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Genetic/ Original Article

Repeatability and number of harvests for selection of Amazon nut genotypes in an agroforestry cultivation system

Abstract – The objective of this work was to estimate the repeatability coefficients of variables related to fruit and nut production, the minimum number of crops required for genotype selection, and the permanent phenotypic correlation coefficients between variables in a cultivated population of Amazon nut. A total of 40 genotypes from seeds, belonging to an agroforestry system implemented in 1995, in the state of Roraima, Brazil, were evaluated during ten years for six variables related to fruit and nut production. The genetic-statistical analyses were performed using the maximum restricted likelihood and best linear unbiased prediction (REML/BLUP) method, based on the basic model of repeatability, without an experimental design, of the Selegen-REML/BLUP software. Genetic variability between genotypes and a moderate regularity in the repetition of the studied variables between different harvests were detected. The number of harvests required to reach a coefficient of determination of 80% ranged from seven, for the variables average nut weight per fruit and number of fruits per plant, to nine, for average nut weight. The correlation results indicate the possibility of indirect selection for nut production through the evaluation of the number and weight of fruits per plant.

Index terms: *Bertholletia excelsa*, nut breeding, plant breeding, selection.

Repetibilidade e número de colheitas para seleção de genótipos de castanheira-da-amazônia em sistema agroflorestal de cultivo

Resumo – O objetivo deste trabalho foi estimar os coeficientes de repetibilidade de variáveis relacionadas à produção de frutos e castanhas, o número mínimo de safras requeridas para seleção de genótipos e os coeficientes de correlação fenotípica permanente entre variáveis, em uma população cultivada de castanheira-da-amazônia. Um total de 40 genótipos provenientes de mudas de sementes, pertencentes à um sistema agroflorestal implantado em 1995, no estado de Roraima, Brasil, foi avaliado por um período de dez anos quanto a seis variáveis relacionadas à produção de frutos e castanhas. As análises genético-estatísticas foram realizadas pelo método da máxima verossimilhança restrita e melhor predição linear não viesada (REML/BLUP), com base no modelo básico de repetibilidade, sem delineamento experimental, do programa Selegen-REML/BLUP. Detectaram-se variabilidade genética entre os genótipos e moderada regularidade na repetição das variáveis estudadas entre as diferentes safras. O número de safras necessárias para atingir um coeficiente de determinação de 80% variou de sete, para as variáveis peso médio de castanhas por fruto e número de frutos por planta, a nove, para peso médio da castanha. Os resultados de correlação indicam a possibilidade de

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seleção indireta para produção de castanhas, por meio da avaliação do número e do peso de frutos por planta.

Termos para indexação: *Bertholletia excelsa*, melhoramento de nozes, melhoramento genético, seleção.

Introduction

The world production of Amazon nut (*Bertholletia excelsa* Bonpl.), a species native to the Amazon region with a great social, ecological, and economic importance, comes almost exclusively from the extraction of natural populations (Homma et al., 2014; Guariguata et al., 2017). One of the main limiting factors for the implantation of crops of this species is the unavailability of cultivars selected and recommended by plant breeding programs (Baldoni et al., 2019).

In the 1960s, the extinct Instituto de Pesquisa e Experimentação Agropecuária do Norte selected 28 Amazon nut genotypes with a high fruit productivity among native crops of the state of Pará. Part of these genotypes are currently conserved in a germplasm bank maintained by Embrapa Amazônia Oriental (Embrapa Recursos Genéticos e Biotecnologia, 2024). In the early 2010s, other genotypes were selected by other Embrapa units in the Brazilian Amazon to start a breeding program for the species, aiming at recommending varieties for fruit production (Pedrozo et al., 2015; Baldoni et al., 2019). In the 2000s, the Research Institute of the Peruvian Amazon began a breeding program with similar objectives (Corvera-Gomringer, 2014).

As Amazon nut is a perennial species, the costs for its maintenance and evaluation in breeding experiments are high. This is why it is essential to define strategies that allow of obtaining genetic gains in the shortest possible time. An alternative is determining the minimum number of measurements (harvests) that must be conducted in a given population for the selection of genotypes of interest, which may lead to a reduction in the required time and labor and, consequently, in the costs involved (Cruz et al., 2004; Pinheiro et al., 2019; Santa Catarina et al., 2020).

A way to determine the number of measurements required for genotype selection in perennial species is through the repeatability coefficient: the higher the value of this parameter, the lower the number of necessary measurements (Cruz et al., 2004). In addition to providing practical information for breeding programs, repeatability estimates can also assist in defining methodologies for the characterization of accessions, aiming at the conservation of the germplasm of a species (Silva et al., 2006).

Repeatability may vary according to the nature of the evaluated character, the genetic properties of the population, and the environmental conditions under which the studied individuals are maintained (Cruz et al., 2004). For natural Amazon nut trees of the states of Roraima, Mato Grosso, and Acre (Pedrozo et al., 2015; Baldoni et al., 2017; Azevedo et al., 2020), for example, the estimates of the repeatability coefficients varied according to the origin of the population, requiring three to ten evaluation harvests for genotypes to be efficiently selected for fruit and nut production.

Another strategy when aiming to shorten the process of selection and, consequently, of the recommendation of varieties is using cultivated Amazon nut trees in the production stage instead of natural trees for the initial selection of genotypes to be used for the formation of the breeding base population (Baldoni et al., 2019).

The objective of this work was to estimate the repeatability coefficients of variables related to fruit and nut production, the minimum number of crops required for genotype selection, and the permanent phenotypic correlation coefficients between variables in a cultivated population of Amazon nut.

Materials and Methods

The study was carried out in an agroforestry system implemented in 1995 in the experimental field of Confiança, belonging to Embrapa Roraima, located in the municipality of Cantá, in the state of Roraima, Brazil (60°3'54"W, 02°15'00"N). The region is characterized by forest vegetation and has an undulating relief, with a predominance of Red-Yellow Ferrosols (Tonini et al., 2006). The climate is of the Ami type according to Köppen's classification, with an annual rainfall varying from 1,795 to 2,385 mm and a concentrated rainy season from May to July.

The agroforestry system contains 71 Amazon nut trees planted in a 24x8 m spacing (56 trees per hectare), which were obtained from open-pollination seeds collected from trees in natural populations. In addition to these trees, the system was initially composed of the following species: cupiuba (*Goupia glabra* Aubl.), pupunha (*Bactris gasipaes* Kunth), cupuaçu [*Theobroma grandiflorum* (Willd. Ex Spreng.) K.Schum.], coffee (*Coffea canephora* Pierre ex A.Froehner), *saman* [*Samanea saman* (Jacq.) Merr.], abiu (*Micropholis venulosa* Pierre), andiroba (*Carapa guianensis* Aubl.), and only gliricidia (*Gliricidia sepium* Kunth ex Steud.) at the border (Ferreira & Tonini, 2009). As of 2012, the pupunha and gliricidia plants were removed from the system.

To prepare the experimental area, four-year-old secondary brushwood vegetation was cut down, without burning afterwards. Then, the area was harrowed, and soil acidity was corrected. For planting fertilization, 40 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} of the FTE BR-12 micronutrients (Nutriplant, Barueri, SP, Brazil) were applied.

A total of 40 Amazon nut trees with at least three years of fruit production between 2010 and 2021 were considered for the study. The genotypes were evaluated during ten harvests/years from 2010 to 2011, 2013 to 2016, and 2018 to 2021. At the end of each harvest, all fruits falling under the crown of each tree were counted and weighed to determine number of fruits per plant (NFP) and fruit weight per plant (FWP). Subsequently, all fruits were opened with the aid of the GDC 150 circular saw (Robert Bosch GmbH, Gerlingen-Schillerhöhe, Germany) in order to obtain the following variables: nut weight per plant (NWP), average fruit weight (FW), average nut weight (NW), and average nut weight per fruit (NWF). For this, hook and precision scales were used, considering the wet weight of the fruits and nuts. The nuts were evaluated with a shell.

The data were collected from all fruits produced by each genotype/harvest and counted, weighed, and evaluated. The genetic-statistical analyses were performed using the restricted maximum likelihood and best linear unbiased prediction (REML/BLUP) method, based on the basic model of repeatability (model 63), without an experimental design, of the Selegen software (Resende, 2007), described as follows: $y = X_m + W_p + e$, where y is the data vector; m is the vector of measurement effects (crop), assumed to be fixed, added to the general average; p is the vector of the permanent effects of plants (genotypic effects + permanent environment effects), assumed to be random; and e is the vector of errors or residues. The uppercase letters represent the incidence and the grids of these effects.

Using the abovementioned model, the deviance analysis of the data was carried out and the following variance and phenotypic parameters were estimated: permanent phenotypic variance between plants, i.e., genotypic + permanent environment variance from one crop to another; temporary environmental variance; individual phenotypic variance; individual repeatability coefficient; repeatability coefficient of the average of m evaluation crops; accuracy of selection, based on the average of m evaluation crops; and general average.

All genetic-statistical analyses were performed using the Selegen-REML/BLUP software (Resende, 2007). Pearson's correlation coefficients between the evaluated traits, considering the permanent phenotypic values, were estimated by the Genes statistical software (Cruz, 2016).

Results and Discussion

The fruit and nut yields obtained per plant were low (Table 1). This could be due to the young age of the plants since, although the experimental period was of ten years, the evaluation only started 16 years after planting, an age when Amazon nut possibly had not yet reached production stability. Furthermore, the evaluated plants came from seeds without any previous genetic selection, differing from those obtained by mass selection carried out in natural populations, such as the clones grown in the state of Amazonas, which presented a higher fruit and nut production of 41 fruits, 22.18 kg of fruits per plant and 6.27 kg of nuts per plant, respectively (Passos et al., 2018). In natural populations in the states of Roraima, Acre, and Mato Grosso, the reported averages were 22 to 111 fruits per plant and 3.55 to 13.76 kg of nuts per plant according to Kainer et al. (2007), Tonini et al. (2008, 2020), and Pedrozo et al. (2015).

The results of the deviance analysis showed that the permanent phenotypic variance between trees was significant, at 1% probability, for all studied variables (Table 1), indicating the presence of a variability that can be exploited in breeding for the selection of the best genotypes. However, it is important to emphasize that, in this case, it is impossible to isolate the variance of the permanent environment from the genetic variance of the considered population.

The estimates for the permanent phenotypic variance among plants for all studied variables were lower than those obtained for the temporary environmental variance component, which corresponds to the temporary variation associated with the occasional environmental effects manifested in each environment, such as climatic fluctuations between the different years and their interactions with the effects verified on the plant (Viana & Resende, 2014). This shows that there was a significant influence of the temporary environment on the phenotypic expression of the evaluated variables, implying difficulties in the selection of superior genotypes through selection methods based only on the plant phenotype. These findings are in alignment with those obtained by Pedrozo et al. (2015), when estimating the components of temporary environmental variance and permanent phenotypic variance for the variables number of fruits per plant and nut weight per plant in the state of Roraima. Similar results were reported by Tonini & Pedrozo (2014), in the same state, where climatic variations had a more significant effect on the pattern of nut production than local factors, such as soil and relief. In both studies, permanent plots delimited the populations of natural Amazon nut trees. In the state of Acre, Azevedo et al. (2020) evaluated native Amazon nut trees and obtained different results for number of fruits per plant, observing a less significant effect of the temporary environment, which is an indicative that, even when they belong to the same species, different populations may present a different behavior in relation to the environmental effect.

Since the methodology of mixed models (REML/BLUP) allows of the analysis of data collected throughout harvests, the temporal repeatability coefficient of the traits was estimated using repeated measures (Resende, 2009). This is an important parameter to evaluate the possibility of reducing the time and effort required to select genetically superior individuals with the accuracy desired by the breeder (Cruz et al., 2012). According to the classification presented by Resende (2009), the estimates obtained in the present study for the individual repeatability coefficient showed moderate magnitudes, indicating a moderate regularity in the repetition of variables from one crop to the other. These results are a consequence of a moderate genetic control over the studied variables, pointing to the need to consider breeding methods with a greater genetic control, as well as to evaluate the possibility of applying indirect selection to other variables that are highly correlated and that present a greater regularity of repetition (Pedrozo et al., 2015).

According to Pereira et al. (2002), low repeatability values can occur if the studied genotypes still need to be stabilized for the variables of interest, a fact that cannot be ruled out for the population under study in the present work, considering that the trees began to be evaluated at 16 years of age. The r-values obtained for number of fruits per plant and nut weight per plant are close to those of 0.3145 and 0.3269 and of 0.2957 and 0.3436, respectively, found for Amazon nut trees

Table 1. Variance components and phenotypic parameter estimates of the variables number of fruits per plant (NFP), fruit weight per plant (FWP), nut weight per plant (NWP), average fruit weight (FW), average nut weight (NW), and average nut weight per fruit (NWF) obtained for 40 genotypes of Amazon nut (*Bertholletia excelsa*) trees grown in an agroforestry system in the state of Roraima, Brazil[(1)].

Component	NFP	FWP (kg)	NWP (kg)	FW (g)	NW (g)	NWF (g)
Vfp	711.3091**	296.5284**	12.8261**	8221.746**	1.6534**	621.1207**
Vet	1108.762	586.3464	25.3988	15277.78	3.3485	993.8725
Vf	1820.071	882.8749	38.2249	23499.52	5.0019	1614.9931
r	0.3908±0.07291[(2)]	0.3359±0.0820	0.3355±0.0819	0.3499±0.0932	0.3305±0.0905	0.3846±0.0976
rm	0.8651	0.8350	0.8347	0.8433	0.8316	0.8621
Acm	0.9301	0.9138	0.9136	0.9183	0.9119	0.9285
m	26.14	17.7	3.64	640.9511	-8.2455	134.982

[(1)]Vfp, permanent phenotypic variance between plants; Vet, temporary environmental variance; Vf, individual phenotypic variance; r, individual repeatability; rm, repeatability of the average of m evaluation years; Acm, accuracy of selection based on the average of m evaluation years; and m, overall average. [(2)]Standard deviation of individual repeatability. **Significant by the χ^2 test, with 1 degree of freedom, at 1% probability of error.

evaluated in permanent plots implanted in two natural populations in the state of Roraima (Pedrozo et al., 2015). These results infer a similar pattern for the behavior of the repeatability parameter in this state, regardless of whether the genotypes are from natural or cultivated populations.

The r estimate obtained for number of fruits per plant was lower than those of 0.5760 and 0.6209 found for two natural populations in the state of Acre (Azevedo et al., 2020). Moreover, the estimates for number of fruits per plant and nut weight per plant were lower than those of 0.50 and 0.49, respectively, obtained in a natural population in the state of Mato Grosso (Baldoni et al., 2017). These repeatability differences between states are expected since this parameter varies with the nature of the variable, the genetic properties of the population, and the environmental conditions under which the genotypes are maintained (Cruz et al., 2004).

The selective accuracy based on the average of the evaluated crops ranged from 0.9119 to 0.9301 for average nut weight and number of fruits per plant, respectively, which reflects excellent precision in genotype evaluation and selection (Viana & Resende, 2014). These high selective accuracy estimates are the result of the high repeatability estimates of the average of the ten study years, since the accuracy values correspond to the square root of the values obtained for the repeatability parameter. These results are similar to those reported by Azevedo et al. (2020) and higher than those found by Pedrozo et al. (2015) when studying Amazon nut trees from natural populations. Determining selective accuracy is important since it is a parameter associated with the accuracy of genotype selection, referring to the correlation between predicted and valid genotypic values, which depends on the magnitude of heritability and repeatability, the quality and quantity of information, and the methodology used to predict genotypic values (Resende & Duarte, 2007; Resende, 2009).

In perennial species, selection for traits related to fruit production is a time-consuming and costly process because field experiments require large areas and are long lasting (Azevedo et al., 2020). For Amazon nut trees, regardless of the propagation method, the time required to reach production stability is even longer (Pedrozo et al., 2023). Therefore, the success of Amazon nut improvement requires strategies that reduce selection cycles and, consequently, the time for the development and availability of cultivars.

According to the obtained results, the number of harvests that must be evaluated to reach a coefficient of determination of 80%, considered the minimum adequate value to compose a breeding population (Resende, 2009; Viana & Resende 2014), is: seven harvests for average nut weight per fruit and number of fruits per plant; eight harvests for fruit weight per plant, nut weight per plant, and average fruit weight; and nine harvests for average nut weight (Table 2). The values obtained for number of fruits per plant and nut weight per plant are similar to those found in two natural populations in Roraima (Pedrozo et al., 2015), but higher than those obtained in natural Amazon nut trees in the states of Acre and Mato Grosso, where the evaluation of only three to four harvests was necessary for the selection of more productive Amazon nut trees (Baldoni et al., 2017; Azevedo et al., 2020). The greater number of crops required in the present work is in alignment with the results of other studies carried out with different perennial fruit species, such as mango (*Mangifera indica* L.) (Maia et al., 2017; Costa et al., 2023), mangaba (*Hancornia speciosa* Gomes) (Pinheiro et al., 2019), and graviola (*Annona muricata* L.) (Sanchéz et al., 2017).

Although production is one of the most important variables for the selection of genotypes in fruit species, other variables related to production can also be studied to evaluate the possibility of obtaining indirect gains through the selection of variables that are easier to measure and that show a lower probability of error (Costa et al., 2023). All the permanent phenotypic correlation coefficients were significate at 5 or 1% probability. In addition, positive and elevated correlation coefficients (>0.77) were obtained for number of fruits per plant x nut weight per plant and for average fruit weight x nut weight per plant (Table 3). Positive and moderate correlations from 0.5032 to 0.6708 were found for fruit weight per plant x nut weight per plant, fruit weight per plant x number of fruits per plant, number of fruits per plant x average fruit weight, and average nut weight x nut weight per plant. Average nut weight per fruit showed negative and moderate correlations with all other traits, meaning that the selection of genotypes with a greater nut weight per fruit will result in lower nut yields and smaller nuts.

Pedrozo et al. (2015) also obtained high permanent phenotypic correlations between number of fruits per plant and nut weight per plant when studying native Amazon nut trees, reinforcing the possibility of selecting genotypes for nut production through indirect selection for number of fruits per plant. From a practical point of view, the latter variable is much less laborious to determine then fruit weight per plant and nut weight per plant, both of which require weighing the fruits and nuts and, in the case of measuring seed weight per plant, opening the fruits (Teixeira et al., 2015). Another possibility is selecting genotypes with a higher nut weight per plant through indirect selection to obtain a higher fruit weight per plant.

Table 2. Genetic parameters related to the repeatability of the variables number of fruits per plant (NFP), fruit weight per plant (FWP), nut weight per plant (NWP), average fruit weight (FW), average nut weight (NW), and average nut weight per fruit (NWF) obtained for 40 genotypes of Amazon nut (*Bertholletia excelsa*) trees grown in an agroforestry system in the state of Roraima, Brazil(1).

	NFP			FWP (kg)			NWP (g)		
m	R^2	Acm	Ef	R^2	Acm	Ef	R^2	Acm	Ef
1	0.3908	0.6252	1.0000	0.3359	0.5795	1.0000	0.3355	0.5793	1.0000
2	0.5620	0.7497	1.1992	0.5028	0.7091	1.2236	0.5025	0.7089	1.2237
3	0.6581	0.8112	1.2976	0.6027	0.7776	1.3396	0.6024	0.7761	1.3399
4	0.7196	0.8483	1.3569	0.6692	0.8180	1.4116	0.6689	0.8178	1.4119
5	0.7623	0.8731	1.3967	0.7166	0.8465	1.4607	0.7163	0.8464	1.4611
6	0.7938	0.8909	1.4252	0.7521	0.8673	1.4965	0.7519	0.8671	1.4969
7	0.8179	0.9044	1.4466	0.7797	0.8830	1.5237	0.7795	0.8829	1.5242
8	0.8369	0.9148	1.4634	0.8019	0.8950	1.5451	0.8016	0.8953	1.5456
9	0.8524	0.9232	1.4768	0.8199	0.9055	1.5624	0.8197	0.9053	1.5629
10	0.8651	0.9301	1.4879	0.8349	0.9137	1.5766	0.8347	0.9136	1.5772
	FW (g)			NW (g)			NWF (g)		
m	R^2	Acm	Ef	R^2	Acm	Ef	R^2	Acm	Ef
1	0.3499	0.5915	1.0000	0.3306	0.5749	1.0000	0.38460	0.62016	1.00000
2	0.5184	0.7200	1.2172	0.4969	0.7049	1.2260	0.55554	0.74534	1.20190
3	0.6175	0.7858	1.3285	0.5970	0.7726	1.3439	0.65216	0.80756	1.30220
4	0.6828	0.8263	1.3970	0.6639	0.8148	1.4172	0.71427	0.84515	1.36280
5	0.7291	0.8538	1.4435	0.7117	0.8436	1.4674	0.75756	0.87038	1.40350
6	0.7635	0.8738	1.4773	0.7476	0.8647	1.5039	0.78946	0.88852	1.43270
7	0.7902	0.8888	1.5029	0.7756	0.8807	1.5318	0.81394	0.90219	1.45480
8	0.8115	0.9008	1.5230	0.7980	0.8933	1.5537	0.83332	0.91287	1.47200
9	0.8289	0.9104	1.5392	0.8163	0.9035	1.5715	0.84905	0.92144	1.48580
10	0.8433	0.9183	1.5525	0.8316	0.9119	1.5861	0.86206	0.92847	1.49720

(1)R^2, coefficient of determination; Acm, accuracy of permanent phenotypic values based on m years of evaluation; and Ef, efficiency of m evaluations, compared to the situation in which only one evaluation was performed.

Table 3. Pearson's correlations of permanent phenotypic values between the variables number of fruits per plant (NFP), fruit weight per plant (FWP), nut weight per plant (NWP), average fruit weight (FW), average nut weight (NW), and average nut weight per fruit (NWF) obtained for 40 genotypes of Amazon nut (*Bertholletia excelsa*) trees grown in an agroforestry system in the state of Roraima, Brazil.

	FWP (kg)	NWP (kg)	NFP	FW (g)	NWF (g)
NWP	0.6170**	-		-	-
NFP	0.6125**	0.7752**		-	-
FW	0.3633**	0.7734**	0.6708**	-	-
NWF	-0.4230**	-0.6867**	-0.5747**	-0.5634**	-
NW	0.2657*	0.5032**	0.4343**	0.4346**	-0.3996**

** and *Significant by the t-test, at 1 and 5% probability, respectively.

Conclusions

1. The variables related to fruit and nut production of Amazon nut (*Bertholletia excelsa*) in an agroforestry system in the state of Roraima, Brazil, show moderate repeatability between different harvests.

2. Depending on the variable, seven to nine evaluation crops are required for the selection of Amazon nut genotypes with a coefficient of determination of 80%.

3. To select Amazon nut genotypes for nut production, a possibility is using indirect selection for number of fruits per plant, average fruit weight, or average nut weight.

4. Genotypes with a higher nut weight per plant also show a higher fruit weight per plant, which is another option for indirect selection of Amazon nut trees with a higher nut production.

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