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## Strategies for the next cycles of intrapopulation improvement of sour passion fruit<sup>1</sup>

### Estratégias para os próximos ciclos do melhoramento intrapopulacional do maracujazeiro-azedo

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#### HIGHLIGHTS:

*The use of flower and fruit morphoagronomic variables was efficient in quantifying the genetic diversity in passion fruit.*

*The Ward-modified location model procedure identified genetic variability and the formation of three groups.*

*Variables related to flowers contributed to the divergence between genotypes.*

**ABSTRACT:** In breeding programs, the identification of genetic variability and knowledge of genetic constitutions existing within segregating populations are of fundamental importance for the success and continuation of the program. The present study aimed to quantify the genetic diversity among progenies of a sour passion fruit population based on morphoagronomic variables of flowers and fruits using the Ward-MLM (modified location model) procedure, which was efficient in quantifying genetic diversity and allowed the identification and consistent formation of three groups within the population. Among the groups formed, group I presented the highest means for the variables related to flowers, group II presented the highest values for the variables related to yield, and group III presented the highest means for the variables related to fruit quality. The androgynous length, nectariferous chamber length, anther surface, nectareous chamber length, and stigma surface were the variables that most contributed to the divergence between the evaluated genotypes. Crosses between groups II and III genotypes are promising for the next cycle, contributing to increased production and improvement of the physical and chemical quality of fruits.

**Key words:** *Passiflora edulis* Sims, Ward-modified location model, recurrent selection

**RESUMO:** Nos programas de melhoramento, a identificação da variabilidade genética e o conhecimento das constituições genéticas existentes nas populações segregantes são de fundamental importância para o sucesso e continuação do programa. Este trabalho teve como objetivo quantificar a diversidade genética entre progênes de uma população de maracujazeiro azedo baseado em variáveis morfoagronômicas de flor e frutos utilizando o procedimento multivariado Ward-MLM (modified location model), que foi eficiente na quantificação da diversidade genética e permitiu a identificação e formação consistente de três grupos dentro da população. Dentre os grupos formados, o grupo I apresentou maiores médias para as variáveis relacionadas a flor, grupo II apresentou os maiores valores para as variáveis relacionadas a produtividade e o grupo III apresentou as maiores médias para as variáveis relacionadas a qualidade dos frutos. O comprimento do androginóforo, comprimento da câmara nectarífera, a superfície das anteras, o comprimento da câmara nectarífera e a superfície do estigma foram as variáveis que mais contribuíram para a divergência entre os genótipos avaliados. Cruzamentos entre os genótipos do grupo II e III são promissores para o próximo ciclo, contribuindo para o aumento da produção, melhoria da qualidade física e química dos frutos.

**Palavras-chave:** *Passiflora edulis* Sims, Ward-modified location model, seleção recorrente

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## INTRODUCTION

Due to its social and economic importance, sour passion fruit (*Passiflora edulis* Sims) has been exploited by several breeding programs, whose research has achieved success in improving production, fruit quality, pulp yield, the introduction of genes that condition resistance to adverse climate conditions, development of hybrid cultivars and various other traits (Faleiro et al., 2021; Fischer et al., 2021).

This advance of genetic improvement in passion fruit allowed the adoption and use of several selection methods, one of which is intrapopulation recurrent selection. This method can increase the frequency of favorable alleles in a population through continuous cycles of evaluation, selection, and recombination of the superior genotypes in each cycle (Cavalcante et al., 2019; Krause et al., 2020; Fischer et al., 2021). However, if there is no carefully planned crossing system, the selection pressure exerted by the recurrent selection method may cause the loss of important alleles, altering or exhausting genetic variability in populations (Grisi et al., 2019; Oliveira et al., 2019).

In this context, the use of multivariate algorithms aimed at quantifying dissimilarity among individuals is considered an essential tool for the breeding program, as it allows the identification of contrasting genotypes, indicating possible promising crosses and maintaining population variability (Paiva et al., 2014; Silva et al., 2014; Krause et al., 2020; Ferreira et al., 2021). Numerous studies have used external and internal fruit traits to study genetic divergence in breeding programs (Torres et al., 2019; Silva Junior et al., 2022); however, few use flower traits combined with fruit traits. Thus, using the Ward-MLM procedure, the present study aimed to quantify the genetic diversity among progenies of a sour passion fruit population based on morphoagronomic variables of flowers and fruits.

## MATERIAL AND METHODS

The experiment was conducted between January 2019 and December 2020 in the experimental area of the Mato Grosso State University (Universidade do Estado de Mato Grosso - UNEMAT), in Tangará da Serra, MT (14° 39' S, 57° 25' W, and altitude of 321 m). The climate of the region is tropical, with a well-defined dry and rainy season and the average annual precipitation ranges from 1,230 to 2,057 mm per year, with an annual temperature ranging from 16 to 36 °C (Fenner et al., 2021).

One hundred ten genotypes from complete sibling families (FIC) of the third selection cycle from the genetic improvement program of UNEMAT under recurrent selection were evaluated. The experimental design used was randomized blocks with three replicates and three plants per plot.

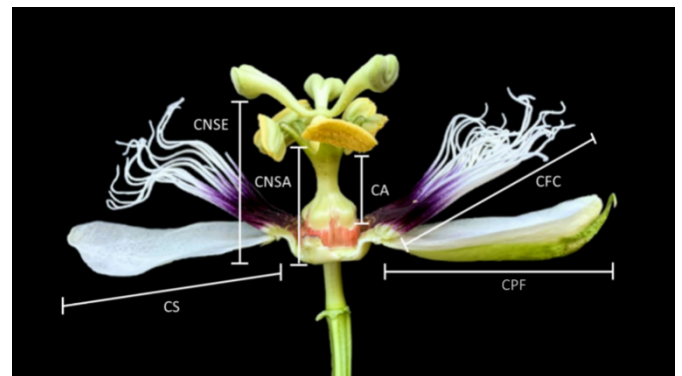
The plant conduction system adopted was spur pruned vsp, with wood posts of 2.5 m, spaced 6.0 m, and a flat wire (12 gauge) 2.0 m above the soil. The distance between the planting lines was 3 m, and 3 m between the pits. The tillage and fertilization of planting and cover were carried out according to the soil analysis. Liming and fertilization for planting and

covering were carried out according to soil analysis, following the recommendations of Borges (2021).

The morphoagronomic variables of the fruits were evaluated from May to September 2020 using nine fruits per plot, which were: Fruit mass in g (MF): With the aid of a digital scale, the fruit mass was obtained by averaging the weight of a sample of nine fruits collected per plot; Pulp percentage (PP): obtained from pulp weighing, this value was divided by the total weight of the fruit and multiplied by 100; Fruit length in mm (CF): was obtained by measuring the longitudinal dimension of the fruits with the aid of a digital caliper; diameter of the fruit in mm (DF): the DF was obtained by measuring the cross-sectional dimension of the fruits with the aid of a digital caliper; fruit shape (FF): carried out by the division of CF/DF; shell thickness in mm (EC): the EC was determined using the arithmetic mean of the measurements of four points of the outer shell. The measurement was performed with a digital caliper; soluble solids (SS): were obtained using a digital refractometer (model RTD-45), with readings in the range of 0 to 45 °Brix. The readings were performed by placing drops of fruit juice individually; titratable acidity (TA): was determined according to the methodology recommended by the AOAC (1990); hydrogen ionic potential (pH): the pH was measured using a digital pH meter (MA-PA200); total soluble solids/ titratable total acidity ratio (SS/TA): it was performed using the simple division of the found SST value by the found att value.

For the evaluation of the variables of the total number of fruits (NF) and yield in kg ha<sup>-1</sup> (Yld), it was considered the first production cycle of the crop in the region, which occurred from November 2019 to November 2020 of this experiment. Yld was obtained by weighing the fruits of the plots during the conduction of the experiment, multiplied by the number of plants per hectare, expressed in t ha<sup>-1</sup>; NF was obtained by counting the fruits of the plots during the conduction of the experiment.

For the evaluation of the flower variables, five open flowers from each plot were collected between 14:30 and 15:30 hours. With the aid of a digital caliper, the following traits were evaluated: length of sepals (CS), length of petals of the flower (CPF), length of corona filaments (CFC), length of androgynous (CA), length of the nectarous chamber on the surface of the anthers (CNSA) and length of the nectarous chamber on the surface of the stigma (CNSE) (Figure 1).



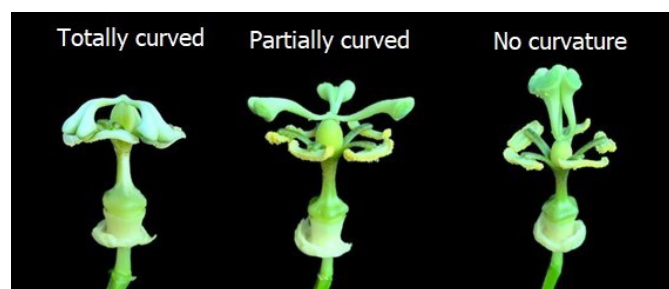
CS - Sepal length; CPF - Flower petal length; CFC - Corona filament length; CA - Androgynous length; CNSA - Nectar length on the surface of the anthers; CNSE - Nectary length on the stigma surface

**Figure 1.** Representation of the flower of *Passiflora edulis* Sims

The percentage of totally curved stigmas (PTCs) of the flowers was also evaluated from 10 flowers of 10 plants between 14:30 and 15:30. For this evaluation, the classification of the curvature of the stigma was proposed by Ruggiero et al. (1976); that is, totally curved stigmas were below and/or at the same level as the anthers. The partially curved ones are those in which the stigmas are located above the anthers, forming an angle of 45°, and those without curvature cover the stigmas that form an approximate angle of 90° concerning the anthers, as shown in Figure 2.

The quantification of genetic divergence was performed using the Ward-MLM (modified location model) method proposed by Franco et al. (1998). For this, the index of Gower generates the estimate of the dissimilarity index that varies by 1 and 0.

The ideal number of groups was defined based on the criteria of the pseudo-F and pseudo-T<sup>2</sup> combined with the likelihood profile associated with the likelihood ratio test. Subsequently, the logarithm chart of the likelihood function was obtained and maximized for different probable numbers of groups according to the MLM method. According to the likelihood peaks for the different numbers of groups formed by the graph, the best number of groups was defined for the analysis. Finally, the complete MLM analysis was performed for the number of defined groups, describing the classification results, with a table of the description of the groups formed and the canonical analysis for the quantitative variables, being used for the latter, the canonical coordinates for the observations. The differences between the groups and the canonical variables (VC) were graphically analyzed. All analyses were performed using the statistical software SAS (SAS, 2022). The diagrams were made using the software SigmaPlot, version 14.0.

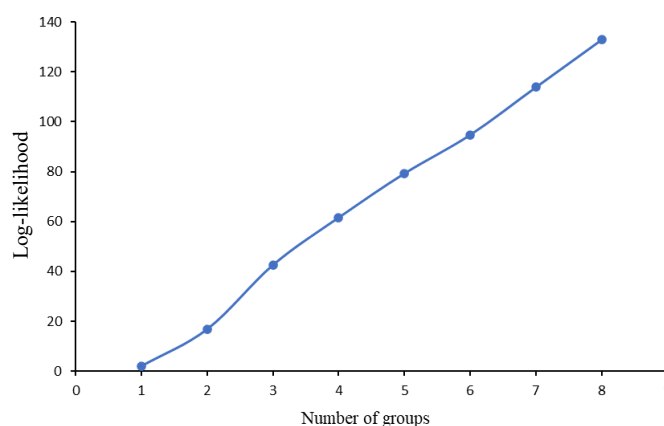


**Figure 2.** Representation of the flower of *Passiflora edulis* Sims in evidence of the curvature of the stylus

## RESULTS AND DISCUSSION

From the logarithmic function of likelihood, according to the criteria of the pseudo-F and pseudo-T<sup>2</sup>, three groups established by the Ward-MLM method were observed. (Figure 3). The ideal number of groups was verified where there was a greater increase in the logarithmic function, with the highest absolute value of 42.61 in the third group.

The formation of three groups found in this study demonstrates that there is variability to be explored in the population; in addition, the formation of the ideal number of groups using the log-likelihood function may vary according to the species, quantity, and types of variables evaluated. Results similar to this study were found by Santos et al. (2014). They



**Figure 3.** Log-likelihood in function of the number of groups

studied genetic divergence in a segregating population of the crossing between *Passiflora edulis* and *Passiflora setacea* using the Ward-MLM method to analyze morphoagronomic data, which allowed the separation of 138 genotypes into three homogeneous groups. On the other hand, Paiva et al. (2014) used the same Ward-MLM method to quantify the diversity in wild and commercial passion fruit species, evaluating qualitative and quantitative morphological traits. They found the greatest increase in probability function was achieved when five groups were considered. Silva et al. (2014) used this same strategy in 81 full-sib progenies of passion fruit. They verified that the Ward-MLM method was efficient in detecting five heterotic groups, providing an indication of possible crosses between divergent groups.

As shown in Table 1, groups I, II, and III were formed by 76, 15, and 19 genotypes, respectively. Group I presented the highest means for the variables SST/ATT ratio, CS, CPF, CFC,

**Table 1.** Means of the variables for each of the three groups formed by the Ward-MLM method and the first two canonical variables

Variables	Groups			Average	Canonical variables	
	I (76)	II (15)	III (19)		VC1	VC2
Yld (t ha <sup>-1</sup> )	18.31	28.94	21.31	22.85	-0.43	0.63
NF (un)	255.70	396.11	299.77	317.17	-0.41	0.57
MF (mm)	238.54	204.87	260.62	234.67	-0.13	-0.65
CF (mm)	94.02	88.56	94.44	92.34	0.12	-0.51
DF (mm)	84.37	80.57	85.5	83.48	0.02	-0.65
FF	1.11	1.11	1.11	1.11	0.14	-0.09
EC (mm)	5.89	5.78	6.07	5.91	-0.07	0.23
PP (%)	39.95	37.35	40.28	39.19	0.05	-0.28
SS (°Brix)	12.75	12.61	12.77	12.71	0.01	-0.08
AT (%)	3.65	3.64	3.73	3.67	-0.10	-0.07
pH	2.97	2.94	3.01	2.97	0.05	0.22
SS/AT	3.56	3.49	3.51	3.52	0.07	-0.04
CS (mm)	40.58	39.14	37.52	39.08	0.06	0.08
CPF (mm)	38.49	36.99	35.15	36.87	0.07	0.10
CFC (mm)	30.43	29.76	28.15	29.44	0.51	0.15
CA (mm)	13.84	13.76	13.61	13.73	0.80	-0.10
CNSA (mm)	22.56	21.99	21.25	21.93	0.54	0.07
CNSE (mm)	30.15	30.04	29.10	29.76	0.78	-0.13
PTC (%)	86.52	85.69	77.43	83.21	0.24	0.19

MF - Fruit mass; CF - Fruit length; DF - Fruit width; FF - Fruit shape; EC - Peel thickness; PP - Pulp percentage; CP - Pulp coloration; SS - Total soluble solids; AT - titratable acidity in citric acid; SS/AT - Ratio; pH - Hydrogen ionic potential; CS - Sepal length; CPF - Flower petal length; CFC - Corona filament length; CA - Length of androgynous; CNSA - Length of the nectareous chamber the surface of the anthers; CNSE - Length of the nectareous chamber the surface of the stigma; CT - Percentage of totally curved stigmas; Yld - Yield; NF - Number of fruits

CA, CNSA, CNSE, and PTC. It is evident that the genotypes that constituted this group presented a higher number of flower-related traits.

However, it is worth mentioning that the variables CA, CNSA, and CNSE are reproductive structures directly linked to pollination and aim to reduce these variables in commercial cultivars. Thus, the lowest means observed for the variables CA (13.61 mm), CNSA (21.25 mm), and CNSE (29.10 mm) were in group III. Oliveira et al. (2020) found that a reduction in the androgynous length in selections of sour passion fruit would allow honeybees and smaller insects to act as pollinator agents, as short androgynous androphores reduce the height of stigmas concerning the crown, favoring pollination.

The genotypes from group II presented intermediate values for almost all variables studied, except for NF (396.11) and Yld (28.94), with the highest values. Therefore, this group can be exploited to increase yield. Group III presented the highest means for MF, CF, DF, FF, EC, PP, SS, AT, and pH, and this group consisted of traits related to fruit quality. Thus, the genotypes of this group have the potential to be explored in crosses because they represent the physical and chemical traits of superior fruits and meet the demands of the consumer market (Borges et al., 2019).

Table 1 shows that the variables that most contributed to genetic diversity based on VC1 were CA (0.80), CNSE (0.78), and CNSA (0.54). These variables show that VC1 is more related to flower traits. Concerning VC2, the variables that most contributed to genetic diversity were Prod (0.63) and NF (0.57), indicating that VC2 is related to crop yield.

The high contribution of the CA variable is explained by the existing relationship of this variable with the formation of the physical traits of fruits; it has a strong relationship with the CNSA and CNSE. Formagio et al. (2021), when working with the correlations between flower and fruit traits of the sour passion fruit, found a strong correlation between CA × CNSA × CNSE, demonstrating that the increase in the length of the androgynous chamber results in the elevation of the length of the nectariferous chamber, the surface of the anthers and consequently also raising the length of the nectariferous chamber the surface of the stigma.

Results similar to this were found by Paiva et al. (2014), who studied with quantification of the existing diversity in wild and commercial species of passion fruit using morphological traits related to the vegetative and reproductive stages of the plants. The authors found that the size of the androgynous (0.53) and the length of the bracts (0.53) were the variables that most contributed to quantifying the genetic diversity among the species.

The shortest distance between the groups was observed between I and II groups, with an estimated value of 17.12, showing similarity between the genotypes that make up this group. The greatest distance occurred between groups II and III, with a magnitude of 22.25 (Table 2). This distance between groups II and III may indicate possible crosses, aiming at the exploration and capitalization of heterosis, increasing the concentration of favorable alleles of the traits of interest. It is noteworthy that the values between the smallest and the greatest distance are close; that is, it reflects a small distance, which may

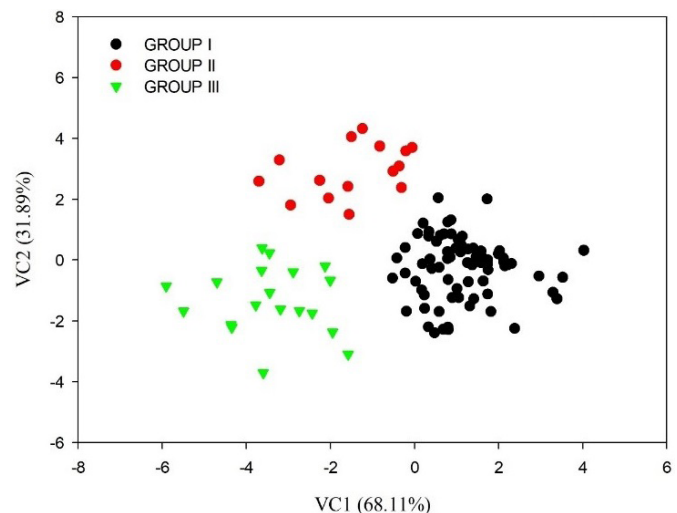
**Table 2.** Number of groups, number of genotypes per group, and distance between groups formed by the Ward-MLM method in the quantification of genetic divergence

Groups	I (76)	II (15)	III (19)
I	-	17.12	22.05
II	-	-	22.25

be due to the selection and recombination processes during the cycles and the formation of the initial base population.

The first two canonical variables could represent 100% of the total variation, so canonical variable 1 was responsible for 68.11% and canonical variable 2 for 31.89% (Figure 4). According to Arevalo et al. (2021), if the first two canonical variables allow estimates above 80% of the total variation, it is possible to satisfactorily explain the variability between genotypes in a two-dimensional dispersion plot. Similar results were found by Fachi et al. (2019) and Krause et al. (2021), in which the first two canonical variables also explained 100% of the total variation.

Obtaining higher populations is one of the main objectives of breeding programs; in this sense, following the principle of choosing genotypes with greater dissimilarity and with high averages for the evaluated traits makes it possible to predict the achievement of promising segregating populations. Thus, based on the data obtained in this study, groups II and III presented important traits for the genetic improvement of passion fruit. Therefore, crossings between the best genotypes of these groups would contribute to increased production (Prod and NF), improvement of much of the physical and chemical quality of fruits (MF, CF, DF, PP, and SST), and would decrease in the main flower structures addressed in this study (CA, CNSA, CNSE).



**Figure 4.** Dispersion of the first two canonical variables (VC1 and VC2) representing the formation of three groups by the Ward-MLM method based on flower and fruit variables of sour passion fruit

**CONCLUSIONS**

1. Multivariate analysis using the Ward-modified location model method based on morphoagronomic flower and fruit variables efficiently quantified genetic diversity and allowed the identification of three groups within the population.

2. Among the groups formed, group I presented higher means for the variables related to flowers, group II presented the highest means for the variables related to yield, and group III presented the highest means for the variables related to fruit quality.

3. The androgynous length, nectariferous chamber length, anther surface, nectareous chamber length, and stigma surface were the variables that most contributed to the divergence between the evaluated genotypes.

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