



ESTIMATES OF GENETIC PARAMETERS AND SELECTION OF THREE-WAY CROSS CUPUAS- SU TREE PROGENIES (*Theobroma grandiflorum*)

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Abstract: In fruit tree breeding programs there is a need for effective selection methods to develop and identify superior genotypes. Thus, this study aimed to estimate genetic parameters and select superior genotypes for a genotypic aggregate in three-way cross progeny of cupuassu tree. As such, 25 three-way cross progeny were evaluated using a randomized block design, with five repetitions and three plants per plot. The following variables were measured over eight harvests: average number of fruits per plant; average fruit production per plant (kg); and incidence rate of witches' broom disease. The mixed model methodology (REML/BLUP) was used to estimate the components of variance and predict the genetic and genotypic values. The Additive Index was used to select families, and within these families select potential clones and genitors. The results showed the existence of genetic variability between families. This allowed the selection of progenies 125, 117, 130, 165, 131, 127, and 143, which resulted in a 14.3% gain in productivity, maintaining extremely low levels of witches' broom. In each progeny, individuals were selected to be recommended to producers in the form of clones, after clonal tests, and others that could be used as parents in the genetic breeding program of cupuassu tree, aiming to obtain more genotypes that are productive and with greater durability of resistance to witches' broom, in the field.

Keywords: fruit tree breeding, genetic selection, REML/BLUP, selection index.

Introduction

The cupuassu tree [*Theobroma grandiflorum* (Willd. ex Spreng.) Schum.] is a species of the Malvaceae family that is native to the Amazon. Currently, two economically important products are processed from the tree: the pulp, or mucilage, that covers the seeds, which is used to make juices, jams, jellies, among others (Pugliese et al., 2013). The oil extracted from the almonds, which is used in the cosmetic and pharmaceutical

industries (Oliveira and Genovese, 2013). It also provides the raw material necessary to produce cupuassu chocolate, or “cupulate”, a product that among other advantages offers an alternative to chocolate made from cocoa for those allergic to caffeine or the theobromine (Genovese and Lannes, 2009).

Brazil is practically the only producer of cupuassu in the world, with the center of production in the state of Pará, in the Amazon

region. In 2018, the state produced more than 27 thousand tons of fruit from 8,500 ha under production (average yield of 3,200 kg/ha), with the municipality of Tomé Açu, Pará, being one of the largest producers (Pará, 2020). In Pará and other cupuassu producing states, such as Amazonas, Rondônia, Acre, and Bahia, the species is socioeconomically significant, as almost exclusively family farmers and small-scale producers cultivate it, and the sale of the fruit and its products provides an alternative source of income for the family (Alves et al., 2014).

With the advent of commercial-scale cultivation, demands for research on the species began to arise, especially in terms of genetic improvement, since producers did not have varieties suitable for plantations. Therefore, the challenge was to quickly develop genotypes that were satisfactorily productive and tolerant to the etiological agent of witches' broom disease, the fungus *Moniliophthora perniciosa* (Stahel) Aime & Phillips Mora, which is the main disease that affects cupuassu crops.

In the 1980s, Embrapa Amazônia Oriental (CPATU) started the cupuassu tree genetic improvement program, with the formation of a germplasm bank that brought together a variety of genotypes with extensive variability as the foundation for the program (Alves et al., 2013). As a result, in 2002, five clonal cultivars were launched by Embrapa Amazônia Oriental: BRS Coari, BRS Codajás, BRS Manacapuru, and BRS Belém. The main characteristics of these cultivars were tolerance to fungus, combined with good productivity and fruit quality. In 2012, the same program made available the BRS Carimbó cultivar, which offered seed propagation, as well as superior levels of disease resistance and productivity than previous cultivars (Alves, 2012). In 2020, the program released the latest cultivars: BRS Careca, BRS Fartura, BRS Golias, BRS Curinga e BRS Duquesa, five clonal cultivars that present the best production and resistance index so far, being recommended for the replacement of unproductive and, or, susceptible plants in aged orchards (Alves and Chaves, 2020).

Despite the technological advances achieved to date, the search for superior genotypes continues, with the goal of consolidating the species as a viable cultivar and improving profitability and crop security. As such, new breeding methods are being implemented, such as the development of three-way cross progeny. This method is well established and routinely used for annual crops such as corn (Sesay et al., 2016), wheat (Whitford et al., 2013), and barley (Mühleisen et al., 2014), as well as for perennial species, as for example eucalyptus (Gomes et al., 2015). However, in breeding programs of perennial fruit trees, discussions of this method in the literature are practically nonexistent.

The three-way cross technique consists of crossing three different genotypes in the formation of progeny that aggregate genes from the three parents in order to exploit the effects between alleles. This enables increases in productivity, stability, adaptability, and resistance to witches' broom disease. It also supports increases in the population's genotypic heterogeneity. The method is a simpler alternative to biotechnological methods that alter the genetic composition or ploidy of individuals. In addition, as with simple crosses, three-way crosses provide more stable individuals than double crosses, and it can enable greater stability and adaptability, depending on the progenitors (Borém et al., 2017).

Although the technique is rarely used for fruit trees and there is no record of its use for cupuassu tree, it is a promising tool that can be employed in genetic improvement. Because cupuassu tree is allogamous and self-incompatible (Venturieri, 2011), it is extremely dependent on genetic variability. It is also a perennial species with a long life cycle. Therefore, each recommended cultivar must offer improved sustainability and consequent longevity in the field. The expectation is that the three-way cross method will promote the development of genotypes with these characteristics. Thus, this study aimed to estimate genetic parameters and use the Additive Index to select superior genotypes for a genotypic aggregate in cupuassu tree three-way cross progeny.

Material and methods

Experimental data

The experiment was installed in February 2007 on a rural property in the municipality of Tomé Açu, Pará, Brazil (geographical coordinates 02° 24' 05,6" S and 48° 00' 08,8" W; elevation of 75 meters), and the experiment was monitored until December 2019. The climate of the region is Am, according to the Köppen classification, with an average temperature during the evaluation period of approximately 27 °C, relative air humidity of 85.5%, and average annual rainfall of 2,729 mm. The soil is deep, well-drained, and classified as Oxisol, with medium texture and low fertility.

To obtain three-way cross progeny, initially, the F1 generation was obtained through crossings between pairs of clones evaluated and

selected based on complementary characteristics from the Cupuassu Tree Active Germplasm Bank of Embrapa Amazônia Oriental (Alves et al., 2013). The obtained progeny (F1) were evaluated in the field for 15 years.

The parent plants, selected among and within these progeny, were cloned and then crossed with four cultivars launched in 2002 [BRS Coari (174), BRS Codajás (186), BRS Manacapuru (215), and BRS Belém (286)], respecting the non-coincidence of parents to avoid inbreeding. Each crossing followed the scheme (P1 x P2) x P3 and consisted of gene contributions from three different clones (Parents 1, 2, and 3), whose genealogy is shown in Table 1. Twenty-five three-way cross progeny were evaluated, using a randomized block design, with five repetitions and three plants per plot.

Table 1. Genealogy of the 25 cupuassu tree three-way cross progeny evaluated over eight harvests in Tomé Açu, Pará, Brazil.

Progeny	Parent 1	Parent 2	Parent 3
117	186 (BRS Codajás)	434	174 (BRS Coari)
118	186 (BRS Codajás)	434	174 (BRS Coari)
121	186 (BRS Codajás)	434	174 (BRS Coari)
123	186 (BRS Codajás)	554	174 (BRS Coari)
124	186 (BRS Codajás)	554	174 (BRS Coari)
125	186 (BRS Codajás)	554	174 (BRS Coari)
126	186 (BRS Codajás)	554	174 (BRS Coari)
127	186 (BRS Codajás)	1074	174 (BRS Coari)
130	186 (BRS Codajás)	513	174 (BRS Coari)
131	286 (BRS Belém)	513	174 (BRS Coari)
132	286 (BRS Belém)	513	174 (BRS Coari)
134	174 (BRS Coari)	286 (BRS Belém)	186 (BRS Codajás)
135	174 (BRS Coari)	286 (BRS Belém)	186 (BRS Codajás)
136	174 (BRS Coari)	286 (BRS Belém)	186 (BRS Codajás)
137	286 (BRS Belém)	513	186 (BRS Codajás)
138	174 (BRS Coari)	186 (BRS Codajás)	215 (BRS Manacapuru)
143	186 (BRS Codajás)	1074	215 (BRS Manacapuru)
144	186 (BRS Codajás)	1074	215 (BRS Manacapuru)
150	186 (BRS Codajás)	434	SEKÓ
152	186 (BRS Codajás)	554	SEKÓ
162	286 (BRS Belém)	215 (BRS Manacapuru)	1074
163	286 (BRS Belém)	215 (BRS Manacapuru)	1074
165	174 (BRS Coari)	286 (BRS Belém)	1074
166	186 (BRS Codajás)	554	1074
169	186 (BRS Codajás)	513	1074

Because cupuassu trees require partial shading, especially in the early years of cultivation, the experiment was installed in consortium with commercial black pepper (*Piper nigrum* L.), which were planted three years before installing the cupuassu tree experiment. The black pepper plants were placed in double rows, at a spacing of (2 x 2) x 4 m, and the cupuassu tree progeny were planted between rows, at a spacing of 6 x 4 m. This consortium lasted for seven years, at which point the pepper plants died due to an attack of fusariosis. The progeny then remained in full sun for the rest of the experiment. Cultivation followed the technical recommendations for both species (Souza, 2007; Lemos et al., 2014).

The collection of experimental data began during the 2010/2011 harvest and continued until the 2018/2019 harvest, and the following variables were evaluated: average number of fruits per plant; average fruit production per plant (kg); and incidence rate of witches' broom disease symptoms.

Statistical analysis

The mixed model methodology (REML/BLUP) was chosen because of its effectiveness to analyze unbalanced data. Although the trial was planned to be balanced, there was death of some plants, which is normal in a 13 years old trial. The method was used to estimate the components of variance and predict genetic and genotypic values. The statistical model was given as:

$$y = Xr + Zg + Wp + e;$$

where, y is the vector of phenotypic data; r is the vector of repetition effects (assumed to be fixed) added to the general average; g is the vector of individual genotypic effects (assumed to be random) [$g \sim N(0, \sigma_g^2)$, where σ_g^2 is the genotypic variance among full-sib progeny]; p is the vector of plot effects (assumed to be random) [$p \sim N(0, \sigma_p^2)$, where σ_p^2 is the environmental variance between plots]; and e is the vector of residuals (random) [$e \sim N(0, \sigma_e^2)$, where σ_e^2 is the residual variance within a plot]. The capital letters (X, Z

and W) represent the incidence matrices for these effects.

The significance of the random effects of the statistical model was tested by the likelihood ratio test (LRT) (Wilks, 1938), with:

$$LRT = -2(\text{Log}L - \text{Log}L_R);$$

where $\text{Log}L$ is the logarithm of the maximum point of the residual likelihood function (L) for the complete model; and $\text{Log}L_R$ is the logarithm of the maximum point of the residual likelihood function (L_R) for the tested model.

The individual phenotypic variance (σ_f^2), individual narrow-sense heritability (h_a^2), coefficient of determination of the plot effects (c_p^2), mean progeny heritability (h_{mp}^2), progeny selection accuracy (r_{gg}), additive heritability within a plot (h_{ad}^2), and coefficient of genetic determination ($CV_g\%$) were estimated, respectively, using the following expressions (Resende et al., 2014):

$$\sigma_f^2 = \sigma_g^2 + \sigma_p^2 + \sigma_e^2;$$

$$h_a^2 = 2\sigma_g^2/\sigma_f^2;$$

$$c_p^2 = \sigma_p^2/\sigma_f^2;$$

$$h_{mp}^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_p^2/r) + (\sigma_e^2/rn)];$$

$$r_{gg} = \sqrt{h_{mp}^2};$$

$$h_{ad}^2 = \sigma_g^2/\sigma_e^2; \text{ and}$$

$$CV_g\% = (\sqrt{\sigma_g^2}/\mu)100;$$

where r is the number of repetitions (5); n is the number of plants per plot (3); and μ is the general average.

The Additive Index (AI) (Resende et al. 2014) was used to select progeny and superior individuals for the genotypic aggregate [average number of fruits per plant and average fruit production per plant (kg)]. This index was given as:

$$AI_i = \sum_{t=1}^2 w_t \frac{V_{Git}}{\sigma_t};$$

where, w_t is the weight attributed to the variable t ; VG_{it} is the predicted genetic or genotypic value of progeny/individual i for variable t ; and σ_t is the standard error of the genetic or genotypic values predicted for variable t . In this index, the coefficients of genotypic variation were used as weights and the selection direction was "higher" for both variables.

The gains predicted with the selection for the genotypic aggregate were obtained directly through the output of the Additive Index. All statistical analyses were performed using the Selegen-REML/BLUP software (Resende, 2016).

Results and discussion

The mixed model methodology (REML/BLUP) used in the present study is widely employed in studies on plant breeding. Resende (2016) highlights the efficacy of this procedure

to model the heterogeneity of genetic variances, as well as to estimate genetic parameters, such as components of variance, heritability, correlation, and selection indices. In the improvement of fruit trees, especially perennials, this method enables researchers to obtain credible results quickly and efficiently, as observed for mango tree (Maia et al., 2017), papaya tree (Cardoso et al., 2017), as well as cupuassu tree (Alves et al., 2020).

Deviance analysis

The deviance analysis (LRT) suggests significant genetic effects ($p < 0.01$) for the variables average number of fruits per plant and average fruit production per plant (kg), indicating the presence of genetic variability among the evaluated progeny (Table 2). On the other hand, the plot effects were non-significant ($p > 0.01$), that is, the estimates related to this effect do not differ from zero (Table 2).

Table 2. Deviance analysis for the variables average number of fruits per plant and average fruit production per plant (kg), evaluated over eight harvests in cupuassu tree three-way cross progeny.

Effect	Average number of fruit		Average fruit production (kg)	
	Deviance	LRT	Deviance	LRT
Progeny	1439.15	20.42**	1792.92	41.88**
Plot	1418.74	0.01 ^{ns}	1751.05	0.01 ^{ns}
Complete model	1418.73		1751.04	

LRT: likelihood ratio test. ** and ^{ns}: significant and not significant, respectively, at 1% probability based on Chi-square test. The null hypothesis was that the complete and reduced models do not differ from each other.

The significant genetic effects obtained here result from the variability of the parental employed, as well as from the cupuassu tree being a self-incompatible allogamous species, which presents most of its loci in heterozygosis (Alves et al., 2007). The non-significance of the plot effects reflects a good experimental control.

Components of variance and genetic parameters

The genotypic variance among the full-sib progeny represented approximately 14% and 22% of the individual phenotypic variance for the variables average number of fruits and average fruit production (kg), respectively (Table 3). These values indicate the presence of variability among the evaluated genotypes, suggesting that the difference in performance can be explained

by dissimilarity between the genetic composition and gene expression of the genetic materials studied herein. Although, there is coincidence in the progenitors of most of these genotypes, the pairing and possible genetic recombination in the formation of reproductive cells of the genitors may have contributed to increased genetic variability (Loidl, 2016).

These results were supported by the estimated coefficients of genetic variation, which were 11.3 and 16.43 for average number of fruits and average fruit production (kg), respectively (Table 3). Comparatively, Pinto et al. (2013) found low CV_g values for quantitative characteristics, when using a population with a narrow genetic base, resulting from crosses between genetically similar materials.

Table 3. Variance components and genetic parameters for the variables average number of fruits and average fruit production (kg) evaluated over eight harvests in cupuassu tree three-way cross progeny.

Component/parameter	Average number of fruit	Average fruit production (kg)
σ_g^2	4.1786	19.4877
σ_p^2	0.0189	0.0953
σ_e^2	24.9001	67.1477
σ_f^2	29.0976	86.7307
h_a^2	0.29	0.45
c_p^2	0.00	0.00
h_{mp}^2	0.72	0.81
r_{gg}	0.85	0.90
h_{ad}^2	0.17	0.29
$CV_g(\%)$	11.30	16.43
μ	18.09	26.87

σ_g^2 : genotypic variance among full-sib progeny; σ_p^2 : environmental variance between plots; σ_e^2 : residual variance within plots; σ_f^2 : individual phenotypic variance; h_a^2 : individual narrow-sense heritability; c_p^2 : coefficient of determination of plot effects; h_{mp}^2 : mean progeny heritability; r_{gg} : accuracy of progeny selection; h_{ad}^2 : additive heritability within plot; $CV_g(\%)$ coefficient of genotypic variation; and μ : overall average.

Consistent with the deviance analysis, the estimated coefficients of determination for the plot effects were practically null (Table 3). This data, together with the high to very high values (Resende and Duarte, 2007) for selective accuracy [0.85 and 0.90 for average number of fruits and average fruit production (kg), respectively] suggests good experimental control and underscores the quality and credibility of the results presented herein. Thus, we can infer that this variability can be further exploited, the experimental conditions were adequate, and the selection of genotypes was effective.

Individual narrow-sense heritability presented moderate magnitudes ($0.15 < h_a^2 < 0.50$) (Table 3). The highest values for heritability were observed for mean progeny heritability, while the lowest were registered for the additive eritability within the plot. The three-way crossing method seems to interfere with the heritability estimates of the studied variables. Working with cupuassu tree genotypes from simple crosses, Alves et al. (2020) observed higher heritability values for the variable average number of fruits. However, the highest values found in the present study were for average fruit production (kg). This trait is more complex as it is dependent on both the average number and average weight of fruits. Therefore, we infer that the use of three parents may result in a greater fixation of alleles related to the average weight

variable, which increased the heritability of average fruit production (kg).

Since these are quantitative variables, it was expected that the estimated heritabilities would not be high. Based on the classification described by Resende and Alves (2020), the individual narrow-sense heritability and additive heritability within a plot can be categorized as moderate, while mean progeny heritability reached values that are considered high. Alves et al. (2020) found higher values for these parameters, again showing the difference between single and triple hybrids.

Progeny selection

The use of selection indices is common in the improvement of fruit trees, with previous analyses focused on papaya (Pinto et al., 2013), açai (Farias Neto et al., 2011), passion fruit (Neves et al., 2011), and peach trees (Silva et al., 2020). To date, the records in the literature describing its use for cupuassu tree are rare; therefore, the present study offers novel insights into the viability of the methodology for the species.

The Additive Index (AI) enabled us to identify the most promising progeny and, within them, the most appropriate individuals to be cloned, aiming at the exploitation of heterosis resulting from three-way crosses (Prazeres et al.,

2016). The goal was to maintain variability between genotypes by selecting a maximum of two individuals from each progeny, given the self-incompatible, allogamous characteristics of the species, which requires diversity to enable effective crossbreeding (Venturieri, 2011).

Taking into account the Additive Index (AI), the 25 three-way cross progeny were ranked in descending order (Table 4). Considering only this value, the first ten progeny would be selected. However, this improvement program aims to obtain not only productive individuals but also genotypes that contain and express resistance to *M. perniciosa*. For this

reason, we also considered the rates of symptomatic plants for witches' broom disease for each progeny.

Thus, the second, third, and tenth ranked progeny based on the AI were discarded due to unsatisfactory performance in terms of witches' broom disease resistance. As such, progeny 125, 117, 130, 165, 131, 127, and 143 were selected. The use of these genotypes will result in an average gain of 14.3% in productivity, resulting in an increase of 2.5 fruits per plant and 3.76 kg of fruits/plant, while maintaining very low incidence rates of witches' broom disease.

Table 4. Classification of 25 three-way cross progeny of cupuassu tree through Additive Index (AI) considering the predicted genotypic values for the variables average number of fruits (VG¹), average fruit production (kg) (VG²), and incidence of witches' broom disease (WB%), evaluated over eight harvests.

Rank	Progeny	VG ¹	VG ²	AI	Gain	Gain (%)	WB%
1	125	20.91	33.57	280.39	280.39	20.10	0.0
2	162	19.65	32.71	268.35	274.37	17.52	28.6
3	169	19.70	32.37	267.26	272.00	16.50	40.0
4	117	20.76	29.37	261.77	269.44	15.41	9.1
5	130	19.51	31.22	261.15	267.78	14.70	0.0
6	165	20.70	28.05	255.84	265.79	13.84	0.0
7	131	19.25	28.86	249.55	263.47	12.85	0.0
8	127	17.80	30.46	246.53	261.35	11.94	0.0
9	143	18.06	29.80	245.52	259.59	11.19	0.0
10	138	18.87	28.03	243.52	257.99	10.50	14.3
11	144	17.76	29.67	242.98	256.62	9.92	14.3
12	163	18.32	28.60	242.23	255.42	9.40	36.4
13	136	19.10	27.30	241.99	254.39	8.96	6.7
14	135	18.53	26.96	236.75	253.13	8.42	0.0
15	166	17.65	25.69	225.60	251.30	7.63	0.0
16	132	17.97	24.92	224.50	249.62	6.92	0.0
17	121	17.71	24.39	220.51	247.91	6.18	14.3
18	124	17.94	23.76	219.38	246.32	5.50	0.0
19	137	16.37	25.21	215.00	244.67	4.80	0.0
20	150	17.26	23.55	213.95	243.14	4.14	10.0
21	152	16.74	22.92	207.89	241.46	3.42	14.3
22	118	15.59	23.54	202.76	239.70	2.67	6.7
23	134	16.73	21.17	200.48	238.00	1.94	7.7
24	126	14.82	20.46	184.73	235.78	0.99	14.3
25	123	14.68	19.11	178.15	233.47	0.00	10.0

Selection of individuals (potential clones)

After selecting the progeny, the best individuals within these progeny were identified, again considering the Additive Index (AI) and incidence of witches' broom disease (WB%). Based on an initial filter, 20 of the 375 individuals were considered suitable for selection, taking into account the highest AI values. Of these 20 individuals, only the descendants of the previously selected progeny were chosen, respecting a maximum of two individuals per progeny to maintain genetic variability. As such, nine plants were identified to be cloned (Table 5). Of these, only progeny 125 and 117 had two individuals each, while the others (130, 165, 131, 127, and 143) included only one individual. The use of these individuals in the form of clones will provide gains of 26.8% in productivity. It is worth mentioning that no selected individual had

symptoms of *M. pernicioso*, despite 12 years of exposure in the field. These clones must be previously evaluated in clonal competition trials, before recommending them to producers.

Selection of individuals (potential genitors)

Based on the same procedure used for the selection of individuals for cloning, seven individuals with potential to act as genitors were identified (Table 6). Of these seven individuals, progeny 125 and 130 had two individuals selected, while the other three progeny (117, 165, and 127) included only one individual. The use of these plants will result in an average gain of 31.3% in productivity. Again, it should also be emphasized that these plants did not present symptoms of witches' broom disease, even after 12 years of exposure to the pathogen in the field.

Table 5. Ranking of the nine selected individuals to be used as clones, based on Additive Index (AI), considering the genotypic values predicted for the variables average number of fruits (VG¹), average fruit production (kg) (VG²), and incidence rate of witches' broom disease (WB%), in a trial of cupuassu tree three-way cross progeny, evaluated over eight harvests.

Rank	Individual (x.y.z) [†]	VG ¹	VG ²	AI	Gain	Gain %	WB%
1	125.5.3	22.49	39.58	241.65	241.65	34.64	0.00
2	127.4.2	20.78	38.02	227.77	235.15	31.01	0.00
3	117.1.3	23.30	33.24	226.28	233.38	30.03	0.00
4	143.3.2	18.71	38.44	218.16	227.84	26.94	0.00
5	125.3.2	20.22	35.21	216.10	226.54	26.21	0.00
6	117.4.3	22.41	30.58	213.31	222.86	24.17	0.00
7	165.1.2	21.84	31.31	212.59	222.13	23.76	0.00
8	131.5.2	20.36	33.09	210.30	220.20	22.68	0.00
9	130.2.1	20.68	32.40	209.80	218.67	21.83	0.00

[†] Individual x.y.z coding: "x" corresponding to the progeny; "y" corresponding to the block; and "z" corresponding to the plant.

Table 6. Ranking of the seven individuals selected to be used as genitors, based on Additive Index (AI) and considering the genetic values predicted for the variables average number of fruits (VG¹), average fruit production (kg) (VG²), and incidence rate of witches' broom disease (WB%), in a test of cupuassu trees three-way cross progeny, evaluated over eight harvests.

Rank	Individual (x.y.z) [†]	VG ¹	VG ²	AI	Gain	Gain %	WB (%)
1	125.2.2	22.29	39.85	268.97	272.92	36.65	0.00
2	125.1.1	22.49	37.96	263.53	269.79	35.08	0.00
3	130.3.2	21.74	37.19	256.48	266.46	33.42	0.00
4	130.1.1	21.77	36.38	253.79	262.63	31.50	0.00
5	117.1.3	22.57	33.54	248.56	258.62	29.49	0.00
6	127.4.2	19.93	36.45	243.34	253.19	26.77	0.00
7	165.3.3	22.59	31.77	242.42	251.96	26.16	0.00

[†] Individual x.y.z coding: "x" corresponding to progeny; "y" corresponding to block; and "z" corresponding to the plant.

The same criteria were used to select suitable individuals to act as genitors in the genetic breeding program. It should be noted that only two plants were selected for both cloning and progenitor purposes. Beyond the inherent value of these individuals, which can be exploited through cloning, they are able to transfer these attributes to their offspring.

The center of origin of cupuassu tree is in the states of Pará and Maranhão (Alves et al., 2007), Brazil, where we find the maximum genetic diversity of the species. However, its main pathogens co-evolved with the species in this environment. Therefore, the establishment of cupuassu tree crops in the region is extremely vulnerable to the diversity of pathogens that naturally occur on intermediate hosts in the native forest. As such, genetic improvement programs that diversify the genetic basis of genetic materials used in plantations are necessary, with the expectation that they will increase the longevity of cultivars in the field and, consequently, the security of production (Borém et al., 2017). Besides the Additive Index, which considered the productive characteristics analyzed herein, the resistance of plants to witches' broom disease was also considered. None of the selected individuals presented

symptoms of attack by the *M. perniciosus* fungus, despite having been exposed to the pathogen in the field for 12 years.

The three-way cross genotypes that have more than one resistant parent should maintain resistance over a longer period of time, offering greater security for the producer. In the case of individuals selected as progenitors, this will enable the transmission of this characteristic to new generations through the pyramidization of resistant genes (Borém et al., 2017) and will help to ensure that future progeny have greater and prolonged resistance to *M. perniciosus* in the field.

Conclusion

The three-way cross progeny studied herein present significant genetic variability for selection. Seven progeny were identified and selected, from which nine individuals with inherent productive potential can be used as clones and made available to producers, after previous clonal trials to testify their potential. Seven individuals were also identified to be used as genitors in the cupuassu tree genetic breeding program, aiming to improve fruit production and obtain genotypes with greater continued resistance to *M. perniciosa* in the field.

References

- ALVES, R.M.; SEBBENN, A.M.; ARTERO, A.S.; CLEMENT, C.; FIGUEIRA, A. 2007. High levels of genetic divergence and inbreeding in populations of cupuassu (*Theobroma grandiflorum*). **Tree Genetics & Genomes**, 3(4):289-298.
- ALVES, R.M. **Implantação de um pomar de cupuaçuzeiro com a cultivar BRS Carimbó**. 2012. Embrapa Amazônia Oriental, Belém.
- ALVES, R.M.; SILVA, C.R.S.; SILVA, M.S.C.; SILVA, D.C.S.; SEBBENN, A.M. 2013. Diversidade genética em coleções amazônicas de Germoplasma de cupuaçuzeiro [*Theobroma grandiflorum* (Willd. ex Spreng.) Schum.]. **Revista Brasileira Fruticultura**, 35(3):818-828.
- ALVES, R.M.; FILGUEIRAS, G.C.; HOMMA, A.K.O. 2014. **Aspectos socioeconômicos do cupuaçuzeiro na Amazônia: do extrativismo a domesticação**. In: SANTANA, A.C. Mercado, cadeias produtivas e desenvolvimento rural na Amazônia. UFRA, Belém.
- ALVES, R.M.; CHAVES, S.F.S.; GAMA, M.A.P.; PEDROZA NETO, J.L.; SANTOS, T.G. 2020. Simultaneous selection of cupuassu tree and Brazilian mahogany genotypes in an agroforestry system in Pará state, Brazil. **Acta Amazonica**, 50(3):183-191.

- ALVES, R.M.; CHAVES, S.F.S. BRS Careca, BRS Fartura, BRS Duquesa, BRS Curinga, and BRS Golias: new cupuassu tree cultivars. **Crop Breeding and Applied Biotechnology**, 20(4):e342920413.
- BORÉM, A.; MIRANDA, G.V.; FRITSCHÉ-NETO, R. 2017. **Melhoramento de Plantas**. Editora UFV, Ed. 7, Viçosa.
- CARDOSO, D.L.; VIVAS, M.; PINTO, F.O.; VIANA, A.P.; AMARAL JUNIOR, A.T.; PEREIRA, M.G. 2017. Diallel mixed-model analysis of papaya fruit deformities. **Ciência Rural**, 47(5):e20151553.
- FARIAS NETO, J.T.; RESENDE, M.D.V.; OLIVEIRA, M. 2011. Seleção simultânea em progênies de açaizeiro irrigado para produção e peso do fruto. **Revista Brasileira de Fruticultura**, 33(2):532-539.
- GENOVESE, M.I.; LANNES, S.C.S. 2009. Comparison of total phenolic content and antiradical capacity of powders and "chocolates" from cocoa and cupuassu. **Food Science and Technology**, 29(4):810-814.
- GOMES, F.J.B.; COLODETTE, J.L.; BURNET, A.; BATALHA, L.A.R.; SANTOS, F.A.; DEMUNER, I.F. 2015. Thorough characterization of Brazilian new generation of eucalypt clones and grass for pulp production. **International Journal of Forestry Research**, 2015:1-10.
- LEMOES, O.F.; TREMACOLDI, C.R.; POLTRONIERI, M.C. 2014. **Boas práticas agrícolas para aumento da produtividade e qualidade da pimenta-do-reino no estado do Pará**. Embrapa Amazônia Oriental, Belém.
- LOIDL, J. 2016. Conservation and variability of meiosis across the eukaryotes. **Annual review of genetics**, 50:293-316.
- MAIA, M.C.C.; OLIVEIRA, L.C.; VASCONCELOS, L.F.L.; LIMA NETO, F.P.; YOKOMIZO, G.K.; ARAÚJO, L.B. 2017. Repetibilidade de características quantitativas de frutos em seleções elite de manga rosa. **Revista Agro@mbiente online**, 11(1):56-62.
- MÜHLEISEN, J.; PIEPHO, H.; MAURER, H.P.; LONGIN, C.F.H.; REIF, J.C. 2014. Yield stability of hybrids versus lines in wheat, barley, and triticale. **Theoretical and Applied Genetics**, 127(2):309-316.
- NEVES, L.G.; BRUCKNER, C.H.; CRUZ, C.D.; VIANA, A.P.; BARELLI, M.A.A. 2011. Predição de ganhos, com diferentes índices de seleção, para características de frutos do maracujazeiro-amarelo. **Revista Brasileira de Fruticultura**, 33(4):1322-1330.
- OLIVEIRA, T.B.; GENOVESE, M.I. 2013. Chemical composition of cupuassu (*Theobroma grandiflorum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats. **Food Research International**, 51(2):929-935.
- PARÁ - Secretaria de Estado de Desenvolvimento Agropecuário e da Pesca. 2020. **Indicadores agropecuários**. Available at <http://www.sedap.pa.gov.br/content/cupua%C3%A7u>. Accessed on Mar 19, 2020.
- PINTO, F.O.; LUZ, L.N.; PEREIRA, M.G.; CARDOSO, D.L.; RAMOS, H.C.C. 2013. Metodologia dos modelos mistos para seleção combinada em progênies segregantes de mamoeiro. **Revista Brasileira de Ciências Agrárias**, 8(2):211-217.

- PRAZERES, C.S.; COELHO, C.; MEDEIROS, M. 2016. Heterose para qualidade fisiológica de sementes na obtenção de híbridos de milho. **Revista Brasileira de Milho e Sorgo**, 15(1):124-133.
- PUGLIESE, A.G.; TOMAS-BARBERAN, F.A.; TRUCHADO, P.; GENOVESE, M.I. 2013. Flavonoids, proanthocyanidins, vitamin C, and antioxidant activity of *Theobroma grandiflorum* (Cupuassu) pulp and seeds. **Journal of agricultural and food chemistry**, 61(11):2720-2728.
- RESENDE, M.D.V.; DUARTE, J.B. 2007. Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical**, 37:182-194.
- RESENDE, M.D.V.; SILVA, F.F., AZEVEDO, C.F. 2014. **Estatística Matemática, Biométrica e Computacional: modelos mistos, multivariados, categóricos e generalizados (REML/BLUP), inferência Bayesiana, regressão aleatória, seleção genômica, QTL-GWAS, estatística espacial e temporal, competição, sobrevivência**. Editora Suprema, Ed. 1, Visconde do Rio Branco.
- RESENDE, M.D.V. 2016. Software Selegen-REML/BLUP: a useful tool for plant breeding. **Crop Breeding and Applied Biotechnology**, 16:330-339.
- RESENDE, M.D.V.; ALVES, R.S. Linear, generalized, hierarchical, bayesian and random regression mixed models in genetics/genomics in plant breeding. **Functional Plant Breeding Journal**, 2(2):a1.
- SESAY, S.; OJO, D.; ARIYO, O. J.; MESEKA, S. 2016. Genetic variability, heritability and genetic advance studies in topcross and three-way cross maize (*Zea mays* L) hybrids. **Maydica**, 61:1-7.
- SILVA, J. O. C.; BRUCKNER, C. H.; CARNEIRO, P. C. S.; RESENDE, M. D. V.; ALVES, R. S.; SILVA, D. F. P. 2020. Estimates of genetic parameters and repeatability coefficients in S₂ peach genotypes grown in a subtropical environment. **Functional Plant Breeding Journal**, 2(1):a3.
- SOUZA, A. G. C. 2007. **Boas práticas agrícolas da cultura do cupuaçuzeiro**. Embrapa Amazônia Ocidental, Manaus.
- VENTURIERI, G.A. 2011. Flowering levels, harvest season and yields of cupuassu (*Theobroma grandiflorum*). **Acta Amazonica**, 41:143-152.
- WHITFORD, R. 2013. Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. **Journal of experimental botany**, 64(18):5411-5428.
- WILKS, S.S. 1938. The large sample distribution of the likelihood ratio for testing composite hypothesis. **Annals of Mathematical Statistics**, 9:60-62.