

Productive performance of elephant grass clones from full-sib families in an advanced stage of breeding

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ABSTRACT: The objectives of this study were to estimate genetic parameters and the repeatability coefficient and to select clones of elephant grass from full-sib families. Fifteen clones and two commercial controls were evaluated in a randomized block design with three replications. Three measurements were taken at regular 10-month intervals. Genotypic variance was significant. The observed heritability based on the mean of the genotypes (0.58), coupled with the results of repeatability (0.53) and selection accuracy (0.76), demonstrates that the genotypes performance is constant between measurements. Thus, only three measurements are necessary to reliably select genotypes and obtain real genetic gains with selection. Among the 15 clones evaluated, six showed higher new means than those of the BRS Capiaçú and BRS Canará controls and can be used as parents and/or to compose value for cultivation and use experiments aimed at the release of cultivars for bioenergy production.

Key words: *Cenchrus purpureus* (Schumach.) Morrone, release of cultivars, REML/BLUP, repeatability.

INTRODUCTION

Concern for energy security and the damage caused by the use of non-renewable sources grow each year. The global energy mix consists mostly of fossil fuels, which, besides being finite, are highly polluting (Lelieveld et al. 2019). In recent years, emissions of atmospheric pollutants from thermoelectric power plants have increased due to higher demand from the agro-industrial sector (Squizzato et al. 2021).

The energy consumption patterns of the world population disturb environmental stability and drive research to reduce the instability of fossil sources; especially oil and its derivatives. With the advancement of research, the goal is to develop and promote the use of renewable energies in order to mitigate pollution and ensure energy security for countries worldwide.

Bioenergy has been advocated as one of the main substitutes for fossil fuels that can serve as a sink for capturing and storing atmospheric carbon (Federico et al. 2022). Taking climate into consideration, Brazil has significant potential for the use of renewable energy sources, with the utilization of biomass as an energy source being one of the alternatives that best align with the country's natural vocation. However, its use falls short of its productive potential (Welfle 2017). One of the most highlighted fields of current research is plant breeding aiming at the selection of genotypes with bioenergy potential (Ferreira et al. 2021).

Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone] is an energy crop that has been widely studied due to its great potential for dry matter production, with high energy quality (Rocha et al. 2015, Vidal et al. 2017, Stida et al. 2018, Silva et al. 2020, Gravina et al. 2020, Vidal et al. 2023) and tolerance to unfavorable weather conditions such as water deficit (Habte et al. 2022). Since 2007, the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) has been developing the elephant grass breeding program, which has driven the improvement of the crop and contributed to promoting its use in the northern region of the state of Rio de Janeiro, Brazil.

Elephant grass is cultivated vegetatively, and its propagation mode carries the advantage of generating uniform populations, maintaining the genetic constitution of the clones (Pereira et al. 2021). In natural conditions, the plant performs cross-pollination, and the mechanism that favors allogamy is protogyny. This feature makes self-pollination in the same panicle difficult and facilitates controlled crossings in plant breeding programs (Silva et al. 2020). For this reason, the breeding of this crop is based on the recombination of divergent genotypes in directed crosses, selection, and cloning of progenies (Pereira et al. 2021).

The elephant grass breeding team at UENF has already conducted several studies involving crosses between genotypes with potential for energy production. The first study began in 2013, when Silva et al. (2020) carried out crosses to obtain full-sib families among eight elephant grass accessions belonging to the UENF germplasm bank, which were selected for late flowering, high dry matter yield, and larger stem diameter. In 2014, Vidal et al. (2022a) implemented an experiment involving 10 parents of elephant grass that had been previously selected based on studies on biomass production potential, originating 15 full-sib families in a circulant diallel system.

Breeding is a continuous process that consists basically of three stages: generation of genetic variability, selection, and testing (Resende et al. 2022). Therefore, preliminary tests must be undertaken to evaluate the superiority of the selected genotypes by comparing them with commercial controls to later compose value for cultivation and use experiments for the release of new elephant grass cultivars. The selection of elephant grass genotypes has been performed based on phenotypic data evaluated in the field using accurate selection approaches, such as mixed models (restricted maximum likelihood/best linear unbiased prediction) (Rodrigues et al. 2017, Stida et al. 2018, Silva et al. 2020, Vidal et al. 2022a, Vidal et al. 2023). This is a precise selection method, as it estimates the components of variance using the restricted maximum likelihood (REML) technique and predicts genotypic values using the best linear unbiased prediction (BLUP) method (Viana and Resende 2014).

The main practical advantages of REML/BLUP include the ability to accurately and unbiasedly predict genetic values, provide specific information about progeny merit and genotypes in progenies, especially in unbalanced experiments. This approach also takes into account the repetition environment where the genotype is located, allowing for the comparison of genotypes or varieties across time (generations, years) and space (locations, blocks). Furthermore, REML/BLUP enables simultaneous correction for environmental effects, estimation of variance components, and prediction of genetic values (Viana and Resende 2014).

Due to the advantages of this method, its use in species improvement has grown significantly and can be applied to different species. Studies conducted by Vivas et al. (2014) to estimate the variance components and genetic value of papaya progenies for the incidence and severity of phoma leaf spot using mixed models allowed the selection of genotypes with negative values for additive genetic effects for both disease severity and incidence on leaves. Quintal et al. (2017) conducted and analyzed, using the REML/BLUP procedure at the genotype level, 17 segregating families of guava. The estimates for genetic parameters revealed excellent selection potential for the population and sufficient genetic variability for both short- and long-term genetic improvement of the population.

In this context, yield is the most important parameter to be considered for any crop. If a clone has high energy efficiency—e.g., high fiber and low ash content—, but it is low-yielding, it will likely not be a commercially released cultivar. Thus, the selection of clones based on quantitative traits such as dry matter yield, via mixed models, may enable the identification of the most promising elephant grass clones.

In view of the foregoing, the present study proposed to estimate genetic parameters and the repeatability coefficient and to select clones of elephant grass from full-sib families in an advanced stage of breeding for dry matter yield.

MATERIAL AND METHODS

Characterization of the experimental area

The experiment was conducted at the State Center for Research in Agroenergy and Waste Use at the Agricultural Research Corporation of Rio de Janeiro, in Campos dos Goytacazes, RJ, Brazil (21°19'23"S, 41°19'40"W; 20 to 30 m altitude). The climate is classified as an Aw type, according to the Köppen system, and the soil is classified as dystric Ultisol ("argissolo"), with a flat topography.

Meteorological data were obtained from an automatic agrometeorological station located near the experimental area. Figure 1 shows the monthly precipitation and temperature data recorded during the experimental period (November 2018 to August 2021).

Genetic material

The elephant grass clones evaluated in the study consisted of 15 hybrids originating from biparental crosses (full-sib families), obtained and selected by Silva et al. (2020) and Vidal et al. (2022a). The 15 clones were previously chosen for their high yield, associated with other traits of interest for bioenergy production. To compose the selection trial in an advanced stage, two commercial cultivars registered in the National Register of Cultivars and used in the region were also evaluated representing controls (Table 1).

Table 1. Identification of 15 elephant grass clones from full-sib families and two commercial controls.

Identification	Year of development	Author	Parent ♀	Parent ♂
Clone 1	2013	Silva et al. (2020)	IJ7139	Cameron
Clone 2	2013	Silva et al. (2020)	IJ7139	Cameron
Clone 3	2013	Silva et al. (2020)	IJ7139	BAG-86
Clone 4	2013	Silva et al. (2020)	IJ7139	BAG-86
Clone 5	2013	Silva et al. (2020)	IJ7139	Cubano Pinda
Clone 6	2013	Silva et al. (2020)	IJ7139	BAG-86
Clone 7	2013	Silva et al. (2020)	IJ7139	BAG-86
Clone 8	2014	Vidal et al. (2022a)	IAC Campinas	IJ7139
Clone 9	2014	Vidal et al. (2022a)	Capim Cana D'Africa	Guaçu/IZ2
Clone 10	2014	Vidal et al. (2022a)	Capim Cana D'Africa	Guaçu/IZ2
Clone 11	2014	Vidal et al. (2022a)	CPAC	Cuba115
Clone 12	2014	Vidal et al. (2022a)	Cameron	Cuba115
Clone 13	2014	Vidal et al. (2022a)	Capim Cana D'Africa	Cuba115
Clone 14	2014	Vidal et al. (2022a)	IAC Campinas	IJ7139
Clone 15	2014	Vidal et al. (2022a)	Capim Cana D'Africa	Guaçu/IZ2
Control – BRS Capiacu				
Control – BRS Canará				

Experimental design and conduct

The experiment was laid out in a randomized block design with three replicates. The experimental unit (or plot) consisted of four 4-m rows spaced 1-m apart. Only the two central rows were considered the usable area of the plot, and the two central meters of these two rows were evaluated, totaling 4 m² of usable area per plot.

Before the onset of the experiment, the soil was plowed, harrowed, and sampled for analysis. Fertilizer application was carried out according to the results of soil analysis and split into four occasions: at planting and once at each harvest for measurements (Freire et al. 2017).

Planting was carried out by distributing stakes (arranged with the base of one plant touching the apex of another) in 5-cm-deep furrows. The first measurement was performed after the establishment of the plot, when a plot-leveling cut was made on February 13, 2019. Subsequent measurements were taken at regular 10-month intervals (Vidal et al. 2022b). The experiment was evaluated by three consecutive measurements, which took place on December 17, 2019, September 29, 2020, and August 16, 2021.

Evaluated trait

The trait under evaluation was dry matter yield (DMY), in $t\cdot ha^{-1}$, which was estimated as follows: a sample was collected from each plot at random, and each part was chopped and packed in a labeled paper bag, weighed, and dried in an oven at $65^{\circ}C$ for 72 h. Then, the samples were weighed again to obtain the air-dried weight. The dried material was ground in a Wiley mill with a 5-mm sieve and packed in plastic bags to determine the oven-dried weight. For this step, 2 g of each ground material were oven-dried at $105^{\circ}C$ for 18 h and then weighed again.

Analysis via mixed models

For the DMY trait, deviance analysis was performed, genetic parameters estimated, and gains predicted. Additionally, repeatability analysis was carried out by the mixed models.

Following the model described in Viana and Resende (2014), deviance analysis was obtained as follows (Eq. 1):

$$D = -2\ln(L)$$

$$\ln(L) = -1/2\ln|X'V^{-1}X| - 1/2\ln|V| - 1/2(y - Xm)'V^{-1}(y - Xm) \quad (1)$$

where: $\ln(L)$: the maximum point of the REML logarithm function; y : the vector of the analyzed variable; m : the vector of observation effects, assumed fixed; X : the incidence matrix of fixed effects; V : the variance-covariance matrix of y .

The likelihood ratio statistical test (LRT) was used to test the significance of the effects, as follows (Eq. 2):

$$LRT = -2\ln(L_{we}) + 2\ln(L_{fm}) \quad (2)$$

where: L_{we} : the maximum point of the maximum likelihood function for the reduced model (without the effects); L_{fm} : the maximum point of the maximum likelihood function for the full model.

The variable was analyzed using Selegen-REML/BLUP software (Resende 2016), which was applied to obtain variance components by REML and individual genotypic values by the BLUP.

The REML/BLUP procedure was executed using model 55 of SELEGEM-REML/BLUP computer software, in which a single location was evaluated in several harvests, in a complete block design (Resende 2016). Genetic values were predicted using the mixed models approach, adopting a model according to the Eq. 3:

$$y = Xm + Zg + Wp + Ti + e \quad (3)$$

where: y : the vector of data; m : the vector of measurement-replication combination effects (assumed fixed) added to the overall mean; g : the vector of genotypic effects (assumed random); p : the vector of permanent environmental effects (plots) (random); i : the vector of genotype \times measurement interaction effects; e : the vector of errors or residuals (random).

The capital letters represent the incidence matrices for the aforementioned effects. Vector m considers all measurements across all replications and adjusts simultaneously for the effects of replications, measurements, and replication \times measurement interaction.

The following phenotypic variance components were provided by the model:

σ_g^2 : genotypic variance;

σ_{perm}^2 : permanent environmental variance;

σ_{gm}^2 : variance of genotypes \times measurements interaction;

σ_e^2 : temporary residual variance;

σ_p^2 : individual phenotypic variance;

$h^2g = h^2$: broad-sense heritability of individual plots, that is, of the total genotypic effects;

r : repeatability at the plot level, given by $(V_g + V_{perm})/V_p$;

c_{perm}^2 : coefficient of determination of permanent environmental effects;

c_{gm}^2 : coefficient of determination of genotypes \times measurements interaction effects;

r_{gmean} : genotypic correlation between measurements;

h_{mg}^2 : mean heritability of genotypes;

Ac_{gen} : accuracy of genotype selection.

The variance components for calculating the repeatability coefficient were estimated using the REML procedure, with repeatability at the plot level (ρ) estimated as follows (Eq. 4):

$$\rho = \frac{\sigma_{2g} + \sigma_{2perm}}{\sigma_{2p}} \quad (4)$$

$$\rho = \frac{\sigma_a^2 + \sigma_p^2 + \sigma_i^2 + \sigma_s^2}{\sigma_a^2 + \sigma_p^2 + \sigma_i^2 + \sigma_s^2 + \sigma_e^2}$$

where: σ_g^2 : the genetic variance between plants; σ_{perm}^2 : the variance of permanent effects; σ_p^2 : the phenotypic variance.

RESULTS AND DISCUSSION

As shown in Table 2, the deviance values reveal the existence of genetic variability between the evaluated genotypes. This evidence is due to the significant difference found by the likelihood ratio test for *genotype* as a source of variation at 5% probability, for the DMY trait.

Table 2. Deviance analysis for dry matter yield in 15 elephant grass clones from full-sib families and two commercial controls evaluated through three measurements for bioenergy production. Campos dos Goytacazes, RJ, Brazil, 2019–2021.

Source		Dry matter yield
Genotype	Deviance	796.43*
	LRT	4.38
Permanent environment	Deviance	793.23ns
	LRT	1.18
Genotype \times measurement	Deviance	796.57*
	LRT	4.52
Full model		792.05

LTR: likelihood ratio test; ns: not significant, by the χ^2 test with 1 degree of freedom; *significant at the 5% probability level (3.84), by the χ^2 test with 1 degree of freedom.

The existence of genetic variability in a selection trial at an advanced stage is extremely important for the breeding program, as it indicates that the selection process has been conducted so as to increase the mean of the genotypes, yet without losing the genetic variability of the population under study. Genetic variability is vital for the selection process, as it makes it possible to estimate components of variance with high precision and to obtain genetic gain with the selection of individuals with high DMY (Muktar et al. 2019).

The significance of the genotype \times measurement interaction reveals that the ranking of genotypes changed when the three measurements were compared. This significant effect demonstrates inconsistency in the DMY of the genotypes between measurements, which makes it difficult to recommend new elephant grass clones. The mixed models (REM/BLUP) methodology allows for selection to be carried out based on the genetic value predicted via BLUP, considering the average performance in each measurement and without the effect of environmental interaction (Viana and Resende 2014).

Genotypic variance (V_g) was considered low-magnitude, contributing only 20% of all phenotypic variation found. However, even with the low V_g found, it is possible to obtain genetic gains with selection for the DMY trait. The BLUP estimators are efficient to select genotypes for low-heritability traits, allowing genetic gains to be achieved with selection (Ferreira et al. 2016). Ambrósio et al. (2023), when evaluating 40 genotypes of elephant grass, also obtained low heritability values. However, even with the low heritability values, it was possible to achieve gains of more than 3 t ha⁻¹.

Environmental effects contributed most to the variation. Temporary residual effects (V_e) predominated, which are the type capable of explaining most of the phenotypic variation found (56%), corroborating a greater environmental influence on the evaluated trait. Permanent environmental variance (V_{perm}) and the genotype \times measurement interaction variance (V_{gm}) contributed 7.3 and 16.63% of phenotypic variation, respectively (Table 3).

Table 3. Components of variance as obtained by individual REML for dry matter yield in 15 clones of elephant grass from full-sib families and two commercial controls evaluated through three measurements for bioenergy production. Campos dos Goytacazes, RJ, Brazil, 2019–2021.

Variance component	Dry matter yield (t ha ⁻¹)
σ^2_g	17.75
σ^2_{perm}	6.51
σ^2_{gm}	14.91
σ^2_e	50.47
σ^2_p	89.63
h^2_g	0.20
R	0.27
c^2_{perm}	0.07
c^2_{gm}	0.17
rg_{mean}	0.54
h^2_{mg}	0.58
Overall mean	30.43

σ^2_g : genotypic variance; σ^2_{perm} : permanent environmental variance; σ^2_{gm} : variance of the genotype \times measurement interaction; σ^2_e : temporary residual variance; σ^2_p : individual phenotypic variance; h^2_g : broad-sense heritability of individual plots; R: repeatability at the plot level; c^2_{perm} : coefficient of determination of permanent environmental effects; c^2_{gm} : coefficient of determination of the genotype \times measurement interaction effects; rg_{mean} : genotypic correlation between measurements; h^2_{mg} : mean heritability of genotypes.

The production of dry matter is a quantitative trait and therefore highly influenced by environmental conditions. In Fig. 1, it can be observed that there was variation in temperature and precipitation between measurements. This variation between measurements resulted in varied productivities, and, consequently, the phenotypic expression of traits varied among different measurements, leading to a significant genotype \times harvest interaction (Pereira et al. 2013).

The interaction between genotypes and the environment directly influences heritability estimates, as well as the type of genetic inheritance that controls the trait. The fact that the inheritance that controls the DMY trait is polygenic and is highly influenced by the environment may explain the low predicted values for genetic variance found in this study. Several studies with the elephant grass crop evaluating DMY for bioenergy production also showed a greater contribution of environmental effects to the phenotypic variations estimated (Stida et al. 2018, Silva et al. 2020, Vidal et al. 2023).

The coefficient of determination of permanent environmental effects (c^2_{perm}) was considered of low magnitude. This shows that the experimental design employed was assertive, providing reduction in environmental heterogeneity within the plots (Cortes et al. 2019).

The mean heritability of genotypes (h^2_{mg}) showed higher estimated values (58%) than V_g . This variable (h^2_{mg}) is calculated based on the means obtained with the decrease in experimental errors provided by the proportional increase in the number of replications (Resende 2009). This parameter allows for advances to be made in plant breeding programs, with a more efficient direction of crossing strategies and selection of genotypes while maintaining gains and the genetic variability of the populations under study (Cruz et al. 2014).

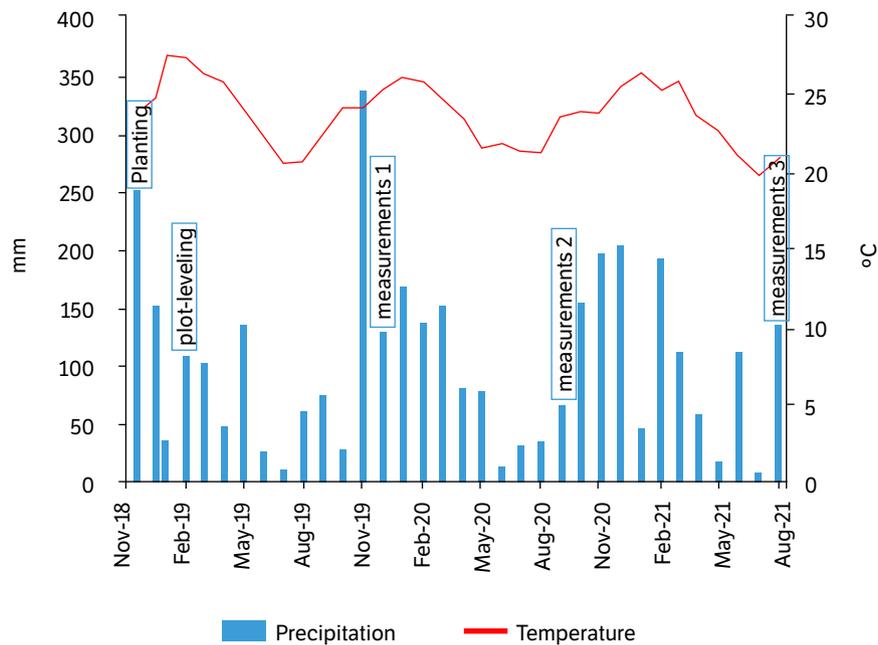


Figure 1. Precipitation and mean temperatures during the experiment with elephant grass. Campos dos Goytacazes, RJ, Brazil, 2019–2021.

Repeatability estimates make possible to predict the stability of response of a trait, by measuring the average correlation between two or more measurements of the same individual (Cruz et al. 2014). According to Resende (2009), the repeatability coefficient can be classified as high ($r > 0.60$), medium ($0.30 < r < 0.60$), or low ($r < 0.30$). In this study, the repeatability estimates for the DMY trait were considered low (0.27), indicating that a single measurement on the individual does not represent its actual DMY, and, therefore, more than one measurement is necessary for an efficient selection.

The coefficient of determination of repeatability, based on the average of three measurements, showed intermediate magnitude (0.53) (Table 4). Based on the estimated increase in repeatability as the number of measurements performed increases, the repeatability level of 60% would be reached with four measurements. These data suggest that increasing precision to values greater than 60% would require a significant increase in the time to recommend new genotypes, given that the interval required between measurements is 10 months (Vidal et al. 2022b). The increase in number of evaluations entails operational expenses, a lot of time, and work. Thus, it is possible to reduce the level of precision to minimize costs and increase gains per time unit (Sánchez et al. 2017).

The use of three measurements allowed a considerable increase in selection efficiency (E_f) for DMY, with a 40% higher result compared with one measurement. The accuracy of permanent phenotypic values (A_{cm} ; used to evaluate experimental precision) based on the three measurements performed was 0.76, classified as high. Ranging from 0 to 1, the most appropriate A_{cm} values are those close to unity. It can be classified as very high ($A_{cm} > 0.90$), high ($0.70 < A_{cm} < 0.50$), moderate ($0.50 < A_{cm} < 0.70$), or low ($A_{cm} < 0.50$) (Resende and Duarte 2007).

High accuracy and effective model selection enhance the efficacy of the whole breeding program (Resende and Alves 2020). The increase in selection efficiency associated with the high values of A_{cm} found in this study demonstrates the reliability of the predicted values relative to the real values. Therefore, the clones evaluated in this experiment can be selected reliably, bringing real genetic gains to the elephant grass breeding program with a view to bioenergy production (Resende and Alves 2020).

Table 4. Efficiency of repeated measures as predicted by BLUP for dry matter yield in 15 clones of elephant grass from full-sib families and two commercial controls evaluated through three measurements for bioenergy production. Campos dos Goytacazes, RJ, Brazil, 2019–2021.

Measurements	Dry matter yield			
	h^2m	Repeatability	A_{cm}	Ef
1	0.34	0.27	0.59	1.00
2	0.50	0.43	0.70	1.25
3	0.58	0.53	0.76	1.40
4	0.64	0.60	0.80	1.49
5	0.68	0.65	0.82	1.55
6	0.70	0.69	0.84	1.60
7	0.73	0.72	0.85	1.63
8	0.74	0.75	0.86	1.66
9	0.76	0.77	0.87	1.69
10	0.77	0.79	0.88	1.71

h^2m : mean heritability of genotypes; A_{cm} : accuracy of permanent phenotypic values based on m measurements; Ef: efficiency of measurements, compared with the situation in which only one measurement is performed.

Among the 15 clones evaluated, six exhibited superior performance (new means) to both tested controls, including the BRS Capiacu control, the latest elephant grass cultivar released in Brazil (Pereira et al. 2017). These were clones 5, 6, 7, 9, 13, and 15 (Table 5). Another five clones performed better than the BRS Canará control, namely, 4, 12, 8, 1, and 14. Among the 15 clones developed, 11 showed higher yield than at least one of the evaluated commercial controls.

Table 5. Ranking, predicted gain, and new mean (BLUP) for dry matter yield in 15 clones of elephant grass from full-sib families and two commercial controls evaluated through three measurements for bioenergy production. Campos dos Goytacazes, RJ, Brazil, 2019–2021.

Genotype	Dry matter yield		
	Gain (t ha ⁻¹)	Gain (%)	New mean
Clone 9	4.26	12.28	34.70
Clone 5	4.01	11.64	34.45
Clone 15	3.73	10.92	34.16
Clone 13	3.57	10.5	34.01
Clone 6	3.45	10.18	33.88
Clone 7	3.22	9.57	33.65
Control – BRS Capiacu	3.05	9.11	33.49
Clone 4	2.89	8.67	33.32
Clone 12	2.74	8.26	33.17
Clone 8	2.32	7.08	32.75
Clone 1	1.9	5.88	32.33
Clone 14	1.54	4.82	31.97
Control – BRS Canará	1.22	3.85	31.66
Clone 3	0.94	3.00	31.37
Clone 10	0.68	2.19	31.11
Clone 11	0.39	1.27	30.83
Clone 2	0	0	30.43

Clone 9 produced 1.2 t ha⁻¹ more than the BRS Capiacu control. When compared with the BRS Canará control, the increase in DMY was 3.02 t ha⁻¹. This increase represents gains of 3.6 and 9.6%, respectively, denoting the potential of the developed genotypes. These six clones can compose the first value for cultivation and use experiment along with other clones that are in the final stage of selection by the elephant grass breeding program at UENF. With the selected clones and

the continuity of the elephant grass breeding program, new and high-yielding elephant grass cultivars are expected to soon be released, aiming to reduce the indiscriminate use of fossil fuels.

CONCLUSION

There was significant genetic variability for selection among the evaluated clones.

The obtained repeatability values show that the genotypes perform with constancy across measurements, which indicates that only three measurements are needed to reliably select elephant grass genotypes.

Six clones were superior to the two tested commercial controls and can be used as parents and/or used to compose value for cultivation and use experiments aimed at the release of cultivars for bioenergy production.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Vidal, A. K. F. and Daher, R. F.; **Methodology:** Vidal, A. K. F. and Ambrósio, M.; **Investigation:** Vidal, A. K. F., Freitas, R. S., Rangel, L. H., Leite, C. L. and Souza, A. G.; **Data curation:** Daher, R. F.; **Formal analysis:** Santana, J. G. S. and Nascimento, M. R.; **Project administration:** Daher, R. F.; **Supervision:** Freitas, R. S.; **Writing – Review & Editing:** Vidal, A. K. F., Ambrósio, M., Santana, J. G. S., Leite, C. L., Souza, A. G., Nascimento, M. R. and Xavier J. V.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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