, and the hybrids presented higher variability than the parents. Pseudo-F and pseudo-t<sup>2</sup> criteria showed that the optimal number of groups was three.

Group I was composed of 118 hybrid genotypes; group II was composed of the 10 *P. setacea* genotypes, and group III was composed of the 10 *P. edulis* genotypes. The longest distance was found between groups II and III (474.96). The shortest distance was detected between groups I and II (198.78), which indicates that the segregating population is genetically closer to *P. setacea* than to *P. edulis*. The Ward-MLM procedure is a useful tool to detect genetic diversity and group accessions using both qualitative and quantitative variables.

**Key words:** Ward-modified location model (MLM) method; Interspecific hybrid; Joint analysis; Morphoagronomic characterization; Genetic divergence

## INTRODUCTION

Passion fruit (*Passiflora edulis* Sims) is a tropical fruit whose cultivation has rapidly evolved in Brazil over the last 30 years (Bernacci et al., 2008; Meletti, 2011). Brazil is the largest passion fruit producer, producing 923.035 ton/ha (IBGE, 2011). Despite its economic relevance, passion fruit production is still limited by diseases in orchards, which lead to losses in fruit quality and production. Passion fruit woodiness virus is caused by Cowpea aphid-borne mosaic virus (CABMV) and is one of the diseases that affects passion fruit crops. It has impaired production in all regions where this fruit is cultivated (Nascimento et al., 2006).

Because of the low genetic variability among the sour passion fruit cultivars that are available for developing resistance to fruit woodiness virus, selected crosses between the cultivated species and resistant wild relatives are used in some strategies that were adopted to control the disease. Among the wild species that were indicated as potential sources of resistance to diseases affecting passion fruit, *Passiflora setacea* DC deserves special attention because it is resistant to CABMV (Junqueira et al., 2006; Bellon et al., 2009). The characterization of a segregating population by means of morphoagronomic descriptors is particularly important for breeders because of the wide variability that is available in the population that remains little known or exploited. Furthermore, it also allows the identification of superior genotypes for advanced generations in the breeding program (Primot et al., 2005).

The use of multivariate statistics is very significant, especially when a large number of descriptors are used to quantify the dissimilarity between individuals. Multivariate procedures unify information from a set of traits and provide robust studies related to genetic divergence, which is relevant to understand the genetic variability of populations (Gonçalves et al., 2008).

There are several available methods to estimate genetic diversity. However, caution is necessary in the selection of a method because it may considerably affect the estimates (Mohammadi and Prasanna, 2003). Determining the method to estimate similarity (and its complement, dissimilarity) depends on the objectives proposed in the research, the characteristics of the variables, and the inherent properties of the distances and coefficients. In general, statistical classification methods only consider continuous or discrete data separately (Padilla et al., 2007). However, new methodologies allow the simultaneous analysis of quantitative and qualitative data.

The Ward-modified location model (MLM) procedure, which was proposed by Franco et al. (1998), is a multivariate method that is an excellent strategy for quantifying variability using quantitative and qualitative variables simultaneously. This strategy involves two stages:

in the first stage, the groups are defined by the Ward clustering method (Ward Junior, 1963) using the Gower dissimilarity matrix (Gower, 1971); in the second stage, the mean quantitative variable vector for each subpopulation is estimated by the MLM procedure, regardless of the values of the qualitative variable. This method has been widely employed in the past five years, especially in studies on genetic diversity aiming to support plant-breeding programs. These procedures have been used by Gonçalves et al. (2009) in *Solanum lycopersicum*; Barbé et al. (2010) in *Phaseolus vulgaris*; and Sudré et al. (2010) in *Capsicum* spp. There are also reports of using this procedure to study banana (Pestana et al., 2011; Pereira et al., 2012) and guava (Campos et al., 2013).

Therefore, this study aimed to characterize parents and hybrids of *Passiflora* based on morphoagronomic descriptors and to estimate the genetic divergence between them based on the joint analysis of qualitative and quantitative variables using the Ward-MLM procedure.

## MATERIAL AND METHODS

### Germplasm

We evaluated 138 individuals from the passion fruit breeding program of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) that included 10 genotypes of *P. edulis*, 10 genotypes of *P. setacea*, and 118 interspecific hybrids that were obtained from crosses between these species. The genotypes of *P. setacea* were from the Active Germplasm Bank (BAG-*Passifloras*) of the Universidade Estadual de Santa Cruz (UESC), Ilheus, Bahia; the genotypes of passion fruit were obtained from the recurrent selection program of the UENF. Hybridizations were performed using *P. edulis* as the female and male parents to obtain the S<sub>0</sub> progenies (Table 1).

**Table 1.** Genotypes of *Passiflora edulis* (*Pe*) and *P. setacea* (*Ps*) used in interspecific crosses to obtain the full-sib progenies of *Passiflora* spp.

Parents/Crosses	Progenies	Genotypes
<i>Pe</i> 139 (40) x <i>Ps</i> 367	UENFH-1	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 14, 16, 19, 20, 22
<i>Pe</i> 139 (38) x <i>Ps</i> 367	UENFH-2	2, 3, 4, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 23
<i>Pe</i> 139 (29) x <i>Ps</i> 367	UENFH-3	2, 4, 6, 7, 8, 9, 10, 11, 13
<i>Pe</i> 89 (11) x <i>Ps</i> (365)	UENFH-4	2, 3, 5, 9, 13, 17, 21
<i>Ps</i> 367 x <i>Pe</i> 139 (40)	UENFH-5	1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23
<i>Ps</i> 367 x <i>Pe</i> 139 (36)	UENFH-6	6, 27
<i>Ps</i> 367 x <i>Pe</i> 89 (11)	UENFH-7	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
<i>Ps</i> 365 x <i>Pe</i> 139 (36)	UENFH-8	3, 8, 13, 16, 28, 45, 47, 48, 55, 58, 61, 62, 63, 68, 74
<i>Ps</i> 365 x <i>Pe</i> 89 (7)	UENFH-9	1, 3, 5, 9, 11, 12, 16, 21, 29, 36, 37, 38, 40, 47, 52, 53, 54, 55, 56, 57

### Interspecific hybridizations

The interspecific hybridizations were held at the UESC campus in a greenhouse from July 14 to August 8, 2010. The temperature inside the house ranged from 28° to 22°C, and the relative humidity was between 60 and 98%. The artificial crosses were carried out at different times, from 1:30 to 2:30 pm for the genotypes of *P. edulis* and from 7:00 to 7:30 pm for the genotypes of *P. setacea*. Flower buds of the parents were protected with paper bags in the morning. The anthers of the donor species were collected, placed on Petri dishes containing

silica gel, and stored in the refrigerator (10°C) until the occurrence of the anthesis of the flowers (anthesis at 12:30 pm in plants of *P. edulis* and from 6:00 to 7:00 pm in plants of *P. setacea*). During pollination, the anthers of the donor species were carefully rubbed on the stigma of the recipient species with the aid of tweezers. After artificial hybridization, the crosses were identified, and the flowers were protected again for up to 24 h. The fruits that resulted from successful hybridizations were protected using nylon net until their full maturity.

### Cultivation conditions

Individuals of different genotypes were planted in November 2011 in the experimental area of the Escola Agrícola Antônio Sarlo, northern State of Rio de Janeiro at the latitude of 21° 45' S and longitude of 41° 20' W and 11 m above sea level. The vertical cordon system was used with stakes that were 2.5 m in height and spaced at 4 m with a number 12 wire that was 1.80 m above the ground. The distance between the crop rows was 3.5 m. The cultural practices recommended for the cultivation of passion fruit were followed (Viana et al., 2003).

The first fruiting occurred in March 2012. The periods of harvesting and evaluation of fruits occurred from March to July 2012. The vegetative and floral morphological characteristics were assessed throughout 2012.

Genotypes were characterized according to the list of descriptors for the registration and protection of passion fruit cultivars that was prepared by the National Service for Cultivar Protection of the Ministry of Agriculture, Livestock and Supply. Thirty-five descriptors were used; 12 were qualitative (Table 2) and 23 were quantitative.

**Table 2.** Qualitative descriptors observed in 10 genotypes of *Passiflora edulis*, 10 genotypes of *P. setacea*, and 118 interspecific hybrids.

Descriptor	Classes observed according to the descriptors for <i>Passiflora</i> spp (Ministério da Agricultura Pecuária e Abastecimento - MAPA)
Color of the branch	1: light green; 2: dark green
Shape of the leaf	7: broken
Division of the leaf blade	3: trilobate
Leaf blade: sinus	1: absent; 2: present
Depth of the sinus	3: shallow; 5: average; 7: deep
Leaf blade: pilosity	1: absent; 2: present
Position of leaf gland	1: adjacent to the leaf blade; 2: close to the middle of the petiole; 3: adjacent to the insertion of the leaf in the branch
Prevailing corona color	1: white; 3: red; 4: purplish-red; 5: purple; 7: dark blue; 8: lilac
Time of anthesis	2: evening; 3: night
Pulp color	2: greenish-yellow; 3: yellow; 4: orange-yellow; 5: orange; 8: light yellow
Prevailing skin color	1: green; 2: yellow; 4: pinkish; 7: greenish-yellow; 8: yellowish-green
Fruit shape	1: oval

### Quantitative descriptors

The following quantitative descriptors and defined measurements were used: stem diameter in mm (SD), at the height of the first node of the main axis; leaf length in mm (LL), the longitudinal extent of the highest edge; leaf width in mm (LW), the transverse measurement of the largest dimension; petiole length in mm (PL), from the insertion of the stem until the insertion of the leaf; flower diameter in mm (FD), from the extreme points of the flower; corona

diameter in mm (CD), from the extreme points of the corona filaments; length of outer series of corona filaments in mm (LC), from the insertion in the flower receptacle up to the apex; petal length in mm (LP), from the insertion of the flower in the apex; sepal length in mm (SL), from the insertion in the flower to the apex; petal width in mm (PW), the largest dimension; sepal width in mm (SW), the largest dimension; floral peduncle length in mm (FP), from the insertion in the receptacle of the flower to the insertion in the stem; androgynophore length in mm (AL), the fullest extent that sustains the sexual organs; bract length in mm (BL), from the insertion in the peduncle to the apex; number of fruits (NF) assessed in weekly harvests (for 4 months), and the total number of fruits per plant is counted at the end of the evaluation period; average fruit weight in g (FW), obtained with a digital semianalytical scale for all mature fruits that were collected during the study period; fruit longitudinal diameter in mm (LD), determined in the longitudinal region of the fruits with the aid of a digital caliper; fruit transverse diameter in mm (TD), determined in the equatorial region of the fruits using a digital caliper; shell thickness in mm (ST), determined by the arithmetic mean of the measures of four points of the outer shell in the middle portion of the fruits (transversely cut, toward the largest diameter) using a digital caliper; pulp weight in g (WP), obtained by weighing the pulp (seeds with arils) with the aid of the semianalytical balance; content of total soluble solids (SST), obtained by refractometry using an ATAGO N1 portable digital refractometer with readings ranging from 0 to 32°Brix; number of seeds (NS), manual counting (average of 10 fruits); and seed size in mm (SS), the ratio between the length and width of a sample of 10 seeds of 10 fruits.

### Statistical analysis

The quantitative and qualitative variables were analyzed simultaneously using the Ward-MLM procedure to form groups of genotypes using the CLUSTER procedure and IML (Interactive Matrix Language) of the SAS software system (SAS Institute, 2000). The distance matrix was obtained by the Gower algorithm (Gower, 1971) to use Ward's clustering method. The optimal number of groups was defined according to the pseudo-F and pseudo-t<sup>2</sup> criteria combined with the likelihood profile associated with the likelihood ratio test (SAS Institute, 2000).

The Gower dissimilarity index was used because the set of variables under study formed a mixed group. The Gower index uses qualitative and quantitative data to generate a single dissimilarity index ranging from 0 to 1. The dissimilarity was given by the following:

$$S_{ij} = \frac{\sum_{k=1}^p W_{ijk} \cdot S_{ijk}}{\sum_{k=1}^p W_{ijk}} \quad (\text{Equation 1})$$

where  $i$  and  $j$  refer to the individuals to be compared with regard to trait  $k$ ,  $p$  is the total number of traits, and  $S_{ij}$  is the contribution of the variable  $k$  to the total distance. If a variable is qualitative,  $S_{ijk}$  takes the value 1 when the concordance is positive or negative for the trait  $k$  between individuals  $i$  and  $j$ . On the other hand, when the variable is quantitative,

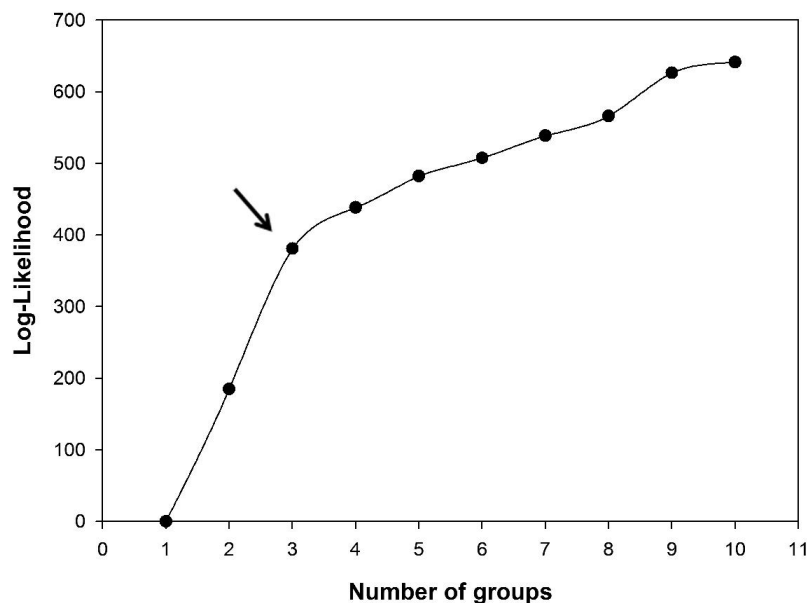
$$S_{ij} = \frac{Y_{ik} - Y_{jk}}{R_k} \quad (\text{Equation 2})$$

where  $R_k$  is the range of variation of the variable  $k$ , with values between 0 and 1. The value of  $W_{ijk}$  was used to define the contributions of  $S_{ijk}$  individuals. Thus, when the value of the variable  $k$  is absent in one or both individuals,  $W_{ijk}$  is 0; otherwise, it is equal to 1.

## RESULTS AND DISCUSSION

### Ward-MLM method

The Ward-MLM procedure, which uses both quantitative and qualitative variables, was used to distinguish 138 genotypes. According to the pseudo-F and pseudo- $t^2$  criteria, three was defined as the optimal number of groups. This is due to the increase in the likelihood function observed in group III, i.e., 196.03 (Figure 1).



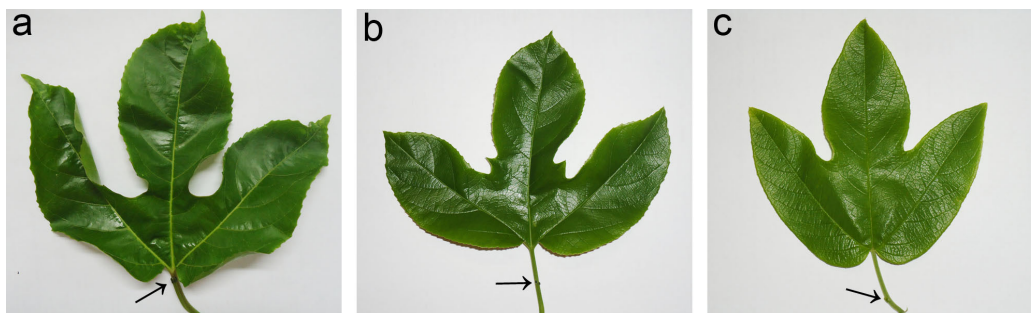
**Figure 1.** Graph of the logarithmic likelihood function (log-likelihood) relative to the number of groups formed by the Ward-modified location model (MLM) strategy in *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids.

The analysis of the likelihood function can define precise criteria to form groups, which results in few subjective groupings. The same is not true for most of the other clustering methods, in which the number of groups is established personally (Gonçalves et al., 2009). Sudré et al. (2010) evaluated 56 accessions of *Capsicum* from the germplasm bank of the

UENF and set five as the optimal number of groups because the maximum value was reached at this point. Pestana et al. (2011) analyzed 74 banana mutants and observed greater increases in the probability function in group III, which agrees with the findings of this study. In turn, Campos et al. (2013) evaluated a population of 138 *Psidium guajava* individuals from the UENF breeding program and found that the greatest increase in the probability function was achieved when five groups were considered. The number of groups may vary according to the species, number of accessions, and number and type of descriptors (Gonçalves et al., 2009).

The Ward-MLM classification strategy for the analysis of the morphoagronomic data allowed the separation of 138 genotypes into three homogeneous groups that were formed according to their similarities. Group I was composed of 118 hybrid genotypes, group II was composed of 10 *P. setacea* genotypes, and group III was composed of 10 *P. edulis* genotypes. The clear separation between the groups indicates that the morphoagronomic traits efficiently discriminated the parents and their hybrids. Santos et al. (2012) evaluated the morphological diversity in *Passiflora subanceolata* (e.g., *Passiflora palmeri* var. *subanceolata*), *Passiflora foetida*, and their hybrids, and the observed clear separation between these species and the segregating population.

The interspecific hybrids, which were placed in group I, had wide variability in all traits under study. All representatives of this group presented leaves that were broken, trilobate, glabrous, and light green and had glands in the middle of the petiole (Figure 2b). Most genotypes (98.30%) had a deep leaf sinus (Table 3 and Figure 2b).



**Figure 2.** Qualitative descriptors for leaf in the genotypes of *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids. **a.** *P. edulis* (glands adjacent to the leaf blade); **b.** interspecific hybrid (glands located in the middle of the petiole); and **c.** *P. setacea* (glands located near the insertion of the leaf on the branch). The arrows indicate the position of the glands.

There was no synchrony in flower opening. Anthesis started around 1:30 pm and went until 3:00 pm, when all the flowers were open. The corona presented a wide variety of colors that included white and dark blue. However, the purplish-red color was the most prevalent in the population (77.11%) and was followed by purple (11.01%), dark blue (8.47%), white (1.69%), and red (1.69%) (Table 3 and Figure 3c-1).

Similar results were obtained by Junqueira et al. (2005) in midwestern Brazil. The authors evaluated 98  $S_0$  plants that were obtained from crosses between *P. edulis* and *P. setacea* using passion fruit as a male or female parent, and they observed light green leaves, high flowering capacity, and corona in shades of lilac in the segregating population.

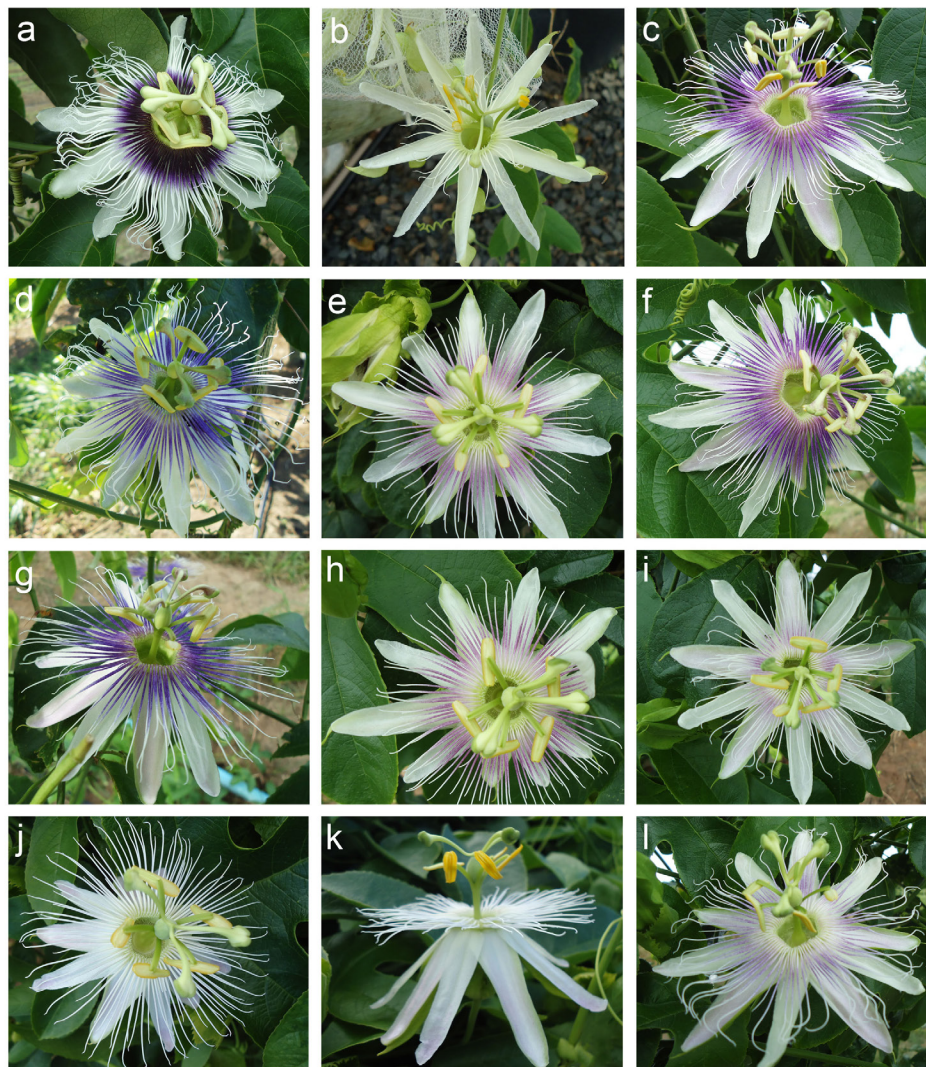
**Table 3.** Descriptors and number of genotypes per group of qualitative traits in each of the three groups (GI, GII, and GIII) formed by the Ward-modified location model (MLM) strategy in *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids.

Descriptors	Groups		
	GI (118)	II (10)	GIII (10)
Color of the branch			
Light green	118	10	-
Dark green	-	-	10
Depth of the sinus			
Shallow	-	10	-
Average	2	-	-
Deep	116	-	10
Position of the gland			
Adjacent to the leaf blade	-	-	10
Middle of the petiole	118	-	-
Adjacent to the insertion of the leaf to the branch	-	9	-
Distributed along the petiole	-	1	-
Color of the corona			
White	2	10	-
Red	2	-	-
Purplish-red	91	-	-
Purple	-	-	10
Lilac	13	-	-
Dark blue	10	-	-
Time of anthesis			
Evening	118	-	10
Night	-	10	-
Color of the pulp			
Greenish-yellow	-	5	-
Yellow	53	-	1
Orange-yellow	33	2	6
Orange	18	-	3
Light yellow	14	3	-
Color of the skin			
Green	43	10	-
Yellow	15	-	7
Pinkish	-	-	2
Greenish-yellow	14	-	-
Yellowish-green	46	-	1
Fruit shape			
Oval	116	4	6
Round	2	-	4
Ellipsoid	-	6	-

Regarding qualitative fruit descriptors, the color of the skin of ripe fruits ranged from green to yellow. However, the yellowish-green color prevailed in genotypes of this group (38.98%) and was followed by green (36.44%), yellow (12.71%), and yellow-greenish (11.86%) (Table 3 and Figure 4a). As for the fruit shape, the oval shape stood out in this group and was observed for 98.30% of the individuals (Table 3 and Figure 4d). The color of the pulp ranged from light yellow to orange, but yellow was predominant in hybrid genotypes (44.90%), followed by yellow-orange (27.96%), orange (15.25%), and light yellow (11.86%) (Table 3 and Figure 5g-j).

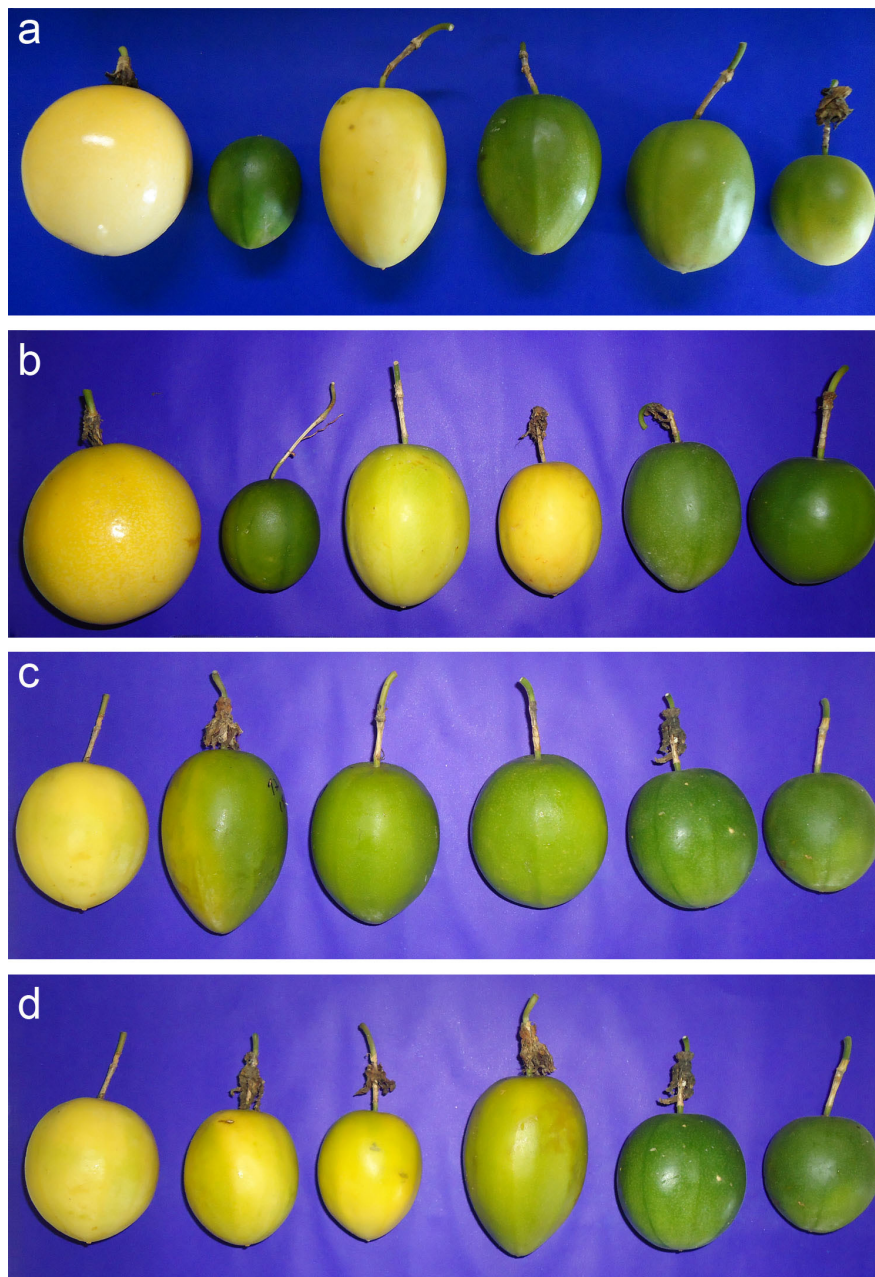
In opposition to the results obtained in this work, Junqueira et al. (2005) observed that the  $S_0$  population (*P. edulis* x *P. setacea*) only produced fruit by hand pollination. The authors attributed this to the fact that plants had a very long androgynophore and that anthesis started around 5:00 pm, which prevented pollination by insects. In this study, the size of the androgynophore and time of anthesis of hybrid plants were not considered to be limiting factors for fruit production.



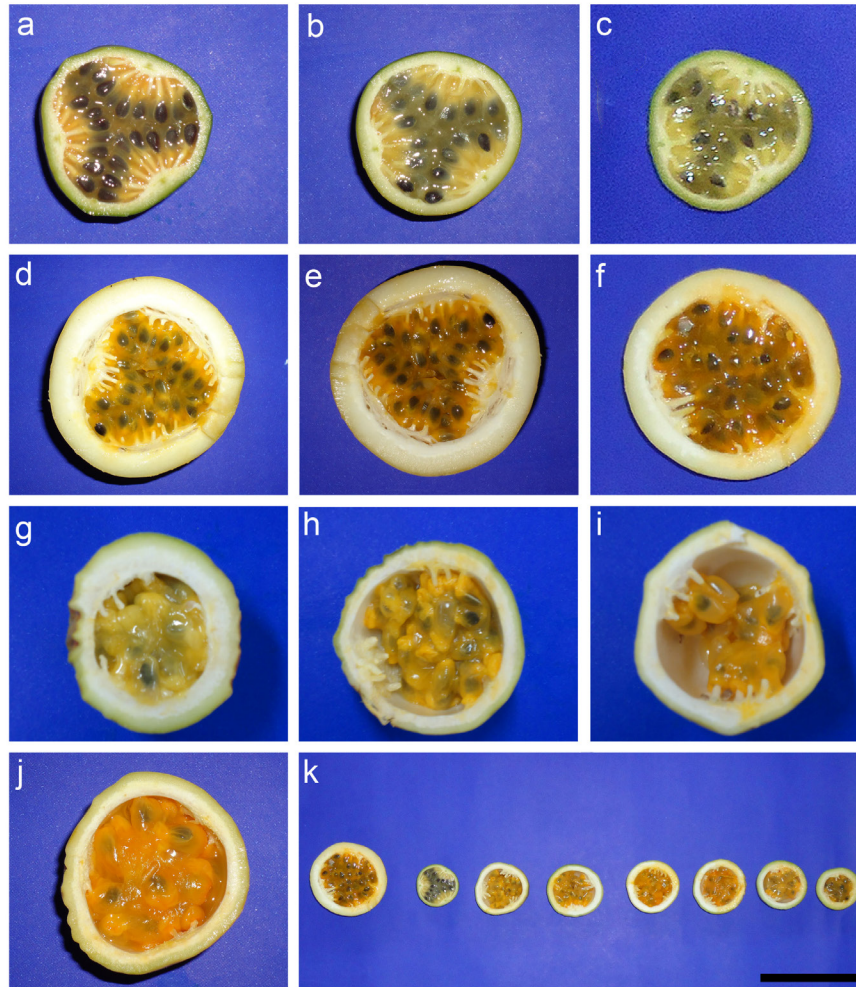


**Figure 3.** Qualitative descriptors for flower in the genotypes of *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids. Predominant color of the corona: **a.** *P. edulis* purple; **b.** *P. setacea* white; **c.-l.** interspecific hybrids: **c** and **f** purplish-red; **d** and **g** - dark blue; **e** and **h** - red; **i** and **l** - lilac; and **j** and **k** - white.

Regarding the quantitative traits, group I contained the genotypes with the highest values for the floral traits FD, CD, LC, LP, SL, and BL (101.44, 79.33, 35.40, 41.01, 46.45, and 28.53, respectively) and the lowest values for the vegetative traits LL (118.02) and PL (44.25). As for the agronomic descriptors of the fruits, the individuals of this group contained high values for SS and low values for NF (13.52), WP (23.00), and NS (57.47) (Table 4). Regarding the traits AL, FW, TD, LD, ST, and SST, the hybrids had values that were intermediate compared to those of the parents (Table 4).



**Figure 4.** Qualitative descriptors for fruits in the genotypes of *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids. **a.** Color of fruit (left to right): *P. edulis* - yellow, *P. setacea* - green, and interspecific hybrids - first: yellow and the last three: yellowish-green. **b.** Fruit shape (left to right): *P. edulis* - round, *P. setacea* - oval, and interspecific hybrids - first three: oval and fourth: round. **c.** Color of fruit: interspecific hybrids - first: yellow, second: greenish-yellow, third and fourth: yellowish-green, and fifth and sixth: green. **d.** Shape of fruit: interspecific hybrids - first five: oval and last: round.



**Figure 5.** Qualitative descriptors for color of the pulp in the genotypes of *Passiflora setacea*, *P. edulis*, and interspecific hybrids: **a.** orange-yellow; **b.** light yellow; **c.** greenish-yellow; **d.** yellow; **e.** orange-yellow; **f.** orange; **g.** light yellow; **h.** yellow; **i.** orange-yellow; **j.** orange; **k.** cross section of fruits of *P. edulis*, *P. setacea*, and  $S_0$  interspecific hybrids. Bar in k equals 10 cm.

The low NF value is probably associated with the elevated height of the stigma in relation to the corona because of the long androgynophore, which hinders pollination. Thus, in this population, it is important to assess the androgynophore length to select plants with a shorter androgynophore.

In passion fruit, pollination affects fruiting because the NS per fruit and pulp yield are correlated with the number of pollen grains deposited on the stigma during pollination (Akamine and Girolami, 1959). Therefore, in this group, the low values for WP and NS may result from the small amount of pollen deposited on the stigma. This may be due to the average length of the hybrid plant androgynophore, which was 16.10 mm. Although smaller than

that of *P. setacea* (24.29 mm, group II), it is still large compared to that of *P. edulis* (6.96 mm, group III). The bees of the genus *Xylocopa*, known as carpenter bees, are the effective pollinators at the time of anthesis of this group. They are known as mamangavas and are the same species that pollinates sour passion fruit. Thus, the distance between the dehiscent front of anthers does not allow precise coupling on the dorsal region of pollinators, which decreases the number of pollen grains deposited on the stigma. This should not occur in individuals of group II because pollinators (bats and moths) are larger and can perform a more efficient pollination. At the time of the anthesis of *P. setacea*, the stigma of hybrid plants may not be receptive, thus hindering pollination. However, this should be further investigated because this group comes from interspecific crosses and other factors may be involved.

**Table 4.** Means of quantitative variables for each of the three groups formed by the Ward-modified location model (MLM) method and eigenvalues of the two canonical variables (CAN) in *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids.

Descriptors	Groups			CAN	
	GI (118)	GII (10)	GIII (10)	CAN1	CAN2
SD (mm)	27.06	16.81	28.96	0.37	0.40
LL (mm)	118.02	118.53	149.12	0.36	-0.16
LW (mm)	148.50	114.90	166.02	0.34	0.21
PL (mm)	44.26	48.98	74.20	0.39	-0.26
FD (mm)	101.44	83.86	89.98	-0.01	0.63
CD (mm)	79.33	52.25	77.59	0.39	0.69
LC (mm)	35.40	22.32	33.18	0.01	0.09
LP (mm)	41.01	32.67	36.32	0.05	0.78
SL (mm)	46.45	39.20	38.64	-0.16	0.63
SW (mm)	14.30	9.44	14.47	0.45	0.63
PW (mm)	10.65	7.77	10.96	0.28	0.37
FP (mm)	75.08	108.54	52.54	-0.63	-0.29
AL (mm)	16.10	24.29	6.96	-0.95	-0.18
BL (mm)	28.53	18.53	25.90	0.24	0.70
NF	13.52	14.89	51.70	0.46	-0.26
FW (g)	63.40	43.35	157.48	0.84	-0.26
TD (mm)	56.97	40.41	73.03	0.79	0.22
LD (mm)	70.94	50.74	81.50	0.58	0.34
ST (mm)	5.40	2.78	6.25	0.56	0.46
WP (g)	23.00	25.38	77.03	0.78	-0.48
SST (°Brix)	13.31	17.84	13.05	-0.46	-0.61
NS	57.47	168.61	317.67	0.58	-0.78
SS (mm)	5.11	4.29	5.06	0.37	0.65

SD = stem diameter; LL = leaf length; LW = leaf width; PL = petiole length; FD = flower diameter; CD = corona diameter; LC = length of the outer series of corona filaments; LP = petal length; SL = sepal length; SW = sepal width; PW = petal width; FP = floral peduncle length; AL = length of androgynophore; BL = length of bract; NF = number of fruits; FW = fruit weight; TD = transverse diameter of the fruit; LD = longitudinal diameter of the fruit; ST = skin thickness; WP = pulp weight; SST = content of total soluble solids; NS = number of seeds; SS = seed size.

Group II consists of the 10 genotypes of *P. setacea* and presents leaves that are broken, trilobate, glabrous, and light green with a shallow leaf sinus. Nine of the 10 individuals studied have petiole glands located near the insertion of the leaf in the branch (Table 3 and Figure 2c). The same description was obtained in a survey of the Passifloraceae family for *P. setacea* in the State of Bahia (Nunes and Queiroz, 2006).

Anthesis is nocturnal and synchronous for all individuals. It starts about 7:00 pm (Table 3). The same floral opening time was reported by Junqueira et al. (2005) for *P. setacea* in the Federal District.

The color of the corona in this group was predominantly white (Table 3 and Figure 3b), which differs from the description of white corona filaments with blue bands that was mentioned by Nunes and Queiroz (2006). The green ripe fruit skin was prevalent in this group (Figure 4a). Concerning fruit shape, 60% of the genotypes have an ellipsoid shape (Table 3 and Figure 4b). The shape and color of fruits were similar to those described by Nunes and Queiroz (2006) for *P. setacea*. With regard to pulp color, three categories have been reported, but the predominant color in this group was greenish yellow (50%), followed by light yellow (30%) and orange-yellow (20%) (Table 3 and Figure 5a-c).

With respect to the quantitative descriptors, this group presented the lowest values for SD (16.81), LW (114.90), and the following traits related to flowers: FD, CD, LC, LP, SW, PW, and BL (83.86, 52.25, 22.32, 32.67, 9.44, 7.77, and 18.53, respectively). The highest values were obtained for FP and AL, which were 108.74 and 24.29, respectively. Regarding the quantitative floral morphological descriptors, the values described by Nunes and Queiroz (2006) for FP (60-120 mm), BL (10-20), FD (50-100), LC (20-25), and AL (25-35) are similar to those obtained in this study (Table 4). Smaller and lighter fruits, as well as thinner skin and smaller seed size were found in group II than in other groups, with low values for FW, TD, LD, ST, and SS (43.35, 40.41, 50.74, 2.78, and 4.29, respectively). On the other hand, the highest mean SST (17.84) was obtained in this group (Table 4).

The sleep passion fruit is an excellent source of genetic resistance to plant pathogens that affect passion fruit crops. Thus, it is widely used in sour passion fruit breeding programs to transfer resistance genes to the commercial species (Cerqueira-Silva et al., 2009). The production of sleep passion fruit is restricted to areas where the species occur and are sold in a small domestic scale. In the municipalities of Curaçá, Uauá, and Canudos in the State of Bahia, this fruit is used for both fresh consumption and the production of jams and jellies that are manufactured in processing industries (Cerqueira-Silva et al., 2012). Thus, studies that seek to characterize wild species morphoagronomically are fundamental in the pre-breeding stage because they generate potentially useful information about the organoleptic properties of fruits and contribute to the dissemination and use of new species for consumption.

In this study, the average values for FW, TD, and LD were similar to those obtained for genotypes of *P. setacea* that were investigated in southwestern Bahia (Cerqueira-Silva et al., 2009). As for the average skin thickness, which is intended to be reduced, the value that was obtained in this study was lower than that found by Cardoso-Silva et al. (2007) and was similar to that reported by Cerqueira-Silva et al. (2009). Higher levels of SST, above 13 °Brix, are desirable for the juice industry (Fortaleza et al., 2005). The average SST reported in this study was similar to that found for sleep passion fruit genotypes that were assessed in southwestern Bahia (Cerqueira-Silva et al., 2009). A thin shell and high soluble solid content are traits that can be included in the passion fruit breeding program to improve fruit quality.

Group III was formed by the 10 cultivars of *P. edulis*. All individuals in this group have leaves that are broken, trilobate, glabrous, and dark green and present a deep leaf sinus and petiole glands adjacent to the leaf blade (Table 3 and Figure 2a); similar observations of *P. edulis* were reported by Cervi and Linsingen (2008). Purple was the predominant corona color in all individuals (Table 3 and Figure 3a), which was also noted by Crochemore et al. (2003) and Nunes and Queiroz (2006), who assessed passion fruit trees from the States of Paraná and Bahia, respectively. The flowers of this group showed no synchrony with floral opening, but flowers started a period of anthesis about 12:00 pm. The beginning of the anthesis period coincides with that found for the sour passion fruit in northern Rio de Janeiro State (Benevi-

des et al., 2009) and in Juazeiro, BA (Siqueira et al., 2009). Souza et al. (2012) studied the reproductive phenology of sour passion fruit from September 2009 to September 2010 under the conditions of the municipality of Campos dos Goytacazes, and they found that flowering occurred from October to March, when the highest average temperatures were observed, with a peak in January and highest percentage of ripe fruit in February.

Regarding the qualitative descriptors of the fruits, yellow was the prevailing skin color (70%), followed by pink (20%) and yellowish-green (10%) (Table 3 and Figure 4a). Cervi and Linsingen (2008) reported wide variation in the fruit skin color of *P. edulis*: yellow, greenish-yellow, red, and purple. The oval fruit shape prevailed in this group and was observed for 60% of individuals (Table 3 and Figure 4b); this observation agrees with the findings of Cervi and Linsingen (2008). Oval passion fruits have always been preferably selected because they are more marketable and have higher yield for juice than other fruit shapes. Meletti et al. (2000) recommend the selection of plants with ovate fruits because they have high juice content. However, Negreiros et al. (2007) found that pulp yield was not correlated with fruit shape.

The pulp color ranged from yellow to orange, but orange-yellow (60%) predominated in the genotypes (Table 3 and Figure 5d-f). Variations in the color of the skin and fruit pulp were also observed by Crochemore et al. (2003) for *P. edulis*. The color of passion fruit skin was used to distinguish the yellow and purple passion fruits. Today, this distinction is not accepted because the external color of the fruit is a trait of complex inheritance, which allows a wide variety of intermediate colors (Bernacci et al., 2008).

Regarding quantitative descriptors, this group presented the highest values for the vegetative traits SD, LL, LW, and PL (28.96, 149.12, 166.02, and 74.20, respectively) and for the flower traits SW and PW (14.47 and 10.96, respectively) (Table 4). The lowest values for floral traits were 52.54 and 6.96 for FP and AL, respectively (Table 4). In this study, the values obtained for the vegetative and floral traits were higher than those reported by Cervi and Linsingen (2008).

The length of the androgynophore is an important trait and should be considered in morphoagronomic assessments of sour passion fruit because it is directly linked to pollination, which, in turn, is related to production. This is the first study that investigated this trait in sour passion fruit. Short androgynophores reduce the height of the stigmas in relation to the corona, thus favoring pollination. At the time of maximum curvature of the stylet, stigmas even touch the corona, and thus, can be easily pollinated by smaller bees (Siqueira et al., 2009).

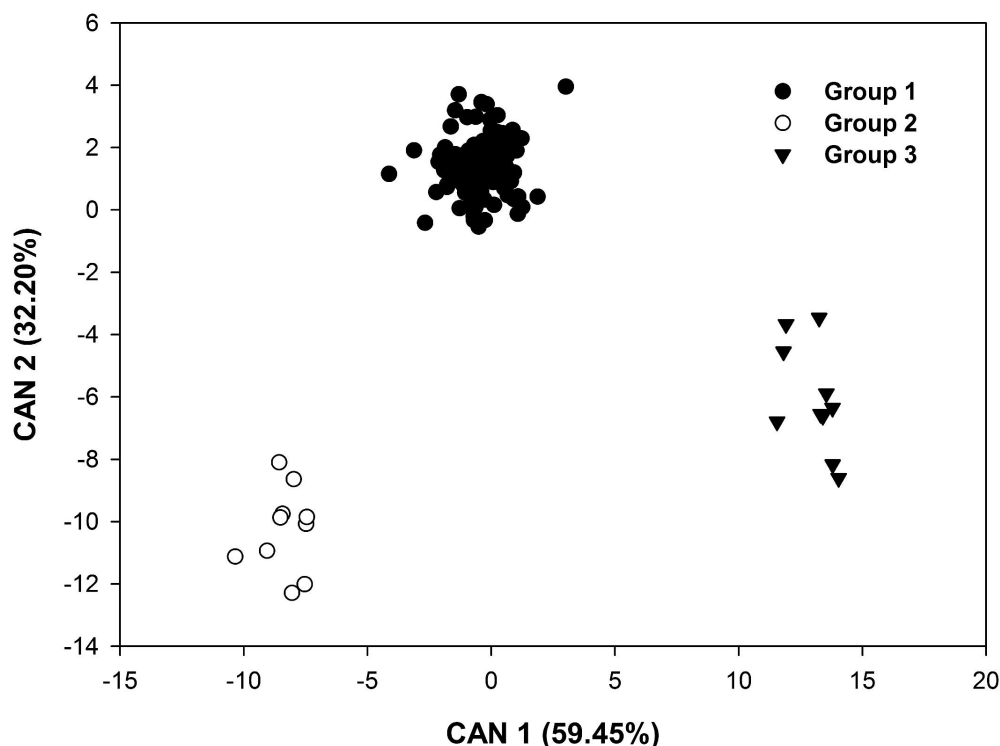
Group III obtained the highest values for NF, FW, TD, LD, ST, WP, and NS (51.70, 157.48, 73.03, 81.50, 6.25, 77.03, and 317.67, respectively). However, the lowest value for SST (13.05) was obtained in this group (Table 4).

Since the 1980s, the genetic breeding of sour passion fruit has led to increased means of agronomic traits, productivity, and fruit quality in each niche market. Heavy, larger, sweeter, and less acidic sour passion fruits are desirable for fresh consumption, while the industry prefers fruits with higher juice yield and high content of soluble solids (Meletti, 2011). The contents of soluble solids indicate the amount of solids dissolved in the pulp; in general, high SST contents are desirable for industrial purposes because they require less sugar (Farias et al., 2005). In this study, we found higher values for FW, LD, and SST than those obtained by Silva et al. (2012) while evaluating 144 full-sib progenies derived from the second cycle of recurrent selection. Abreu et al. (2009) found values that were lower than those obtained in this study for FW, WP, SST, and NS for the cultivar Gigante Amarelo.

When searching for sour passion fruits with improved qualities, thin skin is a desirable trait. Breeding programs seek to select genotypes with reduced skin thickness, which may indicate a greater amount of pulp for the populations studied. Negreiros et al. (2008) report that both the juice industry and the fresh fruit market regard skin thickness as a relevant factor in fruit ranking because it is inversely proportional to juice yield. The hybrid EC-L-7 (Sul Brasil x Marília), which was rated by Abreu et al. (2009), showed an average skin thickness of 7.0 mm and a WP value of 37.75 g. In this study, group III obtained an average skin thickness of 6.6 mm and a WP value of 77.03 g, which indicates that selection over time was effective for these traits.

Akamine and Girolami (1959) found a positive correlation between the NS per fruit and juice content; thus, a larger NS tends to provide higher yields of juice. The average NS per fruit obtained in this study was similar to that found by Santos et al. (2009), who assessed 20 half-sib progenies of sour passion fruit.

The first two canonical (CAN) variables that were obtained by the Ward-MLM method explained 91.65% of the total variation (Figure 6). This high value indicates that a two-dimensional graph is suitable for viewing the relationships between groups and between genotypes within groups. The traits that contributed most to the genetic divergence based on the first CAN variable were AL, FW, TD, and WP (-0.95, 0.84, 0.79, and 0.78, respectively) (Table 4).



**Figure 6.** Graph of the first two canonical (CAN) variables for the three groups formed by the Ward-modified location model (MLM) analysis in *Passiflora edulis*, *P. setacea*, and  $S_0$  interspecific hybrids.

CAN graphical analysis provided the highest dissimilarity at the interspecific level between groups II (*P. setacea*) and III (*P. edulis*) (Figure 6), with a distance of 474.96 (Table 5). The high dissimilarity between genotypes of sour passion fruit and sleep passion fruit may result from the differences between these two groups, which were demonstrated by the morphoagronomic traits. Similar results were obtained by Cerqueira-Silva et al. (2009) when they analyzed intraspecific and interspecific genetic diversity among genotypes of sour passion fruit and sleep passion fruit through physicochemical descriptors of fruits. In turn, the shortest distance between groups was observed between groups I and II (198.78) (Table 5), which showed very similar values for almost all traits. This indicates that the segregating population is genetically closer to *P. setacea* than *P. edulis* and that a higher proportion of the *P. setacea* genome is found in segregating individuals.

**Table 5.** Distance between the groups formed by the Ward-modified location model (MLM) procedure proposed by Franco et al. (1998).

Groups	I	II
II	198.78	
III	236.18	474.96

This study aimed to provide information that will support future crossings between individuals from groups I and III. In this case, to recover the commercial traits of sour passion fruit, we suggest crossing the best individuals of each group, i.e., the genotypes with the best agronomic traits, to aggregate value in fruit marketing and continue the passion fruit genetic breeding program at UENF.

Similar interspecific characterizations have not been reported so far. This is the first research that assesses interspecific genetic diversity through simultaneous analysis of morphoagronomic descriptors using *P. edulis*, *P. setacea*, and their hybrids.

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