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QUANTIFICATION OF THE OPTIMAL NUMBER OF REPLICATES FOR STUDIES OF SORGHUM AGRONOMIC TRAITS

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ABSTRACT – Agricultural experimentation is a crucial step in cultivar recommendation; however, it is necessary to determine how many repetitions are needed to achieve the ideal coefficient of determination for selecting genotypes with high precision and lower costs. This study aimed to estimate the repeatability coefficients and the number of evaluations needed to obtain a coefficient of determination equal to 85% for sorghum genotype variables. The trials were conducted in Balsas, Maranhão, during the second growing season of 2021 and 2022, arranged in a randomized complete block design with three replications and ten treatments: Sudan 4202, AGRI 001-E, AGRI 002-E, BRS 658, BRS 810, BRS Ponta Negra, BRS 373, BR 509, BR 506, and IPA 467-4-2. The following were evaluated: plant height, stem diameter, number of leaves, leaf area, flowering, total cycle, green matter production, dry matter production, relative chlorophyll index, and Brix. The repeatability coefficients (r) and minimum number of repetitions (η_0) were estimated using analysis of variance; principal component analysis of correlations and variances and covariances; and structural analysis of correlations and covariances, using the GENES program. High values were observed for the repeatability coefficient (r) and coefficient of determination (R^2), greater than 0.55 and 78.54%, respectively, with the total cycle variable standing out at 0.99% and 99.65%, respectively, demonstrating high precision in predicting the actual number of individuals from the three replications. Therefore, increasing the number of replications would not result in significant improvements in precision. Experimental trials of sorghum, for the studied cultivars, with three replications, can identify superior genotypes related to all variables studied

Keywords: Evaluations, repeatability coefficient, precision.

Sorghum is a cereal that belongs to the Poaceae family and originates from Africa (Santos et al., 2015). In recent years, it has gained increasing recognition in Brazil due to its versatility, being used for various purposes, such as silage production, green beef, pasture for animals and human consumption (Silva et al., 2021). In addition, it stands out for its remarkable characteristics, such as high photosynthetic efficiency and adaptation to conditions of high temperature and water scarcity, which distinguishes it from most other cereals (Durães et al., 2012).

Crops have been the subject of studies in different environments (Andrade et al., 2016; Bicalho et al. 2018; Carmo et al., 2020), with the objective of identifying genotypes adapted and stable to different cities and regions of the Brazilian Cerrado. However, for the recommendation of improved cultivars, it is important that field experiments have a high degree of experimental precision and, consequently, a high accuracy in inferring the genotypic means of the new materials (Resende and Duarte, 2007).

Thus, to reliably determine genotypic values, it is necessary to use techniques that determine the minimum number of evaluations so that precision goals in the selection process are achieved. To this end, breeders have at their disposal statistical methodologies, such as the estimation of the repeatability coefficient, to help them (Duarte et al., 2022). One of the great uses of the repeatability coefficient is to determine how many measurements are needed for each individual so that phenotypic selection among genotypes can be performed efficiently and with minimum cost and labor (Cruz et al., 2012).

The repeatability coefficient can be estimated using different methods, such as the analysis of variance method, the principal component method and the structural analysis method (Guilhen et al., 2019; Rodrigues et al., 2020).

It is wortwile to note the importance of knowing the repeatability coefficient and the number of replications necessary for the evaluation of the various agronomic traits of sorghum, According to Toebe et al.2022 will allow the performance of experiments with a reliable representation of the population, avoiding the waste of labor and financial resources.

Repeatability has been studied by several researchers on different topics, especially plot size and number of replicates (Magalhães et al., 2023; Palma et al., 2023; Toebe et al., 2022). However, it is important to note that such information still scarce for several sorghum crop traits. Thus, the present study sought to estimate the repeatability coefficients and the number of replications necessary to obtain a coefficient of determination equal to 85% for variables of sorghum genotypes.

Material and Methods

Study area

Two experiments were conducted at the agronomic research unit of the State University of Maranhão (UEMA), in the municipality of Balsas, MA, located in the southern region of the state of Maranhão, latitude 7° 31’ 59’’ S and longitude 46° 2’ 6’’ W) (Figure 1).

According to the Köppen and Geiger classification, Balsas has a tropical hot and humid climate (Aw) and an average temperature of 27.1 °C. The average annual rainfall is 1175 mm, and the heaviest rainfall occurs from November to April, when 85% of the total rainfall occurs during this

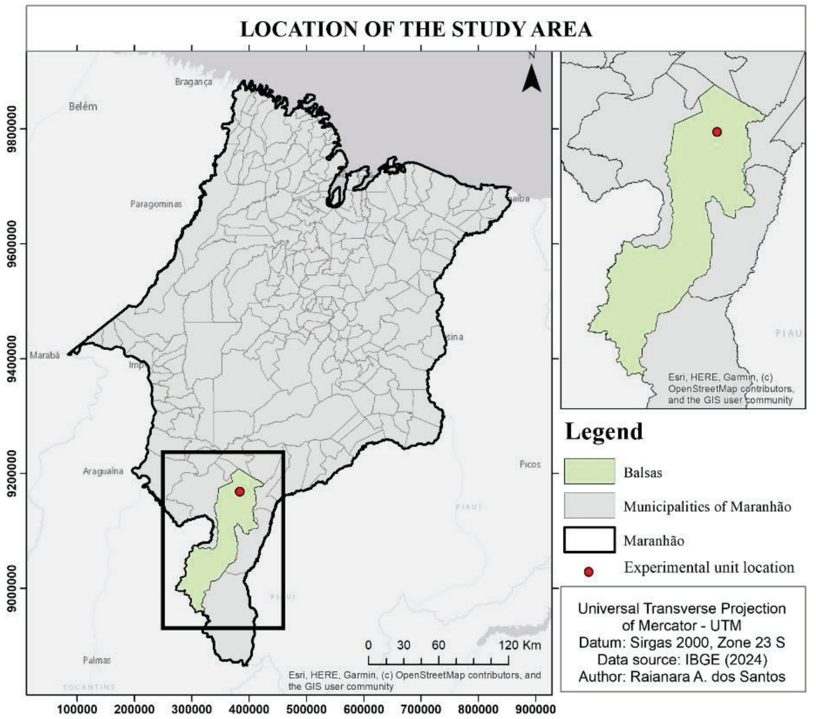


Figure 1 - Study area located in the municipality of Balsas, Maranhão

period (Passos et al., 2017). The meteorological data of temperature and precipitation during the months of experimentation can be observed in Figure 2.

Soil sampling was performed at a depth of 0 to 0.20 m, and the chemical characteristics are shown in Table 1. The results were used to recommend liming and fertilization according to Borém et al. 2014.

Experimental design

The first trial was implemented in the second crop of 2021, and whereas the second was trial implemented in the second crop of 2022; both trials were conducted in a randomized block design with three replicates and ten treatments, which corresponded to 10 sorghum cultivars (Table 2) with different agronomic characteristics.

Each experimental plot consisted of five rows 5 meters in length each, spaced 0.50 meters apart. The two lateral lines served as borders, and the 3 central lines were considered useful evaluation areas, disregarding 0.5 m at each end of these central lines.

Sowing was performed manually, and approximately eight to fifteen seeds were distributed per meter depending on the cultivar. Thinning occurred when the plants reached the stage of three fully developed leaves.

All cultural practices followed the technical recommendations for sorghum presented by (Borém et al.,2014).

The following variables were evaluated from four plants per plot:

Plant height (H) was measured from the soil surface to the panicle apex with the aid of a measuring tape expressed in meters. **The stem diameter (SD)** was measured with the aid of a digital caliper, expressed in millimeters. **The number of leaves (NL)** was

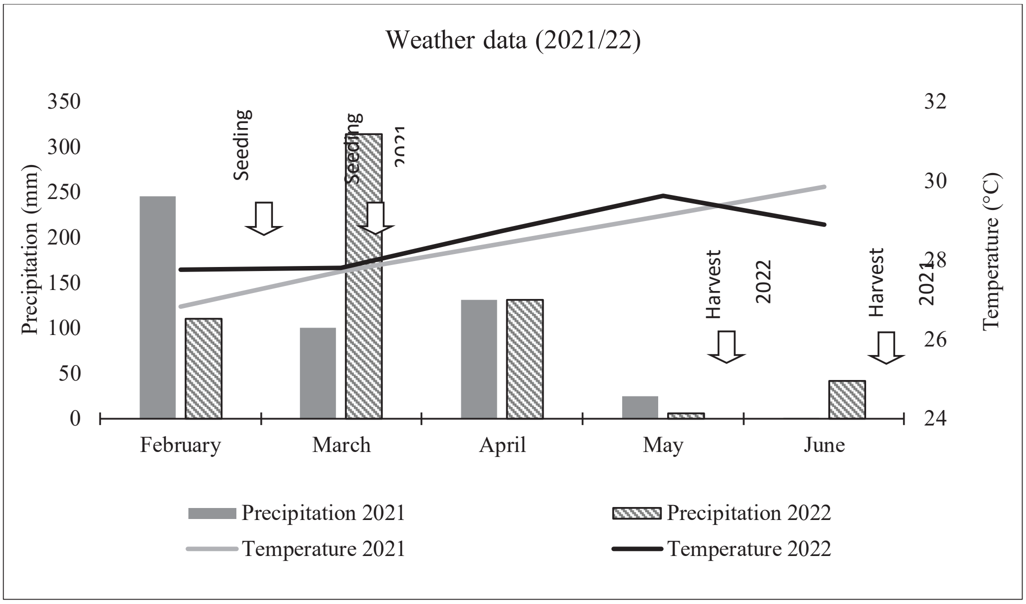


Figure 2 - Mean temperature (°C) and rainfall (mm) data during the months of the experiment in Balsas in 2021 and 2022 (INMET, 2022)

Table 1 - Chemical attributes of the soil in the experimental area.

pH	**OM**	**Q**	**K**	**Ca**	**Mg**	**Al**	**H+Al**	**SB**	**Total CTC**	**V**
in CaCl2	g/dm³	mg/dm³	---------------------------cmol/dm³------------------							%
3,70	9,92	2,70	0,07	0,51	0,03	0,56	3,64	0,61	4,25	14,30

A total of 480 kg of dolomitic limestone (PRNT = 88%) was applied to the area. For planting fertilization, the formulated NPK (5-25-15) was applied at a dose of 30 kg/ha of N, 60 kg/ha of P 2 O 5 and 50 kg of K 2 O. - emergence of the herbicide atrazine. Top-dressing fertilization with N was applied when the plants reached 30 cm deep.

determined by direct counting of fully developed leaves. **The leaf area (LA)** of the plants was determined using a nondestructive method with the formula leaf area (cm²) = length (cm) × width (cm) × 0.75, according to the methodology proposed by Hassan et al. (2010). Leaf length and width were evaluated in the middle third of the plant in four plants per plot with the aid of a measuring tape. **Days to flowering (DF**) consisted of the number of days elapsed from planting to the point at which 50% of the plants in the plot flowered, as assessed by daily monitoring after the onset of flowering. **The total cycle (TC)**, which is the number of days elapsed from planting to harvest, was performed when the plants were at the end of physiological maturation, when

Table 2 - Cultivars evaluated in the 2021 and 2022 harvests in the municipality of Balsas, MA

Cultivate	Type of cultivar	Species	Number of seeds per meter	Maintainer
SUDAN 4202	Sorghum forager	*Sorghum sudanense* (Piper) Stapf	12	IPA
AGRI 001-E	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	8	TROPIGENE
AGRI 002-E	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	8	TROPIGENE
BRS 658	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	10	EMBRAPA
BRS 810	Sorghum graniferous	*Sorghum bicolor* (L.) Moench x *Sorghum sudanense* (Piper) Stapf	10	EMBRAPA
Ponta Negra	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	12	EMBRAPA/ EMPARN
BRS 373	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	12	EMBRAPA
BRS 509	Sorghum graniferous	*Sorghum bicolor* (L.) Moench	10	EMBRAPA
BRS 506	Sorghum saccharine	*Sorghum* ssp	12	EMBRAPA
IPA467-4-2	Sorghum saccharine	*Sorghum* spp.	15	IPA

approximately 75% of the dry matter of the grains accumulated, and when they reached the pasty grain stage. **Green matter production (GMP) was measured in t ha $^{-1}$,** and the plants in the useful area were harvested, identified and taken to the laboratory for weighing of the fresh matter of each treatment. **Dry matter production (DWG) in t ha $^{-1}$**: Samples from each plot were collected, weighed and separated into leaves, stems and panicles. Then, they were placed in paper bags, labeled, and placed in an oven at 65 °C for 72 hours or until a constant weight was reached. **The relative chlorophyll index (SI)** in the leaf was determined using a chlorophyll meter (Chlorophyll Meter SPAD-502) in the middle third of each leaf, avoiding the leaf edges and the midrib region in 4 plants of each plot and in two leaves located in the middle and at the top of the canopy of each plant. **Brix**: The total soluble solids content was evaluated using the °Brix index. For this purpose, a portable digital refractometer (Quimis®) was used.

Repeatability analysis

Initially, the data were subjected to analysis of variance to verify the existence of variability among cultivars. The assessments were done in blocks, considering the measurements for each genotype.

The coefficients of repeatability (r) were estimated via analysis of variance (ANOVA), principal component analysis based on variance and covariance matrix (CP-COV), principal component analysis based on correlation matrices (CO-COR) and structural analysis based on correlation matrices (A.EST.).

Using the ANOVA method, a statistical model with two sources of variation was used:

$$Y_{ij} = \mu + g_i + a_j + e_{ij} \qquad (1)$$

where μ = overall mean; gi = random effect of the i-th individual under the influence of the permanent environment; aj = fixed effect of the environment on the jth measurement; and eij= effect of the temporary environment associated with the j-th measurement on the i-th genotype.

The estimation of the repeatability coefficient, r, for the ANOVA model was performed according to equation 2:

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

in which $\hat{\sigma}_g^2$ is the variance between repeated measures for each genotype and $\hat{\sigma}^2$ is the residual variance.

The principal component analysis method is based on obtaining the correlation matrix between the repeated measures and subsequent estimation of the normalized eigenvalues and eigenvectors. The coefficient according to this method was estimated by:

$$r = \frac{\hat{\lambda}_1 - 1}{\eta - 1} \qquad (3)$$

where $\hat{\lambda}_1$ is the eigenvalue of the correlation matrix associated with the eigenvector whose elements have the same sign and similar magnitude, and η_o is the number of measurements.

Using the method based on the matrix of phenotypic variances and covariances, the coefficient of repeatability was obtained by:

$$r = \frac{\hat{\lambda}_1 - \hat{\sigma}_y^2}{\hat{\sigma}_y^2(n-1)} \qquad (4)$$

where $\hat{\lambda}_1$ is the eigenvalue of the phenotypic variance and covariance matrix associated with the eigenvector whose elements have the same sign and similar magnitude and $\hat{\sigma}_y^2 = \hat{\sigma}_g^2 + \hat{\sigma}^2$ is the phenotypic variance of trait Y.

Using the structural analysis method based on the correlation matrix, the repeatability coefficient was estimated by:

$$r = \frac{1}{n(n-1)\hat{\sigma}_y^2} \sum_j \sum_{\neq j} \hat{\sigma}_{jj'} \qquad (5)$$

In this case, the repeatability coefficient estimator corresponds to the arithmetic mean of the phenotypic correlations between genotypes, considering each pair of measurements. According to Resende., 2002, the estimates of repeatability coefficients are classified as high when they are ≥ 0.6, average when they are < 0.6 and ≥ 0.3 and low when they are < 0.3.

The minimum number of repetitions () to predict the actual value of individuals (genotypes), based on preestablished coefficients of determination (R^2) (0.80, 0.85, 0.90, 0.95 and 0.99), was calculated according to the method described in (Cruz et al.,2012):

$$\eta_O = \frac{R^2(1-r)}{(1-R^2)r} \qquad (6)$$

where η_O is the number of measurements required to obtain the desired coefficient of determination (R^2) and r is the estimated repeatability coefficient.

Statistical analyses were performed using the computer program GENES (Cruz, 2013)

Results and discussion

All variables presented significant genotype effects (P<0.05) in the evaluated experiments, which demonstrates the possibility of applying estimators of the coefficient of repeatability.

Analysis of the coefficients of repeatability (r) of all the variables studied (Table 3) revealed that all of them presented values of high magnitude in all the methods (ANOVA, CP-COV, CO-COR, A.EST), which indicates regularity in the repetition of the performances of the sorghum genotypes from one evaluation to another.

According to Cruz et al. 2012, when repeatability is high, the increase in the number of repetitions in the assay will result in little increase in precision relation related to which would be obtained if an individual were evaluated through a single observation.

In addition, high values for the coefficient of determination (R^2) were also observed, all above 78.54% Table 3, indicating accuracy in the prediction of the real value of individuals from the three measurements performed. These findings corroborate those of Dutra Filho et al. 2020, who reported that three evaluations are necessary for selection with 80% predictability of the actual values of two sugarcane genotypes.

The variables height (H), days to flowering (DF) and total cycle (TC) presented repeatability estimates above 0.79 and precisions above 92% (Table 3). According to Falconer, 1996, a value of 0.79 is considered a high value, which confirms for these characteristics, there was regularity in the measurements performed. In this case, few replicates are required to assess the true value of the genotype regarding this variable, and its superiority can be identified with few measurements. Nonetheless, having a coefficient of determination (R^2) of 92% means that your model explains 92% of all the variation in the observed data. This indicates that it fits reality extremely well, making it a highly reliable tool for making predictions or estimates.

Unlike this scenario, Andrade Júnior et al., 2016 reported low to moderate repeatability estimates for most of the evaluated traits, showing no regularity in the measurements of strawberry yield traits, making it difficult to analyze the superior or inferior performance of the cultivars, which may not be maintained.

Table 3 - Estimated repeatability (r) and coefficient of determination (R^2) of the variables evaluated in sorghum genotypes

		ANOVA	CP-COV	CO-COR	A.EST
H	r	0.84	0.88	0.87	0.87
	R^2	93.88	95.53	95.25	95.23
SD	r	0.7	0.77	0.75	0.74
	R^2	87.64	91.07	89.83	89.7
NL	r	0.79	0.85	0.84	0.84
	R^2	92.01	94.46	94.07	94.06
LA	r	0.59	0.64	0.63	0.63
	R^2	81.22	84.16	83.63	83.47
DF	r	0.95	0.97	0.96	0.96
	R^2	98.14	98.82	98.78	98.77
TC	r	0.98	0.99	0.99	0.99
	R^2	99.17	99.61	99.56	99.55
GMP	r	0.55	0.73	0.71	0.71
	R^2	78.54	89.02	87.86	87.85
DWG	r	0.57	0.71	0.67	0.67
	R^2	79.69	88.02	86	85.9
Brix	r	0.77	0.78	0.78	0.78
	R^2	90.89	91.33	91.39	91.37
IS	r	0.59	0.69	0.66	0.65
	R^2	81.34	87.19	85.11	84.85

CP-COV: principal component based on covariance matrices; CO-COR: principal component based on correlation matrices; A.EST: structural analysis based on correlation matrices; H: height; NL: number of leaves; SD: stem diameter; LA: leaf area; DF: days to flowering; TC: total cycle; GMP: green matter production; DWG: dry matter production; BRIX: total soluble solids content; SI: relative chlorophyll index.

The lowest repeatability estimates, although still considered high (>0.6), were obtained for leaf area (LA), green matter production (GMP), dry matter production (DWG) and the relative chlorophyll index (SI), which ranged from 0.55 to 0.73. This finding demonstrated that the environment had a more significant influence on these traits than on the other traits.

Ferreira et al.,2019 highlighted that a low repeatability coefficient for dry matter production can be attributed to climatic variations throughout the year, such as high temperatures and rainfall, and its decrease during dry periods and due to the transition between dry and rainy periods. Therefore, the author emphasizes the importance of performing evaluations of several harvests because the evaluation of only two successive harvests may not represent the condition of greater genotypic stabilization but rather the occurrence of two very similar harvests.

As the measures of the agronomic traits of the genotypes can oscillate in different ways and intensities, the coefficients of repeatability estimated by analysis of variance may not eliminate this additional component of the experimental error; thus, this estimator would be underestimated (Cruz et al., 2012).

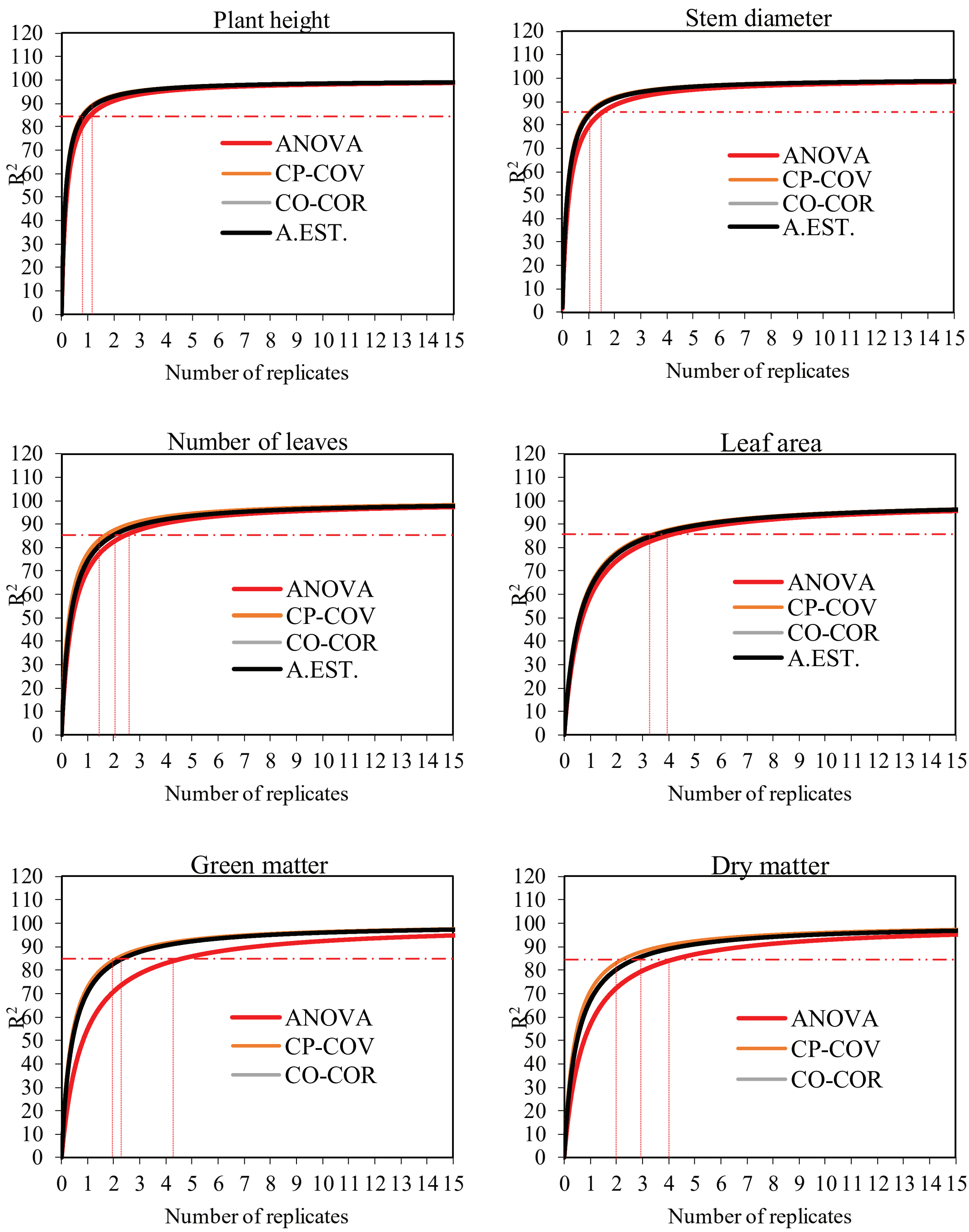
For most features, except for LA, SD, DWG, and IS, the principal component method using a covariance matrix generated the highest repeatability coefficient estimates, which corroborates the results of Alcoforado et al. 2019 and Ferreira et al. 2019. According to Abeywardena, 1972, the repeatability coefficients can be more efficiently estimated using principal component techniques, and the use of this method is more emphasized in situations where the evaluated genotypes exhibit cyclic behavior in related to the studied trait.

According to Resende and Duarte, 2007, for a safe inference, one should aim for a coefficient of determination (R^2) equal to or greater than 81% because, in this case, they have very high experimental precision. However, for the purposes of discussion in this study, we chose to consider an R^2 of 85%.

Figure 3 illustrates the number of replicates required to estimate the actual values of the individuals based on the previously defined R^2 values. With an R^2 of 85%, the three replicates performed in the trials with sorghum genotypes were sufficient for all the variables analyzed. For Leaf area, Green matter, Dry matter and relative chlorophyll index, ANOVA suggested 4 measurements; however, for the other methodologies, 3 measurements were sufficient.

For Green matter production, the evaluated genotypes require an average of four replications to obtain 90% accuracy, Dry matter and Relative chlorophyll require an average of five measurements, and for Leaf area, an average of six measurements, to obtain the same precision (90%) in the prediction of the true value of the individuals evaluated.

To obtain 99% accuracy, 15 measurements were required for H, 19 for SD, 34 for NL, 58 for LA, four for DF, one for TC, 41 for GMP, 48 for DEG, 28 for Brix and 52 for SI. It is possible to observe that to obtain 99% accuracy of the information collected in sorghum assays, a small increase compared to 90% and many repetitions are needed, which would be


Plant height
ANOVA
CP-COV
CO-COR
A.EST.
R^2
Number of replicates
Stem diameter
ANOVA
CP-COV
CO-COR
A.EST.
R^2
Number of replicates
Number of leaves
ANOVA
CP-COV
CO-COR
A.EST.
R^2
Number of replicates
Leaf area
ANOVA
CP-COV
CO-COR
A.EST.
R^2
Number of replicates
Green matter
ANOVA
CP-COV
CO-COR
R^2
Number of replicates
Dry matter
ANOVA
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R^2
Number of replicates

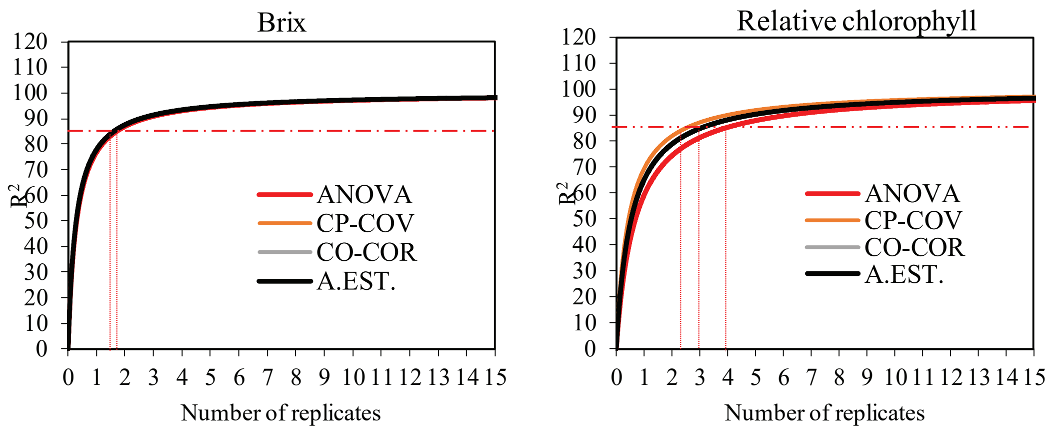

Figure 3 - Minimum number of replicates (η_0) necessary to predict the true value of individuals (genotypes) for each variable, based on the preestablished coefficients of determination (R^2). (CP-COV: Principal components based on covariance matrices; CO- COR: Principal components based on correlation matrices; A.EST: Structural analysis based on correlation matrices).

practically unfeasible for this number of replications, such as GMP, due to the high costs of materials, time and labor.

Ribeiro et al.2015, in their study of the repeatability of maize hybrids with 80% accuracy, determined that nine measurements were sufficient for the traits evaluated (height, ear length, ear diameter and, 100-grain weight.

Considering an R^2 of 85% (higher than the 81% determined by Resende and Duarte.2007, the variables H, SD, DF and TC require one measurement; NL and Brix require two measurements; and LA, GMP, DWG and SI require three measurements. With these results, it is possible to observe a considerable reduction in the evaluation time.

Thus, according to the coefficient of determination (R^2) established by (Resende & Duarte. 2007), equal to or greater than 81%, the 3 replications performed in the experimental trials led to a safe inference of the variables analyzed in the soil and climate conditions of Balsas-MA, taking into account the evaluated gene set.

Conclusions

The experimental assays of sorghum genotypes, conducted with three replications, are able to estimate with 85% accuracy the actual values of the individuals related to the variables height, stem diameter, leaf area, green and dry matter production, days to flowering, total chlorophyll content, and relative chlorophyll content.

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