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BASIC AREAS - Article

Quantification of floral abnormalities in a population generated from sexual polymorphism aiming at recurrent selection in papaya

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ABSTRACT: The aim of this study was to estimate the percentage of deformed fruits (carpelloid, pentandric, and bananoid), summer sterility, repeatability coefficients, and correlations among the variables for floral abnormalities; and select genotypes with the lowest incidence of deformed fruits and summer sterility. In the experiment, which involved 254 plants, traits were evaluated using the digital-phenotypic method and the obtained images were processed and analysed using *ImageJ* v1.48 software with the Cell Counter plugin. Summer sterility had the highest percentage of occurrence in the evaluated periods. Based on the quantification of total abnormalities, the 30 genotypes with the lowest incidence of floral abnormalities were selected. Summer sterility is the main problem of the papaya crop in terms of floral abnormalities. The non-significant correlations between the traits: NDF/TNF+NFN (number of deformed fruits/number of fruits + number of fruitless nodes) and NFS/TNF+NFN (number of fruitless nodes/number of fruits + number of fruitless nodes); NDF/TNF+NFN and NFN/SUM (number of fruitless nodes/sum of abnormalities); and NFS/TNF+NFN and NDF/SUM (number of deformed fruits/sum of abnormalities) indicate that the variables are independent of each other. Of the 254 evaluated progenies, at least 30 were identified as having potential for selection, with lower occurrence of total floral abnormalities.

Key words: *Carica papaya* L., female sterility, carpellody, *ImageJ*.

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INTRODUCTION

Papaya is a trioecious species: staminate or male; hermaphrodite; and pistillate or female plant. However, in commercial terms, most crops consist of hermaphrodite plants. Of the three basic sex forms featured by the plant, hermaphrodites are the most vulnerable to sex reversal, carpellody and pentandry, whereas female plants are stable to floral abnormalities (Damasceno Junior et al. 2008).

Summer sterility or sex reversal appears in situations of elevated temperature, water stress and low nitrogen levels. It is characterized by the sex reversal of a hermaphrodite flower into a functional male flower (Martelleto et al. 2011). In winter temperatures, there is the occurrence of pentandria, in which the fruits are rounded with five deep longitudinal grooves generated by the fusion of the stamens at the ovary (Arkle Junior and Nakasone 1984). Under low or mild temperatures, high humidity, nitrogen deficiency in the soil and water deficit favor carpellody. In this condition, the hermaphrodite flower can convert its stamens into structures similar to carpels (Fig. 1) (Couto and Nacif 1999; Arkle Junior and Nakasone 1984). The occurrence of "bananoid" fruits was also observed, which have their shape and the elongated ovarian cavity, and may be due to a desynchronized pollination.

Floral anomalies reflect negatively on commercial fruit production, so the increased availability of varieties less sensitive to the influence of seasonality on sexual expression is of great importance for the cultivation of papaya and should reduce production fluctuations occurring throughout the year. As a consequence, the producer will have increased chances of success in crop exploitation (Ramos et al. 2011).

The reproduction mode in hermaphrodite papaya is autogamous, with cleistogamy (Damasceno Júnior et al. 2009). As such, it can be self-pollinated without a significant inbreeding depression, which makes it possible to obtain inbred lines in the development of varieties or hybrids (Dantas and Lima 2001). Several methodologies have been adopted in the breeding of this crop (e.g. backcrossing) aiming at the sexual conversion of the Cariflora genotype from dioecious to gynodioecious (Silva et al. 2007a).

Vivas et al. (2012) investigated progeny from dioecious genotypes cultivated by small farmers in the south of Espírito Santo State, Brazil, and concluded that there is a possibility of selection of new progeny, derived from these genotypes, for the formation of future populations via recurrent selection in generation advancement and in the development of new lines of papaya resistant to the black spot. Despite being rarely used in papaya, recurrent selection is a promising methodology, as it aims at increasing the frequency of favorable alleles, preserving genetic variability in the population.

The present study proposes to estimate the percentage of deformed fruits (carpelloid, pentandric and bananoid), summer sterility percentage, repeatability coefficient and correlations among variables for floral abnormalities; and to select genotypes with a lower incidence of deformed fruits and summer sterility.

MATERIAL AND METHODS

Plant material

A segregating population from the recurrent selection program of inbred papaya families was used in the study. The family consisted of 25 plants and was obtained from a cross between five female dioecious progeny (M1, M2, M3, M4 and M5) resistant to the black spot and a pollen mixture of five elite genotypes (UC-SS72-12, UC-JS12, UC-Sekati, UC-36/7 and UC-41/7), four of which were of the Formosa heterotic group and one belonged to the Solo group.

Experimental area

The experiment was carried out in the experimental area of the Caliman Agrícola S.A. company, located in Linhares/ES, Brazil (between 19º06' and 19º18' S; and 39º45' and 40º10' W). The climate in the region is a Koppen's Awi type (humid tropical), with rainy summers and dry winters, and the relief is flat, forming coastal plateaus. During the experiment, the average precipitation, temperature and air relative humidity were 0.08 mm, 23.91 ºC and 73.82%, respectively, considered a high water deficit in the region during the evaluation period (INMET 2017) (Table 1).

Plants were transplanted in September 2016, with rows spaced 3.60 m × 1.50 m, using three seedlings per furrow. Sexing was performed after three months of transplant, maintaining only hermaphrodite plants. The crop treatments applied were the same as those used by the Caliman Agrícola S/A company in commercial crops, under microsprinkler irrigation.

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Table 1. Climatic variables observed in the evaluation months in 2017, in Linhares/ES (INMET 2017).

Period	Average temperature (°C)	Relative humidity (%)	Mean precipitation (mm)
Summer	26.49	70.74	0.06
Fall	24.45	75.40	0.11
Winter	20.80	75.32	0.05
Mean	23.91	73.82	0.08

The following traits were evaluated: percentage of pentandric (%PF), carpelloid (%CF), and bananoid (%BF) fruits (Fig. 1); and percentage of nodes without fruits (%NW), which denotes summer sterility.

The traits were evaluated by the digital-phenotypic methodology, as validated and described by Cortes et al. (2017a), using a Sony DSCHX 300 digital camera. Each plant was photographed in two different positions, one of them perpendicular to the plant, considering the axis of the row, and the other one considering the opposite side of the same plant used in Fig. 1a. The pictures were obtained by taking them at a distance of 2.5 m from the plant row. Both images were used to estimate NFD and NSF. The images were analysed using the public domain image-processing program *ImageJ*. In each plant photographed, a ruler was placed as a reference to facilitate calibration using the software function set scale. The NFD and NSF traits were estimated using the plugin Cell Counter, which is part of *ImageJ*.

The production potential of the genotype was estimated as the number of deformed fruits (NDF) and the number of nodes without fruits (NWF) relative to the sum between total number of fruits, which is the sum of the number of deformed and commercial fruits, and number of nodes without fruits (TNF + NWF). The incidence of floral abnormalities was estimated as NDF and NWF relative to the sum of total abnormalities (SUM).

Three evaluations were carried out: the first at six months after transplanting, in March 2017; the second at nine months, in June 2017; and the last one at twelve months post-transplanting, in September 2017. The harvested fruits correspond to the summer, fall and winter seasons, respectively.

Data analysis

After the collection of data in the three periods, the estimated variables for production potential and incidence of abnormalities were analysed using the linear mixed models methodology, applying the REML (restricted maximum likelihood) procedure to estimate the variance components and genetic parameters and the BLUP (best linear unbiased predictor) procedure to estimate the permanent phenotypic effects.

$$y = Xm + Wp + \varepsilon$$

where y is the vector of observations; m is the vector of measurement effects (assumed as fixed) added to the overall mean; p is the vector of permanent effects of plants (genotypic + environmental effects, assumed as random); ε is the vector of residuals (random); X and W represent the incidence matrices for the said effects.

This is a basic repeatability model for experiments without an experimental design, which is appropriate for the experimental conditions adopted in this study. The said model was analysed using Selegen-REML/Blup software, as proposed by Resende (2016).

Pearson's correlation coefficients among the abnormality variables were estimated and their significances were tested by the t test ($p < 0.01$) using GENES software (Cruz et al. 2014).

The ranking of the 30 progeny with superior potential was obtained from the estimate of total abnormality (TA), obtained as the sum of the product between the means

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Figure 1. Deformed papaya fruits: (a) Bananoid fruit; (b) Carpelloid fruit; (c) Pentandric fruit. Linhares/ES, 2017.

of the estimated variables and the means of variables of greatest influence on the population during the three evaluations; in this case, NDF/SUM ($\mu = 61.20$) and NWF/SUM ($\mu = 38.80$). The estimate of total abnormality was evaluated as described below:

$$TA = [0.612 \times (V1 + V3) + 0.388 \times (V2 + V4)]$$

where: V1 = μ %NDF/TNF + NWF; V2 = μ %NWF/TNF + NFN; V3 = μ %NDF/SUM and V4 = μ %NWF/SUM.

RESULTS AND DISCUSSION

Considering the observations of the three evaluated times, the most frequent abnormality, in average percentage terms, was summer sterility, as inferred by the number of nodes without fruits (Table 2). This finding was also reported in the studies of Damasceno Júnior et al. (2008) and Silva et al. (2007b), who concluded that there is an increase in summer sterility in the months with higher temperatures, between December and March, and a marked increase of carpellody and pentandric in the months with milder temperatures, July, August and September.

The incidence of summer sterility was lower during the winter, inferring that there was a lower sexual reversion in this period, corroborating what was found by Damasceno Júnior et al. (2008), who evaluated the floral behavior of elite hybrids and genotypes of papaya and concluded that the number of hermaphrodite flowers was more severely influenced by environmental factors than by genetic factors in the summer, according to the estimated genotypic determination coefficient (H^2) ($H^2 = 30.72\%$), whereas the number of male flowers was more deeply affected by genetic than environmental factors in both evaluation periods (winter and summer). Thus, they suggested that selection of plants with higher rates and lower rates should be performed in the winter, proposing that environmental fluctuations are responsible for the variation in the number of perfect hermaphrodite flowers.

During the evaluation periods, fruits with elongated shape were also observed, and these had a greater deposition of seeds on one of the sides of the ovarian wall, resembling the shape of a banana, hence their being known as 'bananoid'. The percentage of bananoid fruits was higher in the winter (Table 2), corresponding to the months of June, July and August, which may be due to the water stress occurring in that period.

As regards the carpelloid fruits, the fall was the time of greatest occurrence thereof. This finding corroborates Arkle Junior and Nakasone (1984), who observed that, in the environmental conditions of Hawaii, the highest incidence occurred in the fall and winter, when temperatures are milder.

Tamaki et al. (2011) analysed seasonal variations on the frequency of floral abnormalities, fruit production, pollen germination capacity and reproductive function of pistils. However, observed that summer sterility and floral abnormalities occurred at high (25.6 to 30.5 °C) and low (16.9 to 18.1 °C) temperatures, respectively. Results close to these were also found in the present study, in which the summer sterility occurred at a higher intensity at 20.8 °C to 26.49 °C, and there was a higher frequency of flower anomalies, mainly pentandric.

The average percentage of pentandric fruits was higher in the winter, corroborating Damasceno Junior et al. (2008), who reported that although no significant difference was detected between the two periods, the highest mean was observed in the spring, indicating an increase in carpellody and pentandry rates during the coldest months of the year.

Table 2. Percentages of pentandric (PF%), carpelloid (%CF), and bananoid (%BF) fruits and number of nodes without fruits (%NWF) in the three evaluation periods. Linhares/ES, 2017.

Period	%PF	%CF	%BF	%NWF
Summer	6.14	7.44	13.26	73.16
Fall	20.11	11.23	27.87	40.79
Winter	44.62	5.31	35.16	14.90
Mean	23.63	7.99	25.43	42.95

The temporary environmental variance (Vet) represented the largest proportion of individual phenotypic variance (Vf) for the three traits. Vet represents the temporary environmental variation associated with the ephemeral environmental effects manifested at each measurement, regarded as the evaluations performed, such as the effects of climatic fluctuations from year to year and their interactions with the plant effects (Viana and Resende 2014) (Table 3).

The coefficients of individual repeatability (ρ) was of low magnitude ($0.10 \leq \rho \leq 0.29$) for NDF/TNF + NWF,

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NDF/SUM and NWF/SUM, and intermediate (0.42) for NWF/TNF + NWF. The repeatability coefficient based on the average of the three evaluation periods (ρm) exhibited low magnitude for NDF/TNF + NWF (0.26), suggesting the need for a higher number of repeated measures to increase gains in the accuracy of real estimation of the individuals' genetic value. For NWF/TNF + NWF, NDF/SUM and NWF/SUM, ρm was intermediate ($0.30 \le \rho m \le 0.60$), providing some regularity across the measurements and evidence of random environmental effects on the expression of this trait (Resende 2002).

Cruz et al. (2014) stated that repeatability varies with the nature of the trait, the genetic proportions of the population and the environmental conditions under which individuals are maintained. Therefore, in the present study, the low magnitude can be explained by the fact that the population consisted of plants that were segregating for the evaluated traits, of quantitative nature, which can be greatly influenced by environmental conditions, resulting in variations in their expression from one period of evaluation to another.

Luz et al. (2015) took eight measurements to evaluate morpho-agronomic traits in papaya hybrids, estimating repeatability coefficients by four methods, and obtained results ranging from 0.41 to 0.69 for number of commercial fruits, which are considered medium and elevated magnitudes, respectively. This trait is directly influenced by deformed fruits and summer sterility in final production. The different magnitudes for the repeatability coefficient found by Luz et al. (2015) and in the current study may be due to the nature of the trait and also the genetic structure of the genotypes evaluated in the two studies. Thus, hybrids are more genetically uniform than individuals from a segregating population, given the existence of greater genetic heterogeneity.

In contrast, Cortes (2017b)[3] also evaluated morpho-agronomic traits, but in segregating individuals of an F_2 population of papaya, and obtained low repeatability magnitudes for number of commercial fruits ($0.12 \le \rho \le 0.29$), corroborating the results found here for three of the evaluated traits (NDF/TNF + NWF, NDF/SUM and NWF/SUM) which pertained to deformed fruits. This finding can be explained by the nature of the genetic structure of the segregating population in both studies.

The values found for selection accuracy based on the average of m repeated measures (Acm) ranged from 0.51 to 0.74 (Table 3), characterized as medium. This parameter expresses the correlation between the real genotypic value of the genotypes and that predicted from the information obtained from field experiments, which is higher as the absolute deviations between these values decrease. As stated by Resende and Duarte (2007), the ideal scenario would be one where accuracy values were higher than 0.7, since high accuracies indicate that precision in the selection practiced on the progeny will be high.

Based on the means calculated via REML for the variables analysed in the segregating population, the variable that obtained the highest mean was NDF/SUM (61.20), with fruit deformation being the abnormality of greatest relevance in the three studied periods (summer, fall and winter). This result differs from that found by Damasceno Junior et al. (2008), in which they concluded that summer sterility had a greater influence on the reduction of production, also on the basis of the average found in the summer and spring, however they evaluated the floral behavior of hybrids of papaya, while in the present study the fruit was evaluated.

The traits NDF/TNF + NWF and NFS/TNF + NWF; NDF/TNF + NWF and NWF/SUM; and NWF/TNF + NWF and NDF/SUM had insignificant correlations (Table 4), which suggests that they are dependent variables, i.e., they can be used simultaneously to reduce the occurrence

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Table 3. Repeatability estimates for the productive potential of the phenotypes and floral abnormalities, evaluated via REML, in a base population of recurrent selection of papaya. Linhares/ES, 2017.

Trait	Vp	Vet	Vf	ρ	ρm	Acm	Mean
NDF/TNF+NWF	14.66	125.69	140.35	0.10	0.26	0.51	30.35
NWF/TNF+NWF	120.31	167.74	288.05	0.42	0.42	0.83	24.21
NDF/SUM	83.79	207.85	291.63	0.29	0.55	0.74	61.20
NWF/SUM	83.79	207.85	291.64	0.29	0.55	0.74	38.80

Vp: permanent phenotypic variance between plants; Vet: temporary environmental variance; Vf: individual phenotypic variance; ρ: individual repeatability; ρm: repeatability of the average of m repeated measures; Acm: selection accuracy based on the average of m repeated measures; Mean: overall mean of the experiment.

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(3) Cortes, D. F. M .(2017b). Desenvolvimento de linhagens de mamoeiro assistido por imagens digitais. 159 f. Tese (Doutorado em Genética e Melhoramento de Plantas) – Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes - RJ.

Table 4. Estimate of Pearson's correlation coefficients for the different variables. Linhares/ES, 2017.

Variable	NDF/ TNF+ NWF	NWF / TNF+ NWF	NDF/SUM	NWF /SUM
NDF/TNF+NFN	1	0.066	0.511**	0.028
NFS/TNF+NFN		1	–0.145	0.313**
NDF/SUM			1	–0.369**
NFN/SUM				1

NDF/TNF + NWF = number of deformed fruits/total number of fruits plus number of nodes without fruits; NWF/TNF + NWF = number of nodes without fruits/total number of fruits plus number of nodes without fruits; NDF/SUM = number of deformed fruits/sum of abnormalities; NWF/SUM = number of nodes without fruits/sum of abnormalities; **: significant at the 1% probability level by the t test.

of floral abnormalities. Moreover, we can also infer that they possibly do not have linked or pleiotropic genes, which could minimize the efficiency of reducing both the number of nodes without fruits and deformed fruits in the population. Already, Damasceno Junior et al. (2018) obtained a negative and significant correlation between summer sterility (NWF) and number of carpelloid fruits (NCF), which could lead to selection problems, since individuals showing a lower incidence of NWF can bear more carpelloid fruits.

A significant positive correlation was obtained between NDF/TNF + NWF and NDF/SUM. In other words, more fruit deformity in relation to the productive potential, referring to the total number of fruits (TNF), means a greater proportion of fruit deformity relative to the sum of abnormalities (SUM), a directly proportional relationship (Table 4).

There was also a significant positive correlation between NWF/TNF + NWF and NWF/SUM, which indicates that incidence of summer sterility in the study population is directly linked to the productive potential of the plant, which is expected, since occurs the abortion of the flower and will not be produced fruit, originated the knot without fruit (Table 4).

In the analysis of the variables NDF/SUM and NWF/SUM, a significant negative correlation was found, inferring that they are inversely proportional variables, that is, minimizing the incidence of NDF and NWF at the same time will not be possible since the smaller the NDF, the higher the NWF (Table 4).

In the present study it was possible to observe genotypes that showed greater stability to temperature fluctuations during the three evaluation periods, corroborating with the one found by Silva et al. (2007b), working with sexual expression in segregating populations of papaya (Table 5).

Table 5. Ranking of the 30 progeny according to total abnormalities (TA). Linhares/ES, 2017.

Prog.	µ %NDF/ TNF+ NWF	µ%NWF / TNF+ NWF	µ %NFD/ SUM	µ%NWF/ SUM	TA
137	14.59	10.37	10.42	22.92	28.22
224	13.52	14.50	10.42	22.92	29.17
88	8.98	25.40	9.52	23.81	30.42
227	11.25	25.39	16.67	16.67	33.41
134	16.47	31.15	11.90	21.43	37.77
203	25.61	10.74	25.81	7.53	38.56
228	30.93	17.96	5.56	27.78	40.07
159	9.96	8.54	29.80	36.86	41.95
198	7.79	17.64	26.19	40.48	43.35
138	11.87	12.37	26.86	39.81	43.94
232	34.20	20.98	16.67	16.67	45.73
230	9.48	24.16	22.96	43.70	46.19
226	9.30	16.24	38.26	28.41	46.43
29	9.85	12.37	46.73	19.94	47.16
48	14.08	15.61	32.58	34.09	47.84
229	11.73	16.05	38.63	28.03	47.93
80	17.41	18.40	21.67	45.00	48.51
76	15.94	16.78	30.51	36.15	48.97
140	17.28	10.36	38.62	28.04	49.11
142	18.80	14.04	28.63	38.04	49.24
11	12.17	20.38	37.78	28.89	49.68
193	15.84	21.00	27.27	39.39	49.81
233	18.11	18.55	28.21	38.46	50.46
209	13.49	24.53	32.86	33.81	51.00
202	17.41	25.30	23.02	43.65	51.49
169	19.34	15.69	34.44	32.22	51.50
205	18.15	13.70	41.08	25.59	51.49
34	18.93	15.71	37.11	29.55	51.86
75	20.63	24.58	19.44	47.22	52.38
225	14.44	25.00	41.08	25.59	53.61

µNFD/TNF + NWF = average of number of deformed fruits/total number of fruits plus number of nodes without fruits; µNWF/TNF + NWF = average of number of nodes without fruits/total number of fruits plus number of nodes without fruits; µNFD/SUM = average of number of deformed fruits/sum of abnormalities; µNWF/SUM = average of number of nodes without fruits/sum of abnormalities.

Based on the total abnormalities (TA), 30 individuals potentially superior for the traits evaluated in the segregating population were chosen. Selection of genotypes with minimal occurrence of deformed fruits and summer sterility should be one of the objectives of papaya breeding programs (Table 5).

The ranking of progeny also shows the existence of variability in the segregating population. One of the main goals of papaya breeding is to increase the genetic base of the crop, aiming at the development of superior genotypes and, among other desirable morpho-agronomic traits, a reduced incidence of deformed fruits and summer sterility, satisfying the needs of the internal and external market.

CONCLUSION

Summer sterility, the main problem in papaya crops, was the floral abnormality of highest incidence in the broad-base population under study in the evaluation periods. Repeatability coefficients varied from low to intermediate because of quantitative nature of the characteristics evaluated, which are highly influenced by the environment. The insignificant correlations between the traits NDF/TNF + NWF and NWF/TNF + NWF; NDF/TNF + NWF and NWF/SUM; and NWF/TNF + NWF and NDF/SUM indicate that the variables are independent of each other, that is, genotypes with a low incidence of summer sterility did not tend to a high production of deformed fruits. From the 254 evaluated progenies, at least 30 were identified as having potential for selection as determined by the lower occurrence of total floral abnormalities, which is one of the interests of papaya breeding programs along with elevated agronomic potential for yield and disease resistance.

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AUTHORS' CONTRIBUTION

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