

Estimation of Genetic Parameters of Turiaçu Pineapple Clones and Genetic Correlation between Traits

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Abstract

This study aimed to estimate genetic parameters of Turiaçu pineapple clones using mixed models; and estimate genetic correlation coefficient between traits, to provide important information to breeding programs. For this, 19 pineapple plants were visually selected and its slips were collected and planted in a single environment and nine traits were evaluated. The genetic parameters of most evaluated traits presented significant differences between the 19 clones. Some traits, such as Fusarium wilt resistance, showed high magnitudes of heritability. Clones did not differ in relation to total titratable acidity (TTA) and total soluble solids (TSS). However, these traits, which have implications to fruit quality, showed to be negatively correlated to fruit mass (−0.46 and −0.67). The results indicate that the population of Turiaçu pineapple is suitable for selection, especially for Fusarium wilt resistance. Also, Turiaçu clones can be used in breeding programs of pineapple.

Keywords

Quantitative Genetics, Plant Breeding, *Ananas comosus*

1. Introduction

The pineapple (*Ananas comosus*) is an important fruit crop in the state of Maranhão, Brazil. An area of about 1388 ha was used to grow this fruit in 2014 [1]. The main part of this area is cultivated by family farmers, and the activity is the

primary source of income for many families.

One of the municipalities that stands out on this fruit production is Turiaçu, located in Gurupi region [1]. In Turiaçu, there is a variety of pineapple of unknown origin and genetic identity that has been cultivated for decades by family farmers in the region. This variety of pineapple has unique features such as high content of soluble solids when compared to Pérola cultivar [2]. The quality of fruit enables the variety to gain prominence in marketplaces throughout Maranhão, and the variety is considered popularly the sweetest pineapple of Brazil. The price of Turiaçu pineapple gets up to double the price of other varieties in the market [2].

Turiaçu pineapple is not a registered cultivar. There are no records of genetic breeding for productivity and quality for it, and the crop is normally cultivated with low technology, resulting in low productivity. The current consolidation of the Turiaçu variety in the market indicates that introduction of new varieties may not be a good strategic choice. Therefore, genetic breeding for productivity and quality of fruit may be a good opportunity and would bring economic benefits to farmers.

The Fusarium wilt, a disease caused by the fungus *Fusarium guttiforme* (synonymy *Fusarium subglutinans* f. sp. ananas) affects the production of pineapple in most part of Brazil. According to [2], the Turiaçu pineapple is resistant to this fungus in native growing conditions. However, there are no records of experiments that aimed to verify this information. The lack of infection in Turiaçu can be caused by a genetic resistance or simply by the absence of the pathogen in the region, since no other variety of pineapple susceptible to the Fusarium is grown in Turiaçu.

One way to increase productivity and/or quality of any crop is through genetic breeding. Recently, there has been a large increase in productivity of major crops around the world, and 50% of this increase was due to conventional genetic breeding [3]. To achieve success in genetic breeding, it is necessary to analyze whether there is genetic variation in the trait within the population. Therefore, it is essential to estimate genetic and phenotypic parameters [4].

The prediction of genetic values and selection methods depends, basically, on the variance component estimates. Usually, the variance components are estimated by least squares methods, such as analysis of variance procedures. These procedures allow working with a certain level of unbalance and it is equal of usual variance analysis when data are balanced. On the other hand, there is another situation of genetic values prediction where variances are unknown and the imbalances are such that the method of least squares presents unknown efficiency. In these situations, other estimation procedures of components of variance are demanded, and the prediction of genetic values must be performed simultaneously to the estimation of variance components, *i.e.*, the variance component estimation by restricted maximum likelihood (REML) and the prediction of genetic values by best linear unbiased prediction (BLUP) [5]. Mixed

models by REML/BLUP have been widely used in plant breeding to estimate genetic parameters [6], adaptability and stability of crops [7], genotype \times environment interaction [8], among others.

In addition to genetic parameters, the correlation between traits is also important to plant breeding. Breeding programs must attempt to improve traits of interest without selecting correlated bad traits [9]. Moreover, known correlation estimates allow performing indirect selection of traits that are difficult to evaluate that makes the selection procedure easier [10].

This study aimed to estimate genetic parameters of Turiaçu pineapple clones through mixed models and to estimate the genetic correlation among traits.

2. Material and Methods

To set up the experiment, 19 plants with the better fruit from farms in the rural village of Serra dos Paz, Turiaçu, Maranhão, Brazil (01° 39'48"S; 45° 22'18"W), were visually selected (mass selection) and slips of these plants were collected in 2013. The slips were planted in randomized design scheme in the same municipality, with six replications, in a single environment. The experimental plot was composed of one plant. Eighteen months after planting, the fruits were harvested, the number of slips for each plant was counted and the plant height was measured. The fruits were transported to the Plant and Postharvest Laboratory of Universidade Estadual do Maranhão, where total fruit mass, fruit mass without crown, crown mass and pulp mass were measured individually. Additional procedures were performed as follows.

To chemical analysis, the juice of each peeled fruit was extracted using a blender. Juice samples were used to determine total titratable acidity (TTA) and total soluble solids (TSS) using the following methods:

TTA: 10 g of juice sample were diluted in 60 mL of distilled water. This solution was titrated with sodium hydroxide (NaOH) 0.1N in a burette until pH reaches 8.1. The pH of the solution was monitored with a pH meter Model MPA-210. The results were obtained by the following Equation (1) [11]:

$$\text{TTA}(\%) = (N \times V \times \text{Eq.wt}) / (W \times 10) \quad (1)$$

where: N is the normality of titrant; V is the volume of titrant used; Eq.wt is equivalent weight of predominant acid and W is the volume of the sample.

TSS: one or more drops of juice sample were placed in an analogic refractometer until the surface of the prism was fully covered, and then the measurement was done. This process was done at least two times, until two measurements in sequence presented a difference of less than 0.4° BRIX.

The resistance to Fusarium wilt was verified in the 19 clones. The *F. guttiforme* isolate was obtained from an infected plant on a pineapple farm of another region, located in São Domingos do Maranhão. This isolate was inoculated on D-type leaves collected from the 12 months old clones.

D-type leaves were washed with soap and water, sterilized with a 5% sodium

hypochlorite solution and rinsed with distilled water. After that, the inoculation was done at 2 cm from the base of the leaves by drilling the tissue with a toothpick covered with pathogen.

The leaves were placed on plastic trays wrapped by transparent polyethylene bags. Moistened cotton was placed inside the bags to maintain a humid environment. The trays were kept for 20 days at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and under a photoperiod of 12 hours. The clone resistance was evaluated by measuring the length of the lesion area with a ruler. The clone showed resistance when there was no lesion (length equal to zero).

The software Selegen [5] was used to perform the Genetic-statistical analysis, by Mixed models (REML/BLUP), where the variation components are associated with univariate analysis. The model used was (2):

$$y = Xu + Zg + e \quad (2)$$

where: y is the data vector; u is the scalar of the general mean (fixed effect); g is the vector of genotypic effects (assumed random); and e is the vector of errors (random). The capital letters represent the incidence matrices for these effects.

The mixed model equations used to estimate the general mean and the prediction of genetic values is equivalent to (3):

$$\begin{bmatrix} XX & XZ \\ Z'X & Z'Z + I(\sigma_e^2/\sigma_g^2) \end{bmatrix} \begin{bmatrix} \hat{u} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \quad (3)$$

where I is an identity matrix; σ_g^2 is genotypic variance and σ_e^2 is residual variance.

According to the model, the REML estimators by EM algorithm to obtain estimates of the variance components σ_g^2 (4) and σ_e^2 (5) were:

$$\hat{\sigma}_e^2 = [y'y - \hat{b}'X'y - \hat{g}'Z'y] / [N - r(X)] \quad (4)$$

$$\hat{\sigma}_g^2 = [g'g + \hat{\sigma}_e^2 \text{tr}C^{22}] / N_g \quad (5)$$

where N_g is the number of random elements (individuals); I identity matrix; tr is the matrix trace operator, given by the sum of the diagonal elements of the matrix; N is the total number of data; $r(X)$ is the rank of number of linearly independent columns of X ; and C^{22} is as follows (6):

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{bmatrix} XX & XZ \\ Z'X & Z'Z + I(\sigma_e^2/\sigma_g^2) \end{bmatrix}^{-1} \quad (6)$$

The heritability \hat{h}^2 (7) and the variation components CV_g (8) and CV_e (9) were obtained as shown below:

$$\hat{h}^2 = \hat{\sigma}_g^2 / (\hat{\sigma}_g^2 + \hat{\sigma}_e^2) \quad (7)$$

$$CV_g = \hat{\sigma}_g / \bar{Y} \quad (8)$$

$$CV_e = \hat{\sigma}_e / \bar{Y} \quad (9)$$

where $\hat{\sigma}_g^2$ is the estimated genetic variance; $\hat{\sigma}_e^2$ is the estimated

environmental variance; CV_g is the genetic coefficient of variation; CV_e is the environmental coefficient of variation; \hat{h}^2 is heritability in plot level; \bar{Y} is the mean of the evaluated traits.

To verify the significance of genotypes, the analysis of deviance was used. For this, deviances were obtained running the model with and without heritability values for each trait, and then subtracting them and comparing them to the Chi-square value at 1 and 5% level, with 1 degree of freedom [5]. The genetic correlation between traits (10) was estimated by the Model 102 of Selegen software [5], which adopts the following estimator:

$$\hat{r}_g = COV(\hat{X}_g, \hat{Y}_g) / \sqrt{\hat{\theta}_{\hat{X}_g}^2 \hat{\theta}_{\hat{Y}_g}^2} \quad (10)$$

where:

- \hat{r}_g = estimation of genetic correlation between variables X and Y ;
- COV = covariance operator;
- \hat{X}_g = Estimator of genotypic value of variable X ;
- \hat{Y}_g = Estimator of genotypic value of variable Y ;
- $\hat{\theta}_{\hat{X}_g}^2$ = Genetic variation estimation of the estimated genotypic value of variable X ;
- $\hat{\theta}_{\hat{Y}_g}^2$ = Genetic variation estimation of the estimated genotypic value of variable Y .

3. Results and Discussions

Table 1 shows the deviance analysis of the nine evaluated traits. Number of slips, pulp mass, plant height and crown mass displayed significant differences at the 5% level. Only the TTA and TSS did not showed significant differences at the Chi-square test. These traits are important because the titratable acidity is related to

Table 1. Analysis of deviance for pulp mass (PM), plant height (H), number of slips (NS), fruit mass (FM), fruit mass without crown (FMWC), mass of crown (MC), resistance to Fusarium (RF), total soluble solids (TSS) and total titratable acidity (TTA).

Traits	Deviance	LRT(x^2) ¹
PM	-57.25	6.27*
H	-119.93	3.88*
NS	63.91	6.59*
FM	-32.82	7.00**
FMWC	-35.63	6.89**
MC	-192.09	5.35*
RF	93.13	62.38**
TSS	44.69	1.62 ^{NS}
TTA	-91.27	2.25 ^{NS}

¹LTR(x^2) Chi square of the likelihood ratio test for progeny effect, x^2 tabled: 3.84 and 6.63 for the 5 (*) and 1% (**) levels of significance respectively. NS: non-significant.

tartness and TTS (°Brix) is related to perceived sweetness [12]. Despite the fact that these traits did not showed to be different among the clones evaluated, according to [2] there are differences between the Turiaçu pineapple and other commercial cultivars. This implies that Turiaçu can be used in breeding programs to improve these traits in other cultivars. There is a worldwide tendency of market growth for low acidity fruits, and the development of pineapple cultivars for fresh fruit consumption has been a recent major focus of breeding and selection programs [13]. Considering the renowned sweetness of Turiaçu pineapple, it would be an interesting choice for breeding programs.

The estimates of genetic variance, environmental variance, phenotypic variance, heritability, genetic and environmental variation coefficient, and ratio CV_g/CV_e of evaluated traits in Turiaçu pineapple clones were presented in **Table 2**.

Fusarium resistance showed the higher magnitude of heritability. This result indicates that, for the studied population, genetic breeding may be more effective if focused on this trait of high heritability. In a breeding program for resistance to disease, one of the most important issues is the choice of the parents to obtain populations where the selection will be carried out or they will be used for the obtaining of hybrids [14]. In Turiaçu's pineapple population is possible to choose good parents as source of resistance to fusarium. Resistance to Fusarium wilt is an important trait, as this is a highly destructive disease [15]. The use of resistant cultivars reduces the use of fungicides and, consequently, reduces the cost of production and also environmental pollution, besides to enhance the food security. This result for Fusarium resistance was expected because the inheritance of this trait may be linked to a gene (or a few genes) with dominant

Table 2. Genetic parameters for plant height (H), number of slips (NS), fruit mass (FM), mass of crown (MC), fruit mass without crown (FLWC), pulp mass (PM), resistance to Fusarium (RF), total soluble solids (TSS) and total titratable acidity (TTA).

Traits	$\hat{\sigma}_g^2$	$\hat{\sigma}_e^2$	$\hat{\sigma}_f^2$	\hat{h}^2	$CV_g(\%)$	$CV_e(\%)$	CV_g/CV_e
H (meters)	0.0038	0.0061	0.0099	0.3853	73.483	92.817	0.7917
NS	74.895	12.104	86.999	0.8609	438.155	176.142	24.875
FM (kg)	0.0786	0.0520	0.1306	0.6020	275.219	223.780	12.299
MC (kg)	0.0004	0.0003	0.0007	0.5767	227.577	194.968	11.672
FMWC (Kg)	0.0861	0.0488	0.1350	0.6379	327.812	246.943	132.747
PM (kg)	0.0349	0.0224	0.0573	0.6096	297.258	237.843	12.498
RF (mm)	384.886	0.3156	388.043	0.9918	824.439	74.663	110.421
TSS (Brix)	14.500	0.6061	20.562	0.7052	69.148	44.707	15.467
TTA (%)	0.0103	0.0036	0.0139	0.7419	173.526	10.234	16.956

$\hat{\sigma}_g^2$: estimates of genetic variance; $\hat{\sigma}_e^2$: estimates of environmental variance; $\hat{\sigma}_f^2$: estimates of phenotypic variance; \hat{h}^2 : heritability; $CV_g(\%)$: genetic variation coefficient; $CV_e(\%)$: environmental variation coefficient; CV_g/CV_e : ratio CV_g/CV_e .

character in relation to susceptibility. Therefore, it is a qualitative inheritance that is related to vertical or specific resistance to disease [16]. As the propagation of pineapple is done asexually, both the additive and dominance effects are passed to offspring, so the broad sense heritability has great importance in the genetic breeding of this species.

The CV_g/CV_e ratio is of great importance to genetic breeding because it indicates the possibility of genetic gains by selection. According to [17], when the ratio is greater than 1.0, the situation is favorable to selection. In this study, the estimate ranged from 0.7917 to 11.0421 (as to plant height and resistance to fusarium, respectively). All but one trait showed CV_g/CV_e values higher than 1.0, implying that considerable gains are possible through genetic breeding.

Even the number of slips displayed a high CV_g/CV_e value. This is an important trait for farmers because they use these slips for future plantings, the slips protect the fruits from sunburn [18] and they can be sold, which ensures an extra source of income [19].

Pulp mass can be considered an important trait for pineapple processing industry. In this case, the producers may receive income per pulp mass, and not per fruit, as it is commonly marketed in the region now. This trait also showed CV_g/CV_e greater than 1, indicating that the selection of fruits with higher pulp mass can be a viable strategy.

Table 3 shows results of correlation analysis. Fruit mass, fruit mass without crown and pulp mass are highly correlated (correlation close to 1.0). This indicates that it is not necessary to perform evaluations for these three traits, making the evaluation process easier.

Also, it is possible to observe that TSS and TTA are negatively correlated to fruit mass, *i.e.*, smaller fruits will have more TSS and TTA, and this will have

Table 3. Genetic correlation among pulp mass (PM), plant height (H), number of slips (NS), fruit mass (FM), fruit mass without crown (FMWC), mass of crown (MC), resistance to fusarium (RF), total soluble solids (TSS) and total titratable acidity (TTA). Values above the diagonal are the genetic correlation and values below diagonal are the significance level (p value).

Traits	PM	H	NS	FM	FMWC	MC	RF	TSS	TTA
PM	1	0.65	0.79	0.99	0.99	0.60	-0.39	-0.46	-0.67
H	0.015	1	0.71	0.62	0.65	0.06	-0.11	-0.27	-0.09
NS	0.030	0.019	1	0.79	0.81	0.33	-0.26	-0.17	-0.43
FM	<0.0001	0.031	0.034	1	0.99	0.65	-0.38	-0.52	-0.66
FMWC	<0.0001	0.028	0.047	<0.0001	1	0.60	-0.38	-0.50	-0.64
MC	0.123	0.897	0.389	0.046	0.094	1	-0.25	-0.70	-0.69
RF	0.040	0.140	0.293	0.065	0.045	0.630	1	0.10	0.36
TSS	0.037	0.090	0.709	0.032	0.036	0.013	0.243	1	0.21
TTA	0.002	0.349	0.293	0.0007	0.001	0.017	0.013	0.039	1

influence on consumer's preference to smaller fruits. Variation of pineapple fruit acidity and sweetness are associated with the pineapple clone used, fruit maturation and growing conditions [20] [21], but little research has been carried out on the underlying fruit acid metabolism that determines final pineapple fruit acidity [13].

4. Conclusions

The results of this study showed the existence of genetic variability in the Turiaçu pineapple population. The estimated parameters indicate that the population of Turiaçu pineapple is suitable for selection of the best clones, especially for Fusarium resistance.

Clones did not differ significantly in relation to total titratable acidity (TTA) and total soluble solids (TSS). However, these traits showed to be negatively correlated to fruit mass. Moreover, these traits in Turiaçu pineapple are better than other commercial cultivars, as showed by Araújo *et al.* (2012) compared to the data presented here, implying that Turiaçu can be used in breeding programs for improvement of fruit quality.

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