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Females and hermaphrodites of papaya (*Carica papaya* L.) of the Tainung Nº1 cultivar exhibit the same initial vigor

Plantas fêmeas e hermafroditas de mamão (*Carica papaya* L.) cv. Tainung Nº1 apresentam o mesmo vigor inicial

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ABSTRACT

The sexual types of papaya plants (*Carica papaya* L.), namely female, male, and hermaphrodite, can only be distinguished during their reproductive phase. As a result, numerous studies have been conducted to identify distinctive characteristics among these sexual types in papaya seedlings. One hypothesis suggests that leaf temperature and initial vigor may serve as differentiating factors in female and hermaphroditic plants. Therefore, the objective of this study was to contribute to the morphophysiological characterization of female and hermaphroditic papaya seedlings (cv. Tainung Nº1) by evaluating leaf temperature and initial growth vigor. The analysis was performed using a T-test ($p < 0.05$), employing a predictive approach as well to assess the potential genetic distance between the hermaphroditic and female sexual varieties. The results revealed that both hermaphroditic and female seedlings exhibited an average leaf temperature of 26.76°C, along with similar initial growth vigor. Consequently, the limited genetic differentiation, as well as the evaluated characteristics of the plants during the seedling stage, were insufficient to predict the sexual type of the plants.

Keywords: Sexual differentiation; Multivariate analysis; Seedling stage.

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RESUMO

Os tipos sexuais de mamoeiro (*Carica papaya* L.), nomeadamente feminino, masculino e hermafrodita só podem ser distinguidas durante a sua fase reprodutiva da planta. Como resultado, estudos têm sido conduzidos para identificar características distintivas entre esses tipos sexuais em plântulas de mamoeiro. Uma hipótese sugere que a temperatura foliar e o vigor inicial podem servir como fatores diferenciadores em plantas fêmeas e hermafroditas. Portanto, o objetivo deste estudo foi contribuir para a caracterização morfofisiológica de plântulas de mamoeiro femininas e hermafroditas (cv. Tainung Nº1), avaliando a temperatura foliar e o vigor de crescimento inicial. A análise foi realizada utilizando o teste T ($p < 0,05$), empregando também uma abordagem preditiva multivaridada para identificar a distância genética entre as variedades sexuais hermafrodita e feminina. Os resultados revelaram que, tanto as plântulas hermafroditas quanto as femininas apresentam a temperatura foliar média de 26,76 ºC, bem como o mesmo vigor de crescimento inicial. Consequentemente, a reduzida diferenciação genética, bem como as características avaliadas das plantas enquanto mudas foram insuficientes para prever o tipo sexual das plantas.

Palavras-chave: Diferenciação sexual; Análise multivariada; Estádio de plântula.

INTRODUCTION

Fruit cultivation plays a significant role in the Brazilian economy. Among the fruits grown in Brazil, papaya (*Carica papaya* L.) stands out, with a production exceeding 1.2 million tons and generating over R$ 1 billion in revenue. It is also ranked among the top ten fruits in terms of national production (IBGE, 2020). Moreover, Brazil has been able to meet a portion of the demand for papaya from other countries, including the United States of America, Canada, Argentina, and Uruguay (BRASIL, 2018).

Papaya is an herbaceous plant belonging to the Caricaceae family (USGS, 2013). While wild populations of this species are dioecious, meaning they have separate "male" and "female" plants, whereas domesticated papaya can exhibit dioecy or trioecy, resulting in populations with female, male, and/or "hermaphrodite" plants. In cultivated papaya, these sexual phenotypes can be categorized as follows: 1) Male plants exhibit flowers with functional stamens and rudimentary ovaries. These plants are typically sterile and incapable of fruit production; 2) female plants have flowers with large, rounded ovaries, resulting in a rounded fruit shape, and; 3) hermaphrodite plants possess flowers with functional reproductive organs and are capable of producing elongated fruits.

In any case, these plant sex is complex and determined by sexual chromosomes, with the XX, XY, and XYh combinations determining female, male, and hermaphrodite plants, respectively (MING et al., 2007; VANBUREN et al., 2015; VASHISTHA et al., 2016; WANG et al., 2012). It is important to note that sexual phenotypic reversal is a well-known phenomenon in papaya and is highly influenced by abiotic stresses (INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE, 1988; LIN et al., 2016; TRINDADE, 2000; WANG et al., 2012; ZHOU et al., 2019).

Although sexual expression is influenced by the action of genes on sex chromosomes (WANG et al., 2012), and use of molecular markers allows for the early identification of sexual expression in papaya (OLIVEIRA et al., 2007; VASHISTHA et al., 2016), thereby reducing handling costs. Whereas, other efforts have been made to reduce the costs associated with sex determination in the early stages of cultivation.

Cost reduction is of utmost importance in the context of production and commercialization. Elongated fruits are highly desirable due to their inherent qualities such as enhanced resistance during transportation and the potential to attain high market values (CHAN, 2009). Consequently, in commercial fields, it becomes necessary to

remove plants that do not yield the desired fruits for the market (rounded shape with large cavities). As a result, hermaphrodite plants are preferable in production fields due to their floral organization and composition, which allow for better fruit set (COSTA et al., 2003; SANCHES; DANTAS, 1999).

Among the available hybrids in Brazil, the Tainung Nº 1 cultivar is the preferred choice for cultivation (LUZ et al., 2015; SERRANO; CATTANEO, 2010). However, sexing of plants is a labor-intensive activity in orchards and contributes to increased costs during the establishment phase. Moreover, the plant density up to this point may be three times higher than the ideal density, which hampers soil exploration by the root system of the plants (MING et al., 2007).

Additionally, despite the cost reduction using cloned seedlings and molecular techniques, attempts to early identify the sexual expression of plants and make them more possible to adopte by the producers themselves were carried out. Some studies considered evaluations such as some morphological traits (ROJAS et al., 1985; SÃO JOSÉ; CUNHA, 1988), coloring, and other physical aspects of seeds, as well as the isoenzymatic characteristics of seedlings (ARANGO et al., 2008; SONI et al., 2017).

Chutteang, Yingjajaval and Wasee (2007) propose that physiological differences, such as transpiration rates observed in adult plants, could be indicative of early sex determination in seedlings. Therefore, the main hypothesis suggests that female plants would exhibit higher transpiration rates and, consequently, lower leaf temperatures compared to hermaphrodite plants. Hence, one hypothesis is that morphophysiological characteristics could serve as discriminators between female and hermaphrodite papaya seedlings.

Thus, characteristics that can be easily measured at the seedling stage can be useful for predicting papaya sexual types. To verify it, some predictive methods can be tested for this purpose. Commonly, predictive methods are widely used for studies of genetic diversity, especially based on morphological information (CHIORATO et al., 2007; VASCONCELOS et al., 2017; VIVAS et al., 2018), where diversity is expressed as a coefficient of dissimilarity and measures of genetic distance. Hierarchical representations such as dendrograms are constructed from (dis)similarity matrices and become a way of graphically representing these genetic distances.

Thus, this work aimed to characterize the female and hermaphrodite sexual varieties of papaya seedlings based on characteristics of leaf temperature and growth, and to analyze the putative dissimilarity presented by the seedlings.

MATERIALS AND METHODS

Local and seedlings production

The experiment was conducted from mid-October 2017 to March 2018 in a greenhouse at the Federal Institute of Education, Science, and Technology from Mato Grosso - IFMT, located in Campo Verde. The greenhouse was equipped with a black shade screen, allowing for 50% light incidence. These particular months were chosen due to the climatic characteristics of the region, which experiences a dry winter from May to September, followed by a rainy summer from October to March (MARCO et al., 2014). Consequently, manual watering shifts during seedling acquisition and transplantation were not required. To obtain the seedlings, a sample of 128 commercial seeds of the Tainung Nº1 hybrid papaya was acquired and individually sown at a depth of one centimeter in 280 cm^3 tubes filled with subsurface soil.

Evaluated traits, comparative and predictive analysis

On the 50th day after sowing, the seedlings in the greenhouse were identified, and the following evaluations were conducted. The foliar temperature (ºC) was obtained measuring the temperature of the last fully expanded leaf was measured as the average between two consecutive readings taken with a digital infrared thermometer commonly used for checking human body temperature.

Plant height (cm) was measured on the 50th day and 75th day after sowing (DAS), and the growth differential (cm) was obtained by the difference in cm between the height at 75 DAS and 50 DAS. Subsequently, on the 90th day, the seedlings were transplanted in the experimental area, with a spacing of 0.5 m between plants and one meter between rows, solely for the purpose of determining the sex of the plants, as described by Dantas and Castro Neto (2000).

For analyzing the traits, a two-sample T-test was performed, assuming the null hypothesis that the difference between the two groups (female and hermaphrodite) is zero.

To assess the predictability of clusters among seedlings of the same sexual variety, multivariate analyses were employed to test the analysis of genetic distance. The standardized average Euclidean Distance, obtained following the method proposed by Cruz & Regazzi (2001) was used as a measure of dissimilarity:

$$\text{Euclidean Distance } (d_{ii'}) = \sqrt{\frac{1}{n}\sum_j (X_{ij} - X_{i'j})^2}$$

Where,

$d_{ii'}$: distance between seedlings i and i';

X_{ij}: observation in the *i*-th mute in reference to the *j*-th characteristic;

$X_{i'j}$: observation in the i'-th seedling in reference to the *j-th* characteristic;

n: number of characteristics under study.

The Gower distance matrix (GOWER, 1971) was obtained to assess the predictability of grouping when considering the nominal characteristic (1 for female and 2 for hermaphrodite):

$$\text{Gower's distance } (S_{ij}) = \frac{\sum_k^n w_{ijk} s_{ijk}}{\sum_k^n w_{ijk}}$$

Where,

S_{ijk} indicates the contribution of the *k-th* characteristic and;

W_{ijk} é 1 ou 2 para a *k-ésima* característica.

To show formed groups, the hierarchical representation UPGMA (Unweighted Pair Group Method with Arithmetic mean) (RÉDEI, 2008) were realized.

The analyzes were performed using the R software (R DEVELOPMENT CORE TEAM, 2010) and the figures using the *dendextend* package (GALILI, 2015) and *ggplot2* (WICKHAM, 2016).

RESULTS AND DISCUSSION

Traits female and hermaphrodite papaya seedlings

Considering a sample size of 108 plants that reached the reproductive stage, flower identification was performed following the method described by Dantas & Catro

Neto (2000). The obtained population was characterized as gynoic-andromonoeic, with a 1:1 ratio of female and hermaphrodite plants.

Descriptive statistical of the traits for both female and hermaphrodite groups are shown in Table 1. The one-sample Kolmogorov-Smirnov test indicated a continuous distribution for all traits, except for height at 75 DAS in the female group (Table 1). However, the two-sample T-test revealed that the evaluated traits were not statistically significant ($p > 0.05$) (Figure 1). This suggests that hermaphrodite and female sexual varieties exhibit similar initial growth and leaf temperature.

The overall means are 26.8 ºC, 10.2 cm, 14 cm, and 3.9 cm to foliar temperature, height at 50 DAS, height at 75 DAS, differential growth, respectively.

Table 1. P-value from one-sample Kolmogorov-Smirnov test and descriptive statistic of female and hermaphrodite (herm.) seedling groups of *Carica papaya* cv. Tainung Nº 1.

Trait	Sexing	P-value	Mean	Variance	Standard deviance	Overall Mean
Foliar temperature (ºC)	Herm.	0.85	26.84	0.62	0.79	26.76
	Female	0.98	26.72	0.53	0.73	
Height at 50 DAS (cm)	Herm.	0.09	10.38	4.35	2.09	10.18
	Female	0.11	9.92	4.77	2.18	
Height at 75 DAS (cm)	Herm.	0.47	14.37	4.61	2.15	14.03
	Female	0.05	13.61	4.41	2.10	
Differential growth (cm)	Herm.	0.77	3.99	1.55	1.25	3.85
	Female	0.17	3.69	0.63	0.79	

Despite we did not use gibberellic acid to improve the germination, our means values were similar to Babu et al. (2010), that evaluating the influence of gibberellic acid used to germination in the initial growth papaya cultivars at 60 days after sowing with mean of 10.98 cm.

Chutteang, Yingjajaval & Wasee (2007), reports that female plants show greater vigor when compared to hermaphrodites. In this study, the smaller GD values indicated by variance and standard deviation of the female seedlings suggest less growth, but greater uniformity of seedlings. However, Baiyeri (2006) reports the influence of different types and colors of screenhouse covers on the initial development of papaya plants, indicating that the vigor is highly influenced by environmental conditions.

According to this, the polygenic effect on the growth of papaya plants is widely known (SONDUR et al., 1995).

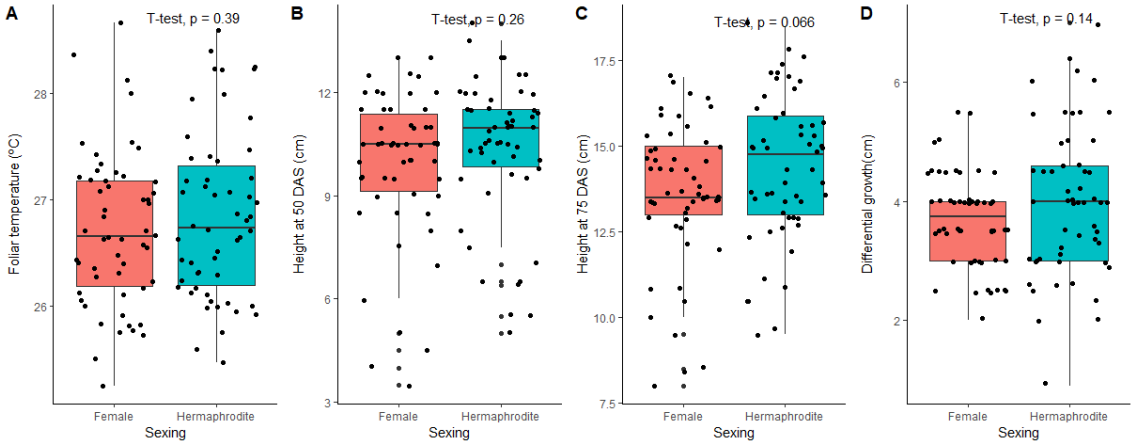

Figure 1. Box plots of the evaluated traits in seedlings of papaya cv. Tainung Nº1. The red box plot indicates the group of female plants identified during sexing, while the blue box plot represents the hermaphrodite plants. The p-value of the T-test is displayed at the top of the figures.

The dispersion values of height can influenced by the difference in vigor between the seedlings, and it is directly influenced by the size and weight of the seeds (WISNER, 2004). Justifying the dispersion values found, since there was no distinction between the sizes and weights of the seeds.

According to the hypothesis of Chutteang et al., (2007) leaf temperature, as well as the temperature differential of female seedlings are lower compared to hermaphrodites, however, they do not differ statistically and it is not possible to select the desired sexual phenotype using this trait. In fact, the mean to foliar temperature is not different, the value obtained by (CHUTTEANG et al., 2007) (27.8ºC), is similar to value we found.

Predictive analysis

We noticed that the evaluated traits in this study are easily assessed; however, it is not possible to predict the sexing of papaya seedlings (cv. Tainung Nº1) using only these traits in a multivariate analysis. Other more complex traits may be necessary to achieve this objective. Additionally, the use of molecular markers or cloning the desired sexual type has been a common and effective way to obtain an appropriate plant density in the field.

The dendrograms considering the dissimilarity matrices obtained by Euclidean Distance and Gower Distance are presenting in Figure 2.

As shown in dendrogram A, it was observing that the individuals do not correspond to the correct grouping indicated by dendrogram B (which, in turn, was obtaining considering sexing at the flowering stage, with the respective nominal information 1 and 2 for female and hermaphrodite, respectively). That observed groups for the evaluated quantitative traits are not sufficient to predict the sexing of this cultivar.

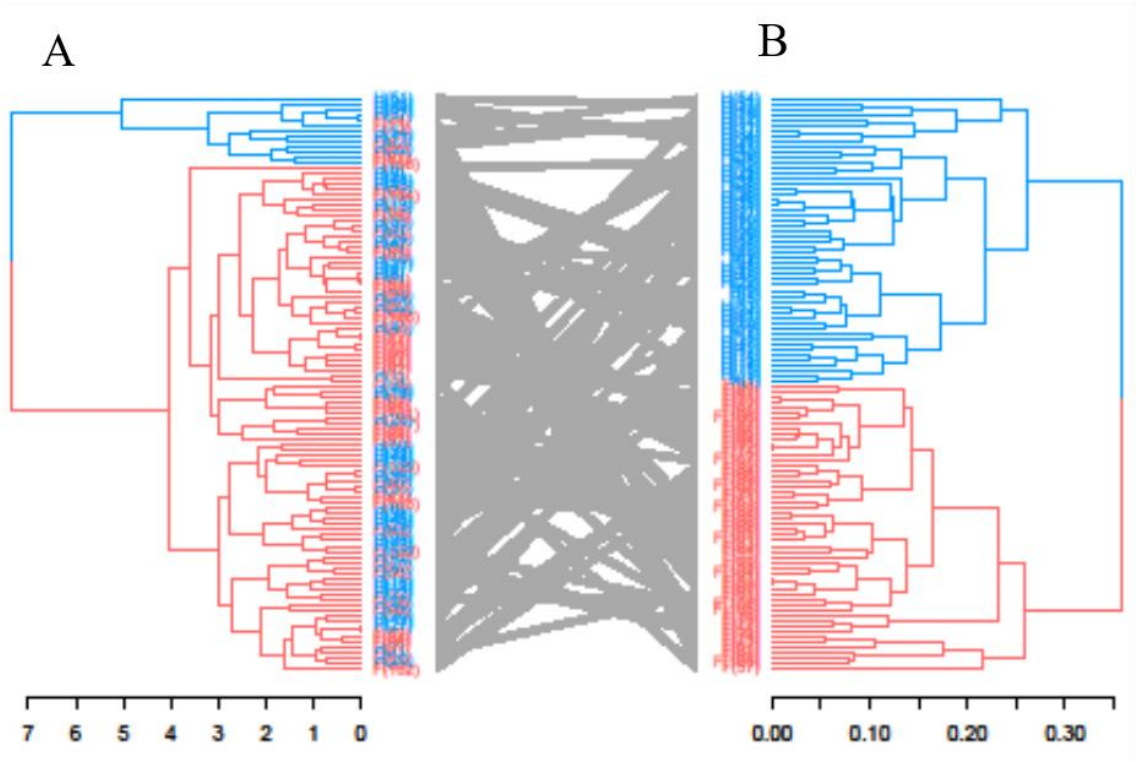

Figure 2. Comparison between the hierarchical representations of genetic dissimilarities among 108 seedlings of Carica papaya cv. Tainung Nº1 considering k=2. A) Dendrogram based on the Euclidean distance, not considering the previous separation of hermaphrodite (blue) and female (pink) plants; B) Dendrogram based on the Gower distance, considering the separation of hermaphrodite (blue) and female (pink) plants using them as nominal characterization.

In order for the hierarchical representations to adequately represent the dissimilarity among the studied individuals, the cophenetic correlation coefficient (CCC) can indicate the reliability of representing genetic distances through dendrograms, with higher CCC values indicating a closer approximation to unity (MONTEIRO et al., 2010).

The cophenetic correlation coefficient considering the dissimilarity matrix obtained by the Euclidean Distance method, which takes into account quantitative traits, showed significance ($p < 0.01$) with a value of $r = 0.70$, demonstrating reliability in the relationship between the dissimilarity matrix and the dendrogram generated by the UPGMA method. However, the CCC of the dissimilarity matrix obtained by the Gower method, which can consider the distinction between female and hermaphrodite seedlings as factors, showed significance ($p < 0.01$) with $r = 0.79$, indicating comparatively better reliability in representing genetic distances through the dendrogram.

However, the range of dissimilarity values based on average Euclidean Distance (Dii') ranged from 0.00 (herm.×female) to 0.65 (herm.×female), indicating that the slight putative difference does not provide evidence of differences between the different sexual types of this cultivar. On the other hand, it should be noted that the population obtained is derived from crosses between complementary parents, resulting in a highly phenotypically uniform F1 progeny, thus making it difficult to identify morphological differences between sexual types of cultivars.

We showed that there is no evidence of success in the use of multivariate analysis based on dissimilarity between females and hermaphrodite seedlings in papaya (cv. Tainung Nº1) because the traits evaluated are not different in seedling stages.

CONCLUSION

According to the results obtained in this experiment, we can conclude that the traits of papaya (cv. Tainung Nº1) seedlings, both for female and hermaphrodite plants, do not differ, and it is not possible to predict the sexual variety based on the considered characteristics.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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