

Repeatability of quantitative characteristics in sweet orange through mixed-model methodology

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Abstract

The objective was to estimate the repeatability coefficient of quantitative traits in multiple harvests of sweet orange, in order to infer the minimum number of evaluations necessary to identify superior genotypes of orange trees through the methodology of mixed models. The experiment was conducted in randomized blocks containing 55 sweet orange genotypes and three replications. The repeatability coefficients were estimated using the maximum residual likelihood method (REML) and the prediction of genotypic values using the best unbiased linear predictor (BLUP). The Selegen software was used to perform the statistical analysis. The average heritability of genotypes in eight seasons, individual and eight seasons repeatability, selection accuracy in one and eight seasons, repeatability determination coefficient, accuracy of permanent phenotypic values based on M years of assessment and efficiency of M assessments compared to situation where only one assessment is carried out. The predictive accuracy of the selection revealed a significant degree of certainty in the inferences made. Evaluation over six seasons can increase accuracy to 70% in selecting sweet orange genotypes for yield-related traits. For the prediction of average fruit mass, seven harvests are enough to obtain 80% accuracy.

Keywords: *Citrus sinensis* (L.) Osbeck, number of measurements, productivity, REML/BLUP, western Amazon

Introduction

The average productivity in Acre, around 14.3 t/ha, is low, around 51% of the national average (Ibge, 2023), and has been attributed, among other factors, to the planting of inadequate cultivars (Ledo et al., 1999). Thus, it is necessary to intervene in the production system by introducing productive cultivars that are acceptable to the consumer market, specifically selected for commercial cultivation (Valente et al., 2017).

One way to efficiently select cultivars is by measuring the repeatability coefficient, which can be estimated from multiple measurements on the same individual, subject to spatial or temporal variations (Maia et al., 2017; Alcoforado et al., 2019). Based on this coefficient, it is possible to establish the most efficient number of evaluations required to ensure the precise selection of superior genotypes, with the lowest cost and labor requirements (Cruz et al., 2014), and furthermore, it

allows selected genotypes to maintain their characteristics over time with minimal environmental influence (Ferreira et al., 2020).

Traditionally, the most commonly used methodology in perennial crop breeding to estimate the repeatability coefficient is the method of least squares (LS) through analysis of variance (ANOVA) (Jesus et al., 2021). However, this analysis, especially when the data is unbalanced or when there is a need to disregard some of its assumptions, does not produce the same efficiency as the analysis of mixed models. Therefore, the inappropriate choice of methodology can lead to imprecise selection of the best genotypes, due to genotypic and environmental biases that may occur, resulting in low selection efficiency (Santos, 2014). Hence, a mixed model methodology is the most effective as it incorporates the estimation of variance components through the residual maximum likelihood (REML) method and predicts genotypic values

through best linear unbiased prediction (BLUP), without confounding genotypic and environmental effects.

The repeatability coefficient has been estimated through mixed models in various perennial fruit crops (Maia et al., 2017; Silva et al., 2017; Rodrigues et al., 2017; Leão et al., 2018; Sales et al., 2019), unlike sweet orange, especially in relation to quantitative traits.

Therefore, the objective of this study was to estimate the repeatability coefficient of quantitative traits in multiple harvests of sweet orange in order to infer the minimum number of evaluations necessary to identify superior orange tree genotypes using the mixed-model methodology.

Materials and Methods

The experiment was installed and conducted in an experimental field in Embrapa Acre, in the municipality of Rio Branco, Acre, from 2002 to 2010. The experiment is located at 11°58'10" S and 68°18'52" W. The climate of the region, according to the Köppen classification, is type Am, with an average maximum and minimum temperature of 31.0 °C and 18.1 °C, respectively and annual precipitation levels between 1.900 – 2.200 mm (Alvares et al., 2013) and relative humidity levels of approximately 84% (Inmet, 2019) (**Figure 1**).

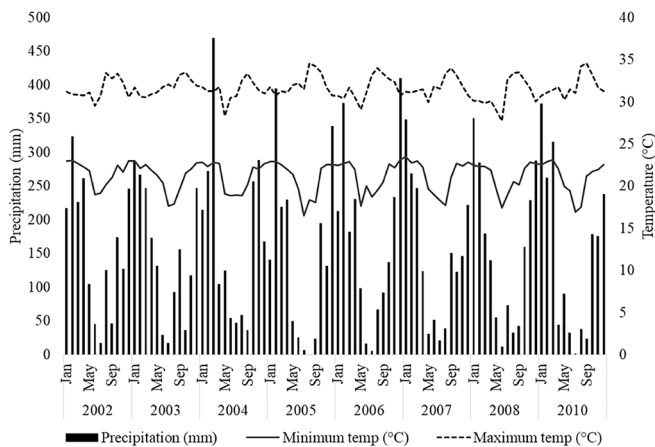


Figure 1. Temperature (°C) and rainfall (mm) during the experimental period (2002 -2010) in Rio Branco - AC, Brazil.

The soil of the experimental area was classified as Oxisol, medium-textured and well-drained, with flat terrain. According Negreiros et al., 2014, chemical analysis of the soil (0 up to 20 cm deep) indicated the following characteristics: pH, 5.2; K, 0.17 cmol_c kg⁻¹; Ca, 3.70 cmol_c kg⁻¹; Mg, 1.49 cmol_c kg⁻¹; Al, 0.14 cmol_c kg⁻¹; H+Al, 2.57 cmol_c kg⁻¹; sum of bases, 5.36 cmol_c kg⁻¹; organic carbon, 7.27 g kg⁻¹; CEC, 5.5 cmol_c kg⁻¹; and base saturation, 68%.

The experimental plant material was collected from March to June 1999, from properties of settlement projects created by the National Institute of Colonization

and Agrarian Reform (Gondim et al., 2001). According these authors, 54 sweet orange matrices in their production phase were selected from the mesoregion of Vale do Acre, plus the local Aquiri cultivar (recommended by Embrapa Acre), totaling 55 genotypes (**Table 1**).

The 55 sweet orange matrices (genotypes), coming from non-grafted plants (produced from seeds) and characterized as vigorous, apparently healthy, an average age of 22 years, productive, and with quality fruits that could meet the demands of the consumer market (Gondim et al., 2001).

According Gondim et al. (2001): i) Five branches, each containing ten buds, were collected from each genotype using pruning shears; ii) the branches were packed in a thermal box, wrapped with wet paper, and taken to the seed laboratory at Embrapa Acre; iii) two vegetative buds were removed from each branch and grafted onto the rootstocks of Cravo lemon plants (*Citrus limonia* (L.) Osbeck) to be evaluated in a clone competition assay and; iv) The genotypes were planted in February 2000, adopting an 8 × 8 m spacing.

The 55 treatments were set up in an experimental design of randomized blocks and three replications. Evaluations were carried out during the 2002 - 2008 and 2010 harvests. The orange trees were cultivated without irrigation. The traits evaluated were total number of fruits per plant, number of fruits per m², average fruit mass (kg), and productivity (kg/m²).

Statistical genetic analyses were performed using mixed-model methods, in which genetic parameters were estimated using REML and genotypic values were predicted using BLUP. For each harvest, individual analyses were performed to verify the heterogeneity of variances, using the following statistical model (Resende, 2002):

$$y = Xb + Zg + e, \text{ in which}$$

y: data vector; b: data vector of fixed block effects, added to the general average; g: data vector of random genotype effects; e: vector of random errors; and X and Z: incidence matrices for vectors b and g.

For cases where the variation in heritability coefficients was verified in a broad sense, the data were standardized using the correction factor obtained, according to the following expression described by Resende (2007):

$$h^2_i / h^2_{ik}, \text{ in which}$$

h^2_{ik} : individual broad-sense heritability for character *i* in evaluation *k*; and h^2_i : average of individual broad-sense heritability of *k* evaluations for character *i*.

After data standardization, joint analysis was

Table 1. Collection sites of sweet orange genotypes [*Citrus sinensis* (L.) Osbeck] in the state of Acre, Brazil

Municipalities	Regional	Coordinates	Genotypes
Brasília	Alto	11°00'01"S, 68°44'59"W	39, 40, 41, 42
Epitaciolândia		11°01'56"S, 68°43'54"W	43, 44, 45, 46, 47
Xapuri	Acre	10°39'11"S, 68°30'03"W	24, 25, 26, 27, 28, 29, 30, 31
Sena Madureira	Purus	09°04'10"S, 68°39'30"W	32, 33, 34, 35, 36, 37, 38
Capixaba	Baixo	10°34'29"S, 67°40'38"W	17, 18, 19, 20, 21, 22, 23
Plácido de Castro		10°19'43"S, 67°10'44"W	1, 2, 3, 4, 5, 6, 7, 8
Porto Acre		09°35'42"S, 67°32'36"W	48, 49, 50, 51, 52
Rio Branco		09°58'29"S, 67°49'44"W	53, 54, 55*
Senador Guiomard		10°09'03"S, 67°44'13"W	9, 10, 11, 12, 13, 14, 15, 16

* Local Aquiri cultivar. Source: Adapted from Negreiros et al., 2014 and Gondim et al., 2001

performed on the genotypes and harvest time, according to the following statistical model:

$$y = Xb + Za + Wc + e, \text{ in which}$$

y: data vector;

b: vector of the block effects (fixed) added to the general average;

a: vector of individual genotypic effects (random);

c: vector of the plot effects (random);

e: vector of errors (random) and;

X, Z and W: incidence matrices for said effects.

Analysis of deviance was performed to test the significance of the variance components, according to the random effects of the model. Using the components of variance, a likelihood-ratio test was used, in which the significance of the model was assessed by a chi-square test with one degree of freedom. For this, deviances were obtained for the complete and reduced model. Afterward, the deviance of the reduced model of each effect was subtracted from the complete deviance of each effect and compared with the chi-square value with one degree of freedom, at 1% and 5% probabilities (Resende, 2007).

From the genotypic and phenotypic variances, genetic parameters were estimated according to Resende (2007): average heritability of genotypes across eight harvests (h^2mg), individual repeatability (r_i), repeatability across eight harvests (r_g), accuracy in the selection of genotypes in one harvest (Ac_1), and accuracy of the selection of genotypes across eight harvests (Ac_g). These parameters were used to verify the number of evaluations necessary to predict the actual value of genotypes accurately.

For the individual analyses, statistical model 20 was adopted to the evaluation of unrelated genotypes obtained in randomized blocks containing one plant per plot, and for the joint analysis model 55 was used to the evaluation of genotypes in the randomized block design with spatial stability and temporal adaptability using Selegen REML/BLUP software to carry out these analyses.

Results and Discussion

The individual repeatability coefficient in one harvest (r_i) ranged from 0.07 for the number of fruits per plant to 0.14 for average fruit mass (Table 2), that is, of low magnitude (<0.3), according to Resende (2002).

Table 2. Estimates of variance components and genetic parameters of the number of fruits per plant (NFP), number of fruits per m² (NFR/m²), average fruit mass (AFM), and productivity in sweet orange genotypes evaluated across eight harvests in Rio Branco - AC, Brazil.

Genetic parameters	Variables			
	NFP	NFR/ m ²	AFM (kg)	Productivity (kg/m ²)
r_i	0.07	0.10	0.14	0.12
r_g	0.38	0.47	0.57	0.52
Ac_1	0.38	0.41	0.49	0.37
Ac_g	0.75	0.76	0.82	0.70

Individual repeatability (r_i), repeatability of eight harvests (r_g), selective accuracy in one harvest (Ac_1) and selective accuracy in eight harvests (Ac_g).

Low repeatability coefficient may be associated with environmental conditions and high coefficient may mean lower influence of them (Brito et al., 2019). In addition, a low coefficient may indicate that the response of a given variable over several harvests may be unstable (Ferreira et al., 2021) and high values indicate regularity in the trait repetition between evaluation times (Catarina et al., 2020).

For repeatability coefficient of eight harvests (r_g) ranged from 0.38 for the number of fruits per plant to 0.49 for average fruit mass (Table 2), that is, of moderate magnitude ($0.3 < r < 0.6$), according to Resende (2002). When applying the REML/BLUP methodology, Catarina et al. (2020) detected that the mean repeatability coefficient of three measures of papaya crops was of medium magnitude for the categories number of marketable fruits and productivity. Regarding the number of fruits per m², the individual repeatability in a harvest was approximately 0.10 (Table 2). Over eight harvests, selective accuracy increased from 41% to 77% (Table 2).

According to Bruna et al. (2012), knowing the repeatability coefficient is important, as it allows the

predictability of the time necessary for the selection of genetically superior individuals with the desired accuracy. According to these same researchers, low repeatability values in a harvest indicate that it is necessary to perform more effective experimentation, by more evaluations in the same individual or with more repetitions than in the previous study, to increase accuracy and achieve satisfactory genotypic determination values.

Average repeatability values from this study indicate that it is not advantageous to increase the number of measurements to select plants. Contrastingly, high values of the character repeatability coefficient indicate that it is possible to predict the actual value of individuals with a small number of measurements, with slight gains in accuracy with an increase in the number of measurements (Cruz et al., 2014). According Pathy & Mohanraj (2021), high repeatability estimates infer that the variance of the trait across measures would have less variation for an individual and, therefore, selection for that trait should be done in the early stages.

The accuracy in eight harvests (Ac_8), ranging from 0.70 to 0.82 for productivity and average fruit mass, respectively (Table 2). Therefore, estimates for productivity and average fruit mass were highly accurate, indicating that the evaluation and predicted genetic value of the individual are reliable. Sánchez et al. (2017) studied soursop and observed selective accuracy of a high magnitude (0.95) based on average harvests, demonstrating high precision and reliability of genotypic selection. Selective accuracy is considered the most important parameter in genotypic evaluation, as it is associated with selection accuracy (Pompeu Júnior et al., 2013).

According to Resende (2007), accuracy greater than 0.50 can be considered ideal in the selection of genotypes and from accuracy values, regularity is observed in the value repetitions from each harvest, allowing the selection of superior genotypes in a few production cycles based on these variables.

Table 3 shows the average heritability (h^2_{med}) and accuracy (Ac_m) that would be obtained using a greater number of measurements than that used in the present study. The coefficient of determination (r) and the selective efficiency (Ef) between the uses of M measured in comparison with the use of only one measure were calculated to establish the optimal number of measurements needed to efficiently select high productivity sweet orange genotypes.

The BLUP prediction for 10 harvests indicated significant increases in the coefficient of determination, accuracy and selective efficiency from the first to the

tenth evaluation (M) for all traits analyzed (Table 3).

The significant increases in the coefficient of determination, accuracy, and selective efficiency observed in the present study are also verified by Sánchez et al. (2017) who found increases in estimates of the repeatability coefficient and the optimal number of measurements needed in the selection of superior soursop genotypes.

Regarding the number of fruits per plant, the individual repeatability in a harvest was approximately 0.07 or 7% (Table 2 and Table 3). When investigating the individual repeatability from a harvest, Pompeu Júnior et al. (2013) observed that the repeatability coefficient increased from 10% in one harvest to 35.15% in five harvests, with a selective accuracy of 84.59%. Moreover, they highlighted the difficulty in quantifying this variable precisely, largely because it is a quantitative character. Hence, they suggest that for precise selection, several replications of each genotype are necessary, in addition to cropping several harvests. If repeatability

Table 3. Efficiency of repeated measures predicted by BLUP for number of fruits per plant, number of fruits per m², productivity, and average fruit mass in sweet orange genotypes evaluated in eight harvests in Rio Branco - AC, Brazil

M	Number of fruits per plant				Number of fruits per m ²			
	h^2_{med}	r	Ac_m	Ef	h^2_{med}	r	Ac_m	Ef
1	0.14	0.07	0.38	1.00	0.16	0.10	0.41	1.00
2	0.25	0.13	0.50	1.33	0.28	0.18	0.53	1.34
3	0.33	0.18	0.57	1.55	0.37	0.25	0.60	1.58
4	0.40	0.23	0.63	1.71	0.43	0.30	0.65	1.75
5	0.45	0.27	0.67	1.83	0.48	0.35	0.69	1.88
6	0.49	0.31	0.70	1.93	0.52	0.40	0.72	1.99
7	0.53	0.35	0.73	2.01	0.56	0.43	0.75	2.09
8	0.56	0.38	0.75	2.08	0.59	0.47	0.76	2.16
9	0.59	0.41	0.77	2.14	0.61	0.50	0.78	2.35
10	0.61	0.43	0.78	2.18	0.63	0.52	0.79	2.29
M	Average fruit mass (kg)				Productivity (kg/m ²)			
	h^2_{med}	r	Ac_m	Ef	h^2_{med}	r	Ac_m	Ef
1	0.24	0.14	0.49	1.00	0.13	0.12	0.37	1.00
2	0.38	0.25	0.61	1.32	0.23	0.21	0.48	1.33
3	0.47	0.33	0.68	1.52	0.30	0.29	0.55	1.55
4	0.53	0.40	0.73	1.66	0.36	0.35	0.60	1.71
5	0.58	0.46	0.76	1.77	0.40	0.40	0.63	1.83
6	0.62	0.50	0.79	1.86	0.44	0.45	0.66	1.93
7	0.65	0.54	0.80	1.93	0.47	0.48	0.68	2.01
8	0.67	0.57	0.82	1.98	0.49	0.52	0.70	2.08
9	0.69	0.60	0.83	2.03	0.51	0.55	0.72	2.14
10	0.71	0.63	0.84	2.07	0.53	0.57	0.73	2.18

Number of measurements/evaluations (M), average heritability (h^2_{med}), coefficient of determination of repeatability (r), accuracy of permanent phenotypic values based on M years of evaluation (Ac_m), and efficiency of the M evaluations compared with the situation in which only one evaluation is performed (Ef).

is high, increasing the number of measurements would moderately increase the experimental accuracy (Cruz et al., 2014).

Leão et al. (2018), by the BLUP/REML methodology, found that grape vine hybrids may be selected from the fourth year of evaluation, as the variables of production and number of bunches per plant presented coefficient of determination and accuracy greater than 0.8.

The highest value of the individual repeatability (r_1) coefficient was found for average fruit mass (14.59%), although it is still of low magnitude. Over eight harvests, selective accuracy increased from 49.21% to 82.38% (Table 2).

According Negreiros et al. (2008), the repeatability coefficient ranged from 0.29 to 0.68, requiring approximately 13 measurements by analysis of variance (ANOVA) to predict with 85% efficiency which genotypes would be the most productive. Coefficients of determination greater than 60% were observed for 10 measurements of average fruit mass. Six measurements were required to predict the actual values of individuals with 79% certainty (Table 3).

Productivity also showed a low individual coefficient of repeatability (Table 3), probably, due to the low additive variance compared to residual variance, which precludes the selection of genotypes based on few measurements with only individual information (Bergo et al., 2019). On the other hand, over eight harvests the repeatability coefficient increased from 0.12 to 0.52 with a selective accuracy of 70.53% (Table 3).

Therefore, the alternation of production or the systematic and random variations of fruit production between years may be a possible explanation for the low values of the repeatability coefficient found in this study. Climatic variations throughout the experimental period should also be considered (**Figure 1**), since the average heritability was high, and all traits are highly influenced by the environment. However, these values are considerable for perennial species (Viana & Resende, 2014). Selection accuracy depends on heritability, repeatability coefficient, and trial quality (Ferreira et al., 2021).

Quintal et al. (2017), after evaluating the productivity of 95 guava genotypes over three harvests, estimated repeatability coefficients ranging from 0.14 to 0.29, not making it possible to select valuable genotypes. They concluded it possible to obtain a coefficient of 0.50 from five harvests. Sánchez et al. (2017) obtained moderate to high estimates of individual repeatability for fruit production and high values of average repeatability in studies with soursop. Similar results were also reported

in other fruit crops such as peach (Bruna et al., 2012) and sweet orange trees (Negreiros et al., 2014).

When considering estimated individual repeatability, adopting seven harvests was found to lead to accuracy levels of approximately 70% when predicting the permanent phenotypic values of individuals (Acm), for all evaluated characteristics (Table 3). The efficiency of using six harvests compared with the use of only one ranged 1.86 or 86% to 1.99 or 99% for the evaluated characters, corroborating the results of Pompeu Júnior et al. (2013), who stated that orange production stability is achieved in the fifth harvest.

The selection of citrus plants based on five evaluations should be carried out with caution, especially when considering the evaluations each year, as there is not always a correction between annual and accumulated productions over a long period. In addition, the position of cultivars can change during the early years owing to the production time of each canopy (Souza, 2010).

The values for the coefficients of determination were consistent with those reported by Souza (2010), who tested the selection of Pera orange clones and found that to achieve an average of 85% efficiency, seven evaluations would be necessary.

Negreiros et al. (2008) used the principal components method to obtain a coefficient of determination greater than 90% for average fruit mass in orange trees, from five measurements. Further, to predict the actual values of the individuals, with a degree of certainty above 95%, 10 measurements are necessary. By this same methodology, Souza (2010) found that to achieve 95% efficiency in the selection of sweet orange trees, 25 harvests would be required. Therefore, the ideal number of evaluations to be performed in selecting sweet orange trees should be defined by the researcher, according to the stage of the breeding program and the desired accuracy. However, a reduction in the number of harvests implies a reduction in selective accuracy.

In this study, a greater number of measurements were necessary for the characters number of fruits per plant and productivity, owing to the lower estimates obtained for the repeatability coefficient. However, Souza (2010) found that the repeatability coefficient for the production of Pera orange can already be determined in the first five harvests, owing to greater stabilization of fruit production in these evaluations. Sánchez et al. (2017) used data from individual soursop plants and found that eight harvests were sufficient to obtain a maximum coefficient of determination of 80% with high precision and selective efficiency. Alves et al. (2018) verified that

it would take 10 years of evaluation to obtain 80% of the determination coefficient in physic nut (*Jatropha curcas*) plants by using the REML/BLUP methodology.

In the present study, we found that more than 10 measurements were required for a coefficient of determination higher than 80% to select genotypes with greater efficiency. However, genotypic selection can also be performed using an average of six to eight evaluations, leading to less time and cost expenditure.

Conclusions

It's possible to conclude that the predictive accuracy of the selection for the four variables evaluated in sweet orange genotypes revealed a significant degree of certainty in the inferences, accuracy, and gain with selection.

Evaluation over six harvests can increase accuracy to 70% in the selection of sweet orange genotypes for production-related characteristics. For the prediction of average fruit mass, seven harvests are sufficient to obtain 80% accuracy.

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