ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392201602623

Repeatability of biometric and fruit and seed yield traits of sacha inchi

Mágno Sávio Ferreira VALENTE[1*], Maria Teresa Gomes LOPES[1], Francisco Célio Maia CHAVES[2], Ariane Mendes OLIVEIRA[3], Diego Rodrigo Bilby de FREITAS[4]

[1] Universidade Federal do Amazonas, Departamento de produção Animal e Vegetal, Avenida General Rodrigo Octávio, 6200, CEP: 69077-000. Manaus, AM, Brasil.

[2] Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, Embrapa Amazônia Ocidental, Rodovia AM-10, Km 29, CEP: 69010-970. Manaus, AM, Brasil.

[3] Universidade Federal do Amazonas, Departamento de Engenharia Florestal, Avenida General Rodrigo Octávio, 6200, CEP: 69077-000. Manaus, AM, Brasil.

[4] Universidade Nilton Lins, Departamento de Ciências Biológicas e Saúde, Avenida Professor Nilton Lins, 3259. CEP: 69058-030. Manaus, AM, Brasil.

* Corresponding author: magnosavio@yahoo.com.br

ABSTRACT

Repeatability allows an estimation of the number of evaluations needed to optimize the selection of superior genotypes, with consequent effects on the research costs in terms of financial and human resources. The objective of this study was to estimate the coefficient of repeatability of biometric and yield traits, related to fruits and seeds of sacha inchi (*Plukenetia volubilis*), and to define the number of evaluations required for an efficient selection and evaluation of genotypes of the species. A total of 37 non-domesticated accessions were evaluated for 19 months in a randomized block design with 5 replications and 2 plants per plot. The total number of fruits, total number of seeds, total fruit weight, mean fruit weight, and number of seeds per fruit of the accessions were evaluated by monthly sampling. Additionally, seed biometry was assessed in a sample of 30 seeds per accession. Repeatability coefficients were estimated by analysis of variance, principal components and structural analysis. The principal component method based on the covariance matrix was the most appropriate for establishing repeatability estimates of sacha inchi, due to the cyclical nature of the crop. Superior genotypes of the species can be selected for yield-related traits with about 90% accuracy, from 5 harvests (months) onwards. To ensure this accuracy level, it would be necessary to evaluate a minimum of 5 and 25 fruits to determine mean fruit weight and number of seeds per fruit, respectively, and 39 seeds would be required to evaluate the biometric traits.

KEYWORDS: breeding, accuracy, selection efficiency, *Plukenetia volubilis* L.

Repetibilidade de caracteres biométricos e de produção em frutos e sementes de sacha inchi

RESUMO

A repetibilidade permite estimar o número de avaliações para selecionar genótipos superiores com maior eficiência, o que tem reflexo direto sobre os gastos com recursos humanos e financeiros da pesquisa. O objetivo deste trabalho foi estimar o coeficiente de repetibilidade de características biométricas e de produção relacionadas aos frutos e sementes de sacha inchi (*Plukenetia volubilis*) e definir o número de avaliações necessárias para um eficiente processo de seleção e avaliação de genótipos da espécie. Um total de 37 acessos não domesticados foram avaliados em blocos casualizados com 5 repetições e 2 plantas por parcela durante 19 meses. Colheitas mensais avaliaram o número total de frutos, número total de sementes, peso total de frutos, peso médio de frutos e número de sementes por fruto dos acessos. Adicionalmente, foi realizada a biometria das sementes através de uma amostra de 30 sementes de cada acesso. Os coeficientes de repetibilidade foram estimados por meio dos métodos da análise de variância, componentes principais e análise estrutural. O método de componentes principais com uso da matriz de covariância se mostrou o mais indicado para obtenção de estimativas de repetibilidade em sacha inchi devido ao comportamento cíclico da cultura. Genótipos superiores da espécie podem ser selecionados, com acurácia de 90%, a partir de 5 colheitas (meses) para os caracteres de produtividade. Para mesma acurácia, seria necessária a avaliação de no mínimo 5 e 25 frutos para determinação do peso médio de frutos e número de sementes por fruto, respectivamente, e de 39 sementes para avaliação dos caracteres biométricos.

PALAVRAS-CHAVE: melhoramento genético, acurácia, eficiência de seleção, *Plukenetia volubilis* L.

INTRODUCTION

The Amazon is the world's most important center of biodiversity. However, many Amazonian plant species with promising economic value are still little known, underexploited and overlooked by science. One example is sacha inchi (*Plukenetia volubilis* L.), a semi-woody vine of the family Euphorbiaceae (Krivankova *et al.* 2007).

Sacha inchi is a new and promising crop, native to the Amazon region. The seeds contain about 54% oil and 27% amino acid- rich protein. The oil contains high levels of unsaturated fatty acids, which are essential fatty acids because they are not synthesized by the human body (Follegatti-Romero *et al.* 2009). The intake of these fatty acids is important for the prevention of cardiovascular and neuromuscular diseases and also has a hypocholesterolemic effect when used as food supplement (Ramaprasad *et al.* 2006).

While the above properties of sacha inchi indicate it as an excellent food supplement, the species also has desirable characteristics for reforestation and slope protection and is an interesting option for family agriculture programs (Bordignon *et al.* 2012). Although the composition and properties of sacha inchi are relatively well-known and its cultivation has already expanded to the regions of Alto Solimões and Manaus in the Brazilian Amazon, as well as to São Paulo State in southeastern Brazil (Rodrigues *et al.* 2014), the available literature provides little information to facilitate the identification of superior genotypes.

For commercial cultivation of sacha inchi, genotypes must be selected with desirable and phenotypically stable agronomic characteristics. When a genotype is selected for commercial cultivation, it is expected to maintain its superior or above-average initial performance throughout its productive life. This expectation can be confirmed by estimating the repeatability coefficient of relevant traits for agro-industry (Lira Júnior *et al.* 2014).

Based on the repeatability coefficient of a trait, it is possible to establish the number of phenotypic observations of each plant necessary to ensure an efficient discrimination or phenotypic selection of genotypes, requiring less time, cost, and labor (Cruz *et al.* 2012). Repeatability coefficient estimates have been used in studies of traits of several perennial species such as guarana (Nascimento Filho *et al.* 2009), oil palm and American oil palm (Chia *et al.* 2009), Surinam cherry (Danner *et al.* 2010), peach palm (Bergo *et al.* 2013), and sweet orange (Negreiros *et al.* 2014), providing a basis to determine adequate numbers and periods of genotype evaluation to improve the efficiency of breeding programs. For the species under study, there are no research reports of this nature.

Consequently, the objective of this study was to estimate the repeatability coefficient of biometric characters and of fruit and seed-yield-related traits of sacha inchi, and to define the number of evaluations required for an efficient selection and evaluation of genotypes of the species.

MATERIALS AND METHODS

The study was carried out with plant material from a sacha inchi (*in vivo* conservation) germplasm bank maintained in the medicinal plant sector of Embrapa Amazônia Ocidental - CPAA, in Manaus, state of Amazonas, northern Brazil. The sacha inchi germplasm bank of CPAA currently contains 37 non-domesticated accessions, of which 25 were collected in the interior of the State of Amazonas and 12 on the rural property "Nova Jerusalém" in Careiro Castanho (3º31'45.0" S and 59º49'07.9" W).

Seed samples from the 37 accessions were sown in 1 L plastic bags filled with sifted earth. After seedling formation in January 2013, the plantlets were transplanted to the field, at 3 x 3 m spacing. The soil was classified as Oxisol and 2.0 t ha^{-1} lime was applied for acidity correction. The crop was grown on a vertical trellis structure, and treated daily with all cultural practices required for the development, as recommended by Céspedes (2006), and irrigated by drip irrigation.

The experiment was evaluated in a randomized block design with five replications and two plants per plot. The plot mean was used for all calculations. The fruits were collected approximately every four weeks. At each monthly harvest, the following yield traits were measured: total number of fruits (fruit $plant^{-1}$); total number of seeds (seed $plant^{-1}$); total fruit weight (g $plant^{-1}$); mean fruit weight (g $fruit^{-1}$), as the ratio between total fruit weight and total number of fruits; and number of seeds per fruit (seed $fruit^{-1}$), as the ratio between the total number of seeds and total number of fruits. The experimental plantation was monitored for 19 consecutive months (from June 2014 to December 2015) after the beginning of the fruiting period and stabilization of production.

In addition, the seed biometry was analyzed in a sample of 30 seeds of each accession. Seed length, width, and thickness were measured with a digital caliper (0.01mm), and seed fresh weight was determined with an analytical balance (0.0001 g).

To evaluate the genetic variability of the genotypes, all studied traits were analyzed by ANOVA. The following statistical model was used for the yield-related traits:

$$Y_{ijk} = \mu + g_i + a_j + ga_{ij} + b_{(i)j} + \varepsilon_{ijk}$$

where, Y_{ijk} is the observation of the i^{th} genotype in the j^{th} environment and the k^{th} replication; μ is the general mean; g_i the effect of the i^{th} genotype under the influence of the permanent environment (i = 1, 2,..., p); a_j is the effect of the j^{th} environment (j = 1, 2,..., n); ga_{ij} the effect of the i^{th} genotype interaction in the j^{th} environment; $b_{(i)j}$ the effect of the k^{th}

block (K = 1,2, ..., k) within the j[th] environment; and ε_{ijk} the experimental error. In this study, each of the 19 harvest months was considered as a different environment in the model, and the genotype-environment (GE)interaction refers to the effects of different harvest months on the agronomic performance of the 37 genotypes.

For the analysis of seed biometric traits, the following statistical model was adopted:

$$Y_{ij} = \mu + g_i + \varepsilon_{ij}$$

The repeatability coefficients (*r*) were estimated by the methods analysis of variance (ANOVA), principal components based on correlation matrices (PCcor) and on phenotypic variances and covariances (PCcov), and by the structural analysis method, based on the intraclass correlation matrix (SAcor).

For the estimation of repeatability in yield traits, the method considered a reduced model of the general model of analysis of variance, as proposed by Cruz *et al.* (2012):

$$Z_{ij} = \mu + g_i + a_j + \delta_{ij}$$

where

$$Z_{ij} = 1/k \sum_{k=1}^{n} Y_{ijk}$$

where *k* is the number of replications; μ the general mean, g_i the effect of the i[th] genotype associated with the permanent environmental influences; a_j the effect of the j[th] measurement and $\delta_{ij} = ga_{ij} + \varepsilon_{ijk}$, where ga_{ij} is the effect of the genotype-environment interaction and ε_{ijk} the experimental error.

The repeatability for yield traits was given by:

$$\hat{r} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_\delta^2} = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_{ga}^2 + (\sigma^2/k)}$$

where the genotypic variance ($\hat{\sigma}_g^2$) is obtained from the reduced model and $\hat{\sigma}_{ga}^2 + (\sigma^2/k)$from the full analysis model.

For the biometric traits of seeds, the repeatability coefficient was calculated by:

$$\hat{r} = \frac{\hat{\sigma}_g^2}{\sigma^2 + \hat{\sigma}_g^2}$$

where $\hat{\sigma}_g^2$ is the genotypic variance and σ^2 the residual variance.

Periodicity is a factor that cannot be isolated by analysis of variance. In this case, the repeatability coefficient can be more efficiently estimated by principal component analysis, which is better suited for cases in which the studied traits of the genotypes have a cyclical behavior. This method allows an estimation of the repeatability coefficient in a two-fold approach: first by the correlation matrix (PCcor) and second by the phenotypic variance and covariance matrix (PCcov) (Abeywardena 1972).

By the PCcor method, the estimator of the repeatability coefficient ($\hat{r}$) was used as proposed by Rutledge (1974), given by:

$$\hat{r} = \frac{\hat{\lambda}_1 - 1}{n - 1}$$

where $\hat{\lambda}_1$ is the eigenvalue of the parametric correlation matrix between genotypes in each measurement pair (*n*)

For the method PCcov, the estimator of the repeatability coefficient was computed by:

$$\hat{r} = \frac{\hat{\lambda}_1 - \hat{\sigma}_Y^2}{\hat{\sigma}_Y^2\ (n - 1)}$$

where $\hat{\lambda}_1$ is the eigenvalue obtained by the matrix of phenotypic variances and covariances, and where $\hat{\sigma}_Y^2$ is the phenotypic variance.

The method of structural analysis to estimate the repeatability coefficient was proposed by Mansour *et al.* (1981), with some conceptual differences in relation to methods based on principal components. In this method *R* is the parametric correlation matrix between genotypes in each pair of evaluation and $\hat{R}$ its estimator. An estimator of the repeatability coefficient based on structural analysis is expressed by:

$$\hat{r} = \frac{\alpha' \hat{R} \alpha - 1}{n - 1}$$

where α' is the eigenvector with parametric elements associated with the highest eigenvalue of the uniform correlation.

The number of measurements (*n*) required to obtain efficiency in the selection process, based on pre-determined determination coefficients - R^2 (80, 85, 90, 95 and 99%), was calculated as described by Cruz *et al.* (2012), by the following expression:

$$n = \frac{R^2(1-r)}{(1-R^2)r}$$

where r is the repeatability coefficient, obtained according to one of the different methods used.

All statistical analyses were performed with software GENES (Cruz 2013).

RESULTS

Significant differences were found by the F test at 1% probability for all characters (Table 1), indicating variability among genotypes. However, the successful selection of these genotypes depended on the degree of repeatability of the analyzed phenotypic traits.

The significant effect of the genotype-environment interaction indicated high heterogeneity in the genotype behavior across the different environments (harvests). The only exception was the number of seeds per fruit, with a non-significant effect for the GE interaction.

The sacha inchi fruit and seeds were harvested monthly, with an approximate mean of 7 fruits, 27 seeds and a weight of 24.3 g per harvested plant^{-1}, corresponding to a mean fruit weight of 3.36 g and a mean number of 3.82 seeds per fruit. High coefficients of variation were recorded for the total number of fruits (84.35%), total number of seeds (83.35%), and total fruit weight (91.76%), suggesting strong environmental influence. However, these high values of coefficient of variation were also accompanied by a high level of genetic variation, thereby reducing the environmental effect on the genotypes.

The ratios between the coefficients of genetic variation and of experimental variation were 0.34; 0.33; 0.35; 0.66, and 0.18 for total number of fruits (TNF), total number of seeds (TNS), total fruit weight (TFW), mean fruit weight (MFW), and number of seeds per fruit (NSF), respectively, indicating the possibility of higher gains with selection for mean fruit weight, lower gains for number of seeds per fruit and intermediate gains for the other yield traits. For the biometric seed traits, higher selection gains may be obtained for seed thickness, followed by length and mean weight. The estimates of the maximum heritability value were considered high (> 75%) for all traits, enabling the selection of superior plant material.

Estimates of the repeatability coefficient obtained for the traits based on different statistical procedures are listed in Table 2. The repeatability coefficients estimated by principal components based on covariance matrices (PCcov) showed higher values than those estimated by other methods, while by the method of analysis of variance, these estimates were

Table 1. Analysis of variance and estimates of genetic parameters with data of mean squares of genotypes (MSG), mean square of genotype-environment interaction (MSGxE), overall mean ($\bar{X}$) coefficient of genetic variation (CV), heritability (h^2) and the ratio between the coefficient of genetic variation and the coefficient of experimental variation (b) for nine fruit and seed traits evaluated in 37 genotypes of sacha inchi (*Plukenetia volubilis* L.).

Traits	MSG	MSGxE	$\bar{X}$	CV (%)	h^2 (%)	b
Total number of fruits (n)	1117.686*	173.463*	6.983	84.35	84.48	0.34
Total number of seeds(n)	15495.535*	2473.604*	26.683	83.35	84.04	0.33
Total fruit weight (g)	16314.018*	2488.017*	24.310	91.76	84.75	0.35
Mean fruit weight (g)	9.721*	0.310*	3.360	14.24	96.81	0.66
Number of seeds per fruit (n)	0.500*	0.112^{ns}	3.822	9.52	77.61	0.18
Mean seed weight (g)	0.161*	---	0.948	15.99	85.70	0.45
Seed length (mm)	15.076*	---	17.625	8.20	86.15	0.46
Seed width (mm)	2.417*	---	15.176	4.71	78.86	0.35
Seed thickness (mm)	6.319*	---	8.460	10.15	88.33	0.50

^{ns},*: non-significant and significant at 1% probability by the F test, respectively.

Table 2. Estimation of repeatability coefficients and their determination coefficients (in brackets) for nine fruit and seed traits evaluated in 37 genotypes of sacha inchi (*Plukenetia volubilis* L.), obtained by analysis of variance (ANOVA), principal component analysis based on the covariance matrix (PCcov) and the correlation matrix (PCcor), and structural analysis based on the theoretical eigenvalue of the correlation matrix (SAcor).

Traits	ANOVA	PCcov	PCcor	SAcor
Total number of fruits (n)	0.223 (84.48)	0.663 (97.40)	0.343 (90.86)	0.221 (84.37)
Total number of seeds(n)	0.217 (84.04)	0.653 (97.28)	0.350 (91.08)	0.218 (84.15)
Total fruit weight (g)	0.226 (84.75)	0.678 (97.56)	0.340 (90.72)	0.246 (86.08)
Mean fruit weight (g)	0.615 (96.81)	0.650 (97.24)	0.638 (97.09)	0.628 (96.97)
Number of seeds per fruit (n)	0.154 (77.61)	0.269 (87.51)	0.235 (85.39)	0.156 (77.87)
Mean seed weight (g)	0.167 (85.70)	0.186 (87.30)	0.190 (87.55)	0.175 (86.44)
Seed length (mm)	0.172 (86.15)	0.190 (87.53)	0.188 (87.42)	0.172 (86.13)
Seed width (mm)	0.111 (78.86)	0.240 (90.47)	0.176 (86.50)	0.130 (81.81)
Seed thickness (mm)	0.202 (88.33)	0.227 (89.79)	0.215 (89.14)	0.199 (88.14)

always lower or equal to those resulting from multivariate analysis. For most traits, the difference between the results of the different methods increased when the estimates of the repeatability coefficient were low (<0.3). In the analysis of all studied traits and methods, the repeatability estimates ranged from 0.111 to 0.678. However, it must be emphasized that the determination coefficients were mostly above 80%, regardless of the methodology used for their estimation.

For the discrimination of genotypes, at a minimal accuracy of 90% of the predicted plant value, the estimated number of harvests for total number of fruits, total number of seeds and total fruit weight varied from 5 (PCcov) to 33 (ANOVA) harvests or months of evaluation (Table 3). In this case, the estimates established by the method PCcov were more realistic, for taking the cyclical production pattern into consideration.

Table 3. Number of necessary evaluations associated with different coefficients of determination (R^2) for the total number of fruits (TNF), total number of seeds (TNS), total fruit weight (TFW), mean fruit weight (MFW), number of seeds per fruit (NSF), mean seed weight (MSW), seed length (SL), seed width (SW), and seed thickness (STh) evaluated in 37 genotypes of sacha inchi (*Plukenetia volubilis* L.), obtained by analysis of variance (ANOVA), principal component analysis based on the covariance matrix (PCcov) and the correlation matrix (PCcor), and structural analysis based on the theoretical eigenvalue of the correlation matrix (SAcor).

R^2	Traits								
	TNF	TNS	TFW	MFW	NSF	MSW	SL	SW	STh
					ANOVA				
0.80	14	15	14	3	22	20	19	32	16
0.85	20	21	20	4	32	28	27	46	22
0.90	32	33	31	6	50	45	43	72	36
0.95	67	69	65	12	105	95	92	153	75
0.99	346	358	339	62	543	496	477	796	392
					PCcov				
0.80	3	3	2	3	11	17	17	13	14
0.85	3	4	3	4	16	25	24	18	19
0.90	5	5	5	5	25	39	38	28	31
0.95	10	11	10	11	52	83	81	60	65
0.99	51	53	47	54	269	432	423	313	338
					PCcor				
0.80	8	8	8	3	13	17	17	19	15
0.85	11	11	12	4	19	24	24	27	21
0.90	18	17	18	6	30	38	39	42	33
0.95	37	36	37	11	62	81	82	89	69
0.99	190	185	193	57	322	422	427	463	362
					SAcor				
0.80	15	15	13	3	22	19	19	27	16
0.85	20	21	18	4	31	27	27	38	23
0.90	32	33	28	6	49	42	43	60	36
0.95	67	69	59	12	103	89	92	127	77
0.99	349	355	305	59	535	466	478	660	400

These estimates indicated that the evaluation of yield traits in 37 accessions of sacha inchi genotypes during five consecutive months ensured satisfactory accuracy of selection. Similarly, the evaluation of at least 39 seeds (value established by the PCcov method for mean seed weight) ensured an accuracy of 90% in a combined evaluation of all biometric seed traits of the species.

The minimum number of fruits needed for a reliable selection (with about 90% accuracy), based on mean weight, varied from 5 to 6 fruits among the estimation methods. For the number of seeds per fruit, the required number of evaluated fruits was higher, ranging from 25 (PCcov) to 50 (ANOVA) fruits.

DISCUSSION

The significant effect of the genotype-environment interaction was due to the occurrence of periods considered favorable and unfavorable for the crop, normally related to rainy periods and dry spells, along with the irregular genotype performance over the months. High coefficients of variation for yield-related traits were expected, since, in our preliminary tests in Manaus, the reproductive stage of sacha inchi lasted on average 86 days, while, according to Céspedes (2006), the plant production does not end after the first harvest, but a next harvest must occur within three to four weeks. Thus, although only ripe fruit is collected in the monthly harvests, various fruits in different reproductive stages are observed, contributing to the observed differences in traits among months of collection.

In addition, environmental conditions are not stable throughout the year in the study area, and although the genotypes were treated daily with the same care in terms of cultural practices (irrigation, fertilization and staking), the high temperatures in certain periods of the year had a strong influence on yield-related traits. Although sacha inchi is native to the Amazon, where temperatures are typically high, its characters may be strongly affected by heat (Cardoso *et al.* 2015).

The permanent environmental variance is a source of error that reduces the precision in genetic studies. Consequently, breeders always try to keep it as low as possible by a careful management, thus enabling the repeatability coefficient to approach the heritability estimate (Shimoya *et al.* 2002). High heritability values, as found in this study, allow significant gains in all traits. Knowledge of the maximum heritability in the case of perennial and semi-evergreen crops, as is the case of sacha inchi, is fundamental, because the selection of promising genotypes depends on long experiments of retreated trait measurements on a same plant (Oliveira *et al*. 2011).

As in our analysis, other studies that compared repeatability estimates of variables of perennial plants by different analysis methods showed that the estimates by ANOVA were always lower than those of other methods, e.g. yellow mombin (Silva *et al.* 2015), macaw palm (Manfio *et al.* 2011), Surinam cherry (Danner *et al.* 2010), American oil palm, oil palm (Chia *et al.* 2009), and bacuri fruit (Silva *et al.* 2009). The reason is that the origin of the genotypic variance used to estimate the repeatability by ANOVA is not only genetic. In this case, the permanent environmental variance component among plants is mingled with the genotypic variance, so repeatability is underestimated (Danner *et al.* 2010). We obtained the highest repeatability estimates using principal component analysis with the covariance matrix. The principal component method allows an isolation of the alternating effect, unlike in ANOVA, so this component is included in the experimental error (Chia *et al.* 2009).

The differences among the results obtained by different methods increased as the repeatability coefficient was reduced, which was also observed in West Indian cherry (Lopes *et al.* 2001) and in biometric traits of macaw fruit (Manfio *et al.* (2011). Since the evaluation by principal component analysis is the best method for the evaluation of traits of genotypes that have a cyclical behavior, our results indicate that the repeatability coefficients for yield-related traits of sacha inchi are higher than 0.65, except for biometry and number of seeds per fruit.

Estimates of the repeatability coefficient are considered high when ≥ 0.6, medium when between <0.6 and ≥ 0.3 and low when <0.3 (Resende 2002). However, since we evaluated quantitative characteristics, and sacha inchi has a semi-perennial behavior, the magnitude of our estimates can be considered reasonable, even when below 0.3, due to the determination coefficients, which were mostly higher than 80%, regardless of the methodology used for their estimation. These higher values of determination coefficients indicate that the number of assessments used in this study was sufficient to predict the actual values of the genotypes and that their characteristics can be evaluated with good reliability. Another objective would be the reduction of the required number of evaluations, for the sake of cost and time-effectiveness.

For the yield-related traits, we estimated that superior genotypes of sacha inchi can be selected with a reliability of 90% from the evaluation of five monthly harvests onwards using the PCcov-based analysis. In an assessment of 18 half-sib progenies of jatropha (*Jatropha curcas*), a species of Euphorbiaceae that is also oleaginous, Teodoro *et al.* (2016) proposed that four measurements would be required to ensure an accurate evaluation (minimum 80%) of the analyzed traits, including productivity. These authors also indicated PCcov as the most accurate method to estimate the repeatability coefficient.

During the selection process of genotypes with a view to the release of cultivars or to the choice of parents for recombination, it is important to confirm the genetic superiority of some relevant traits. For sacha inchi the number of seeds is an essential trait, since the main commercial value of the species is in the oil extracted from the seeds. Sacha inchi fruits usually have four lobes, with one oilseed per lobe (Krivankova *et al.* 2007). In the 37 studied accessions, there was a variation of three to seven loci per fruit. However, this does not necessarily indicate the presence of seeds, since in many cases withered or empty locules were observed, thus increasing the number of evaluations needed to estimate this trait accurately.

The analysis of the biometric data of the seeds also showed great variability among the genotypes under study, however, the mean values of these characteristics were similar to those observed in other studies with the species (Cardoso *et al.* 2015). Biometric data of seeds are related to the traits of seed dispersal and seedling establishment, apart from being used to differentiate pioneer from non-pioneer species in tropical forests (Baskin and Baskin 1998). For this analysis, a recommended, predetermined number of seeds is normally used. With the objective of a combined assessment covering all commonly evaluated traits, our results indicated that the selection of superior genotypes of sacha inchi with an accuracy of 90% requires at least 39 seeds.

Sacha inchi is a non-domesticated species with a lengthy production cycle. Due to the high cost of collecting expeditions, knowing the number of necessary evaluations will also be useful in the planning of future expeditions (Manfio *et al.* 2011), to optimize the time and space required for exploration and transport of promising plant material to be incorporated in breeding programs.

CONCLUSIONS

Our results indicate that the principal component method based on the covariance matrix is the most suitable strategy to estimate repeatability of sacha inchi, due to the cyclical behavior of the crop. The evaluation of five monthly harvests for yield traits, and a minimum of five, 25 and 39 evaluations for mean fruit weight, number of seeds per fruit and biometric seed traits, respectively, allow the selection of superior genotypes at an accuracy of approximately 90%.

ACKNOWLEDEGEMENTS

The authors are indebted to the Brazilian Council for Scientific and Technological Development (CNPq) and the Research Foundation of the State of Amazonas (FAPEAM), for funding this study.

REFERENCES

Abeywardena, V. 1972. An application of principal component analysis in genetics. *Journal of Genetics,* 16: 27-51.

Baskin, C.C.; Baskin, J.M. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego, 666p.

Bergo, C.L.; Negreiros, J.R.S.; Miqueloni, D.P.; Lunz, A.M.P. 2013. Estimativas de repetibilidade de caracteres de produção em pupunheiras para palmito da raça putumayo. *Revista Brasileira de Fruticultura*, 35: 829-836.

Bordignon, S.R.; Ambrosano, G.M.B.; Rodrigues, P.H.V. 2012. Propagação in vitro de sacha inchi. *Ciência Rural*, 42: 1168-1172.

Cardoso, A.A.; Obolari, A.M.M; Borges, E.E.L.; Silva, C.J.; Rodrigues, H.S. 2015. Environmental factors on seed germination, seedling survival and initial growth of sacha inchi (*Plukenetia volubilis L.*). *Journal of Seed Science*, 37: 111-116.

Céspedes, I.E.I.M. 2006. *Situación y avances del cultivo de sacha inchi en el Peru. Cultivo de sacha inchi en Dirección de Investigacion Agraria.* INIA Subdireccion de Recursos Genéticos y Biotecnologia, Estación Experimental Agrária "El Povenir", Tarapoto, 11p.

Chia, G.S.; Lopes, R.; Cunha, R.D.; Rocha, R.D.; Lopes, M.T.G. 2009. Repetibilidade da produção de cachos de híbridos interespecíficos entre o caiaué e o dendezeiro. *Acta Amazonica*, 39: 249-254.

Cruz, C.D; Regazzi, A.D.; Carneiro, P.C.S. 2012. *Modelos Biométricos Aplicados ao Melhoramento Genético - Volume 1.* 4th ed. Editora UFV, Viçosa, 514p.

Cruz, C.D. 2013. GENES - A software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum*, 35: 271-276.

Danner, M.A.; Raseira, M.C.B.; Sasso, S.A.Z.; Citadin, I.; Scariot, S. 2010. Repetibilidade de caracteres de fruto em araçazeiro e pitangueira. *Ciência Rural*, 40: 2086-2091.

Follegatti-Romero, L.A.; Piantino, C.R.; Grimaldi, R.; Cabral, F.A. 2009. Supercritical CO_2 extraction of omega-3 rich oil from sacha inchi (*Plukenetia volubilis* L.) seeds. *The Journal of Supercritical Fluids*, 49: 323-329.

Krivankova, B.; Polesny, Z.; Lojka, B.; Lojkova, J.; Banout, J.; Preininger, D. 2007. Sacha Inchi (*Plukenetia volubilis*, Euphorbiaceae): A Promising Oilseed Crop from Peruvian Amazon. In Conference on International Agricultural Research and Development. University of Kassel, Germany. (http://www.tropentag.de/2007/abstracts/links/Krivankova_NnQmCSMU.pdf). Accessed on April 07, 2016.

Lira Júnior, J.S.; Bezerra, J.; Moura, R.; Santos, V.D. 2014. Repetibilidade da produção, número e peso de fruto em cirigueleira (*Spondias purpurea* L.). *Revista Brasileira de Fruticultura*, 36: 214-220.

Lopes, R.; Bruckner, C.H.; Cruz, C.D.; Lopes, M.T.G.; Freitas, G.D. 2001. Repetibilidade de características do fruto de aceroleira. *Pesquisa Agropecuária Brasileira*, 36: 507-513.

Manfio, C.E.; Motoike, S.A.; Dos Santos, C.E.M.; Pimentel, L.D.; De Queiroz, V.; Sato, A.Y. 2011. Repetibilidade em características biométricas do fruto de macaúba. *Ciência Rural*, 41: 70-76.

Mansour, H.; Nordheim, E.V.; Ruledge, J.J. 1981. Estimators of repeatability. *Theoretical and Applied Genetics*, 60: 151-156.

Nascimento Filho, F.J.; Atroch, A.L.; Cruz, C.D.; Carneiro, P.C.S. 2009. Repetibilidade da produção de sementes em clones de guaraná. *Pesquisa Agropecuária Brasileira*, 44: 605-612.

Negreiros, J.R.S.; Andrade Neto, R.C.; Miqueloni, D.P.; Lessa, L.S. 2014. Estimativa de repetibilidade para caracteres de qualidade de frutos de laranjeira-doce. *Pesquisa Agropecuária Brasileira*, 49: 40-48.

Oliveira, T.N.; Santos, M.V.F.; Lira, M.A., Mello, A.C.L.; Ferreira, R.L.C.; Júnior, L.; Silva, N.G.M. 2011. Estimativa de coeficientes de repetibilidade para mancha ocular em clones de Pennisetum. *Archivos de zootecnia*, *60*: 797-808.

Ramaprasad, T.R.; Bhaskaran, V.; Debnath, S.; Sambaiah, K.; Lokesh, B.R. 2006. Spray-dried Milk Supplement with α-Linolenic Acid and Eicoesapentaenoic Acid and Docosahexaenoic Acid Decreases HMG Co a Reductase Activity and Increases Biliary Secretion of Lipds en Rats. *Steroids*, 71: 409-415.

Resende, M.D.V. 2002. *Genética biométrica e estatística: no melhoramento de plantas perenes.* Embrapa Informação Tecnológica, Brasília, 975p.

Rodrigues, P.H.V.; Bordignon, S.R.; Ambrosano, G.M.B. 2014. Desempenho horticultural de plantas propagadas *in vitro* de sacha inchi. *Ciência Rural*, 44: 1050-1053.

Rutledge, J.J. 1974. A scaling which removes bias of Abeywardena's estimator of repeatability. *Journal Genetics*, 61: 247-250.

Shimoya, A.; Pereira, A.V.; Ferreira, R.P.; Cruz, C.D.; Carneiro, P.C.S. 2002. Repetibilidade de características forrageiras do capim-elefante. *Scientia Agricola*, 59: 227-234.

Silva, C.A., Detoni, J.L., Costa, P.R., Schmildt, O., Alexandre, R.S., Schmildt, E.R. 2015. Estimativa de repetibilidade em características de cajá-mirim no Norte do Espírito Santo. *Revista Agro@mbiente*, 9: 284-291.

Silva, R.G.; Chaves, M.C.L.; Arnhold, E.; Cruz, C.D. 2009. Repetibilidade e correlação fenotípicas de caracteres do fruto de bacuri no estado do Maranhão. *Acta Scientiarum Agronomy*, 31: 587-591.

Teodoro, P.E.; Costa, R.D.; Rocha, R.B.; Laviola, B.G. 2016. Número mínimo de medições para a avaliação acurada de características agronômicas de pinhão-manso. *Pesquisa Agropecuária Brasileira*, 51: 112-119.

Received: 14/09/2016
Accepted: 13/06/2017