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Adaptability and stability via mixed models in elephant-grass (*Cenchrus purpureus* (Schumach.) Morrone) varieties for energy purposes

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ABSTRACT: Elephant grass stands out among lignocellulosic biomass plants utilized for second-generation biofuel production due to several advantageous characteristics compared to other raw materials. Its short production cycle and ability to thrive in adverse soil and climate conditions contribute to its appeal. Additionally, breeders seek genotypes with high productivity potential and adaptability to various favorable cultivation environments. This study aimed to estimate genetic parameters, predict genetic values using mixed models (REML/BLUP), and evaluate stability and adaptability for energy biomass production in elephant grass genotypes. The experiment was conducted in Campos dos Goytacazes, RJ, Brazil, utilizing a two-replicate experimental block design that included 40 elephant grass genotypes. Four harvest assessments were performed between 2016 and 2019. Genetic parameter estimation and selection of superior genotypes based on genetic value using the REML/BLUP procedure were performed using Selegen software. Stability and adaptability analyses were obtained through the harmonic mean of genotypic values (HMGV), enabling the identification of stable and highly productive genotypes. Genotypes 17, 18, 32, 16, 36, 6, 15, 31, and 34 exhibited outstanding performance in terms of HMGV, indicating enhanced stability, adaptability, and simultaneous productivity, thus ensuring robustness in cultivation. These selected genotypes hold potential for future breeding programs aimed at improving elephant grass yield for biomass production.

Key words: bioenergy, biomass, genetic parameters, REML/BLUP, yield.

INTRODUCTION

Elephant grass (*Cenchrus purpureus* (Schumach.) Morrone) is widely recognized as one of the most utilized tropical forages in Brazil (Cunha et al. 2011). This perennial grass, belonging to the Poaceae family, exhibits dual aptitude with exceptional performance (Cavalcante et al. 2012). Notably, elephant grass is renowned for its high dry matter yield potential (Fedenko et al. 2013). It is extensively cultivated in warm climate regions, serving multiple purposes such as cutting, grazing, ensilage, and bioenergy production (Tibayungwa et al. 2011). In terms of forage aptitude, it can provide up to 15.85 $t \cdot ha^{-1}$ of forage mass when interspecific-hybridized with millet (*Pennisetum glaucum*) (Emerenciano Neto et al. 2019). Furthermore, for bioenergy purposes its energy potential reaches up to 61.6 $t \cdot ha^{-1} \cdot year^{-1}$ of dry matter (Vidal et al. 2019; 2022). Elephant grass possesses a short growth cycle of five to seven months, characterized by rapid leaf area expansion after planting or cutting, leading to its high biomass production potential. This remarkable biomass production results from various factors such as efficient sunlight interception, photosynthetic efficiency, regrowth and tillering capacity, reserve carbohydrate storage, nutrient absorption, and water use efficiency (Marafon et al. 2014; Pereira et al. 2021).

In studies focused on energy production, certain traits are of paramount importance. Notably, traits such as higher growth rate, increased yield, and enhanced energy efficiency are crucial considerations. These traits are dependent on the chemical composition and contents of cellulose, lignin, high calorific value, high carbon/nitrogen ratio, in addition to low levels of moisture, ash, and nitrogen (Jaradat 2010; Quirino et al. 2005). Elephant grass is highly productive in smaller areas, has a lower production, allows total mechanization, and provides renewable energy, greater carbon assimilation, and increased productivity by increasing the applications of nitrogen and potassium (Gravina et al. 2020; Silva et al. 2020; Woodard et al. 2015[1]).

Over the past four decades, the energy sources in Brazil and other parts of the world have undergone significant structural transformations. This shift has led to the emergence of a new paradigm in energy generation and consumption, driven by concepts of sustainability and the increasing attractiveness of renewable energy sources (Alves et al., 2018; Fontoura et al. 2015; Paterlini et al. 2013; Sant'Ana et al. 2018). Elephant grass (*Cenchrus purpureus* (Schumach.) Morrone) stands out as one of the available renewable energy sources. This species exhibits high photosynthetic efficiency, possesses a remarkable capacity for dry matter accumulation, and features a high fiber percentage. These characteristics make it a potential candidate for energy purposes (Quesada et al. 2004).

Moreover, it is worth noting that the actions described in this study align with the Sustainable Development Goals (SDGs) recommended by the United Nations (UN) (Moreira et al. 2020). Utilizing elephant grass for energy production presents an opportunity to harness renewable energy and mitigate the impact of carbon dioxide emissions resulting from the use of fossil fuels and their derivatives, ultimately contributing to environmental preservation and restoration. Therefore, elephant grass exhibits significant promise as an important alternative renewable energy source for regional, national, and global development (Borges et al. 2016).

Over the course of the last 15 years, the Universidade Estadual do Norte Fluminense (UENF) has been dedicated to conducting studies aimed at obtaining, evaluating, selecting, and indicating high-quality genotypes of elephant grass, with a focus on its application in the field of bioenergy. Throughout this period, the results obtained have been encouraging in terms of the enhancement of this cultivation, as evidenced in studies carried out by Silva et al. (2020), Gravina et al. (2020), Vidal et al. (2022), as well as Vidal et al. (2023a) and Santana et al. (2023). Simultaneously, the Empresa Brasileira Pesquisa Agro Pecuária (EMBRAPA) has played a significant role in the field of elephant grass studies, concentrating on the characterization and evaluation of germplasm, with an emphasis on biomass quality. These activities promote its utilization as a renewable energy source. In this context, the analysis of genetic variability and the careful selection of elephant grass genotypes for bioenergy purposes have the potential to generate superior combinations capable of optimizing direct biomass combustion. Furthermore, these initiatives can effectively broaden the contribution of elephant grass to the sustainable diversification of the energy landscape, as highlighted by Rocha et al. (2017) and Pereira et al. (2021).

Elephant grass can generate 21 units of energy for each unit of fossil fuel (21:1) consumed during its production (combustion), while sugar cane, converted into ethanol, only reaches a ratio of 9:1 (Ferreira et al. 2021). According to Rocha et al. (2017), high total dry biomass ($Mg \cdot ha^{-1} year^{-1}$) is the main factor to consider in the production of bioenergy via the direct combustion of biomass. Elephant grass has been used as a raw material for thermal energy generation, cellulosic ethanol production, and other high-value biotechnological applications (Fontoura et al. 2015). For example, private companies in Brazil and other parts of the world are using elephant grass as a substrate for biogas production and electricity generation (Fontoura et al. 2015). The genetic selection of high-yield genotypes of elephant grass is important to increase its use in bioenergy production (Ferreira et al. 2022; Silveira Júnior et al. 2022).

Furthermore, during the process of plant selection, accurate estimation of genetic superiority is essential (Negreiros et al. 2008). Mixed models (REML/BLUP) have gained popularity in plant breeding as they enable the evaluation of individual genotypes, estimation of variance components (Restricted Maximum Likelihood - REML), and prediction

[1] Woodard, K.R., L.E. Sollenberger Production of Biofuel Crops in Florida: Elephant grass SS-AGR-297, Agronomy Department, University of Florida UF)/Institute of Food and Agricultural Sciences (IFAS) Extension, Gainesville, Florida, USA (2015) Available at: https://edis.ifas.ufl.edu/ag302.

of individual genetic values (Best Linear Unbiased Prediction - BLUP). These models maximize the capture of additive variance, enhancing the desired genetic gains and facilitating a more precise selection process (Viana and Resende 2014; Resende 2016; Viana and Resende 2014).

To address stability, adaptability, and productivity simultaneously in breeding studies, Resende (2009) developed the harmonic mean of the relative performance of genotypic predicted values (HMRPGV-BLUP) method. This approach incorporates stability and adaptability analyses into a single statistical analysis, accounting for correlated errors within locations and aiding in the selection of superior genotypes. The method offers advantages such as providing genetic values discounted for instability, applicability to any number of environments, and simultaneous consideration of stability and adaptability (Ambrósio et al. 2023; Rosado et al. 2012; 2019; Silva et al. 2011).

Simultaneous selection for productivity, stability, and adaptability using mixed models (REML/BLUP) has been successfully employed in various crops, including sugarcane (Bastos et al. 2007), common bean (Carbonell et al. 2007), rice (Borges et al. 2010), carrot (Silva et al. 2011), cowpea (Santos et al. 2016), grugru palm (Rosado et al. 2019), maize (Krause et al. 2020), and safflower (Oliveira Neto et al. 2021). However, there is limited research utilizing the REML/BLUP method in elephant grass breeding, representing an innovative approach to successfully select potential genotypes for bioenergy production. Therefore, this study aims to estimate genetic parameters, predict genetic values using mixed models (REML/BLUP), and assess stability and adaptability for energy biomass production in elephant grass genotypes.

METHODS

Location, design, population, and evaluated traits

The experiment was carried out at the Colégio Estadual Agrícola Antônio Sarlo Research Farm, in Campos dos Goytacazes/RJ, Brazil (321º45S, 41º20W, 11 m asl). The experimental design employed in this study was a randomized complete block design with two replications. The experimental plots were arranged in rows that were 1.5 cm apart and 3 m long. The use of two replications in elephant grass cultivation has been widely supported by previous studies conducted by Souza et al. (2017), Stida et al. (2018), Daher et al. (2020), Rodrigues et al. (2020) and Vidal et al. (2023b).

The utilization of two replications is crucial as it facilitates the application of mixed models analysis, as a smaller number or lack of replications hinders the use of traditional analysis methods. In the present study, the selection process was conducted using mixed models due to the following reasons: a) Extrapolation: Two replications enable the extrapolation of sample values (variance and mean) to represent the entire population; b) Improved Predictions: The adoption of mixed models leads to more accurate predictions, particularly when dealing with missing data. The predictions are based on genetic values rather than phenotypic values, thereby resolving issues related to unbalanced data resulting from varying numbers of replications, treatments, or experiments conducted across multiple locations. This methodology effectively handles complex data structures, including repeated measurements, different years, and diverse experimental designs. By adopting the BLUP methodology, selection accuracy can be maximized while minimizing prediction errors (Resende 2004; Resende et al. 2014; Viana and Resende 2014).

Forty elephant grass genotypes from the elephant grass germplasm bank of UENF were used in this study (Table 1). These were selected based on previous research on biomass production, incorporating traits such as late flowering, dry matter yield, stem diameter, and number of tillers (Rossi et al. 2014). Table 1 provides the code number and origin of the genotypes studied. The evaluated genotypes are highly heterozygous clonal varieties.

Throughout the experiment, fertilization was carried out following the recommended practices outlined in the manual of the state of Rio de Janeiro, taking into consideration the results of soil analysis (Almeida et al., 1988). Fertilizers were applied on five occasions: during planting and once at each assessment harvest. The application consisted of 100 kg·ha^{-1} of P_2O_5 (single superphosphate) prior to sowing, and 25 kg·ha^{-1} of N (ammonium sulfate) and 25 kg·ha^{-1} of K_2O (potassium chloride) during the harvesting period, over the course of four years. The crop was irrigated as required, and weed control was managed manually (Freire et al. 2013).

Table 1. Identification and origin of the 40 elephant grass accessions belonging to the germplasm bank (Universidade Estadual do Norte Fluminense Darcy Ribeiro, municipality of Campos dos Goytacazes, RJ, Brazil, 2023).

Code	Genotype	Origin
1	Elefante de Colômbia	Colombia
2	Mercker	Brazil
3	Três Rios	Brazil
4	Mercker Santa Rita	Brazil
5	Pusa Napier nº 2	Índia
6	Gigante de Pinda	Brazil
7	Napier nº 2	Brazil
8	Mercker S.E.A.	Brazil
9	Taiwan A-148	Brazil
10	Porto Rico 534-B	Brazil
11	Albano	Colombia
12	Híbrido Gig. da Colômbia	Colombia
13	Pusa Gigante Napier	Índia
14	Costa Rica	Costa Rica
15	Cubano Pinda	Brazil
16	Mercker Pinda	Brazil
17	Mercker Pinda México	Brazil
18	Mercker 86 – México	Colombia
19	Taiwan A-144	Brazil
20	Napier S.E.A.	Brazil
21	Taiwan A-143	Brazil
22	Elefante de Pinda	Colombia
23	Mineiro	Brazil
24	Mole de Volta Grande	Brazil
25	Napier	Brazil
26	Teresópolis	Brazil
27	Taiwan A-46	Brazil
28	Duro de Volta Grande	Brazil
29	Merckeron Comum Pinda	Brazil
30	Cameroon - Piracicaba	Brazil
31	Taiwan A-121	Brazil
32	P-241-Piracicaba	Brazil
33	IAC – Campinas	Brazil
34	Elef. Cach de Itapemirim	Brazil
35	Roxo	Brazil
36	Guaçu/IZ.2	Brazil
37	King Grass	Brazil
38	Roxo Botucatu	Brazil
39	Vruckwona Africana	Africa
40	Pasto Panamá	Panama

Source: Elaborated by the authors.

The plant was cut near ground level and weighed in the field. Subsequently, to determine dry matter yield (DMY), a sample was taken from each randomly chopped plant, placed in a labeled paper bag, and weighed. The samples were then oven-dried at 65 °C for 72 h to obtain the air-dried weight (ADW) (Menezes et al. 2016). Afterward, the samples were ground using a Wiley mill with a 5 mm sieve and packed in plastic bags to determine the oven-dried weight (ODW). For ODW determination, 2 g of each ground material were kept in an oven at 105 °C for 18 h and then weighed again.

Dry matter yield was measured based on the performance of each plot. The plants were harvested for evaluation on four occasions: twice during the summer (rainy season) and twice during the winter (dry season). Hence, a total of 40 elephant grass genotypes were evaluated across four harvesting seasons between 2016 and 2019.

Adaptability and stability analysis via mixed models

The analysis of deviance, estimation of genetic parameters, prediction of gains, and assessment of repeatability, adaptability, and stability of genotypes were carried out for the aforementioned traits. Following the model proposed by Viana and Resende (2014), the analysis of deviance was performed as follows:

$$D = -2ln(L) \tag{1}$$

$$\ln(L) = -\frac{1}{21n\left|X'^{V^{-1}}X\right|} - \frac{1}{21n|V|} - \frac{1}{2(y-Xm)'^{V^{-1}}(y-Xm)} \tag{2}$$

where ln(L) is the maximum point of the restricted maximum likelihood logarithm function (REML); y is the vector of the analyzed variable; m is the vector of observation effects, considered fixed; X is the incidence matrix of fixed effects; and V is the variance-covariance matrix of y.

The statistical LRT (likelihood ratio test) was used for testing the significance of the effects, as shown below:

$$LRT = \left| -21n(L_{we}) + 21n(L_{fm}) \right| \tag{3}$$

where L_{we} is the maximum point of the maximum likelihood function for the reduced model (without the effects) and L_{fm} is the maximum point of the maximum likelihood function for the full model. Variables were analyzed by Selegen-REML/BLUP software (Resende 2016), which was used to obtain the components of variance by the restricted maximum likelihood (REML), and the individual genotypic values using the best linear unbiased predictor (BLUP).

To investigate the genotype x environment interaction, adaptability and stability analyses were combined using the REML/BLUP mixed model in Selegen-REML/BLUP software (Resende 2016). The present study adopted the statistical model no 55. This model consists of the evaluation in a single location across several harvests, in a complete block design with temporal stability and adaptability (HMRPGV method).

It is noteworthy that Resende (2009) developed the Selegen-REML/BLUP (Statistical System and Computerized Genetic Selection via Mixed Linear Models) software with the aim of enhancing genetic selection methodologies through the statistical analysis of field experimental data. The REML/BLUP procedure is currently regarded as the optimal selection approach in plant breeding. It enables the adjustment of a wide range of models, including complex ones, to suit the characteristics of the study population. Furthermore, it is easy access and facilitates efficient handling of various typical situations encountered in plant breeding, making it highly accessible and interpretable (Resende 2009; Viana and Resende 2014).

This model is applied to an experiment with a Complete Block design with Temporal Stability and Adaptability (HMRPGV method), with evaluation in a single location across several harvests:

$$y = X_m + Z_g + W_p + T_i + e \tag{4}$$

where *y* is the vector of data, m is the vector of the measurement-replication combinations effects (assumed as fixed) added to the overall mean, g is the vector of genotypic effects (assumed as random), p is the vector of permanent environmental effects (plots, in this case) (random), i is the vector of the genotype x measurement interaction effects, and e is the vector of errors or residuals (random). The uppercase letters represent the incidence matrices for the aforementioned effects. Vector m comprises all the measurements across all replications and adjusts simultaneously for the effects of replications, measurement, and replication x measurement interaction.

The distributions and structures of means (E) and variances (Var) were assumed as shown next:

$$E\begin{bmatrix} y \\ g \\ p \\ i \\ e \end{bmatrix} = \begin{bmatrix} Xm \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix};\ Var\begin{bmatrix} g \\ p \\ i \\ e \end{bmatrix} = \begin{bmatrix} I\sigma_g^2 & 0 & 0 & 0 \\ 0 & I\sigma_p^2 & 0 & 0 \\ 0 & 0 & I\sigma_i^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix} \tag{5}$$

The adjustment of the mixed model equation was obtained from the following equations:

$$\begin{bmatrix} X'X & X'Z & X'W & X'T \\ Z'X & Z'Z + I\lambda_1 & Z'W & Z'T \\ W'X & W'Z & W'W + I\lambda_2 & W'T \\ T'X & T'Z & T'W & T'T + I\lambda_3 \end{bmatrix} X \begin{bmatrix} \hat{g} \\ \hat{p} \\ \hat{\imath} \end{bmatrix} = \begin{bmatrix} Z'y \\ W'y \\ T'y \end{bmatrix} \tag{6}$$

The following parameters were estimated: Vg: genotypic variance; Vperm: permanent environmental variance; Vgm: genotype x measurement interaction variance; Ve: temporary residual variance; and Vp: individual phenotypic variance.

where $\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{(1 - h_g^2 - h_a^2)}{h_g^2}$; $\lambda_2 = \frac{\sigma_e^2}{\sigma_{perm}^2} = \frac{(1 - h_g^2 - c_{perm}^2)}{c_{perm}^2}$; $\lambda_3 = \frac{\sigma_e^2}{\sigma_{gm}^2} = \frac{(1 - h_g^2 - c_{gm}^2)}{c_{gm}^2}$ denote $h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{perm}^2 + \sigma_{gm}^2 + \sigma_e^2}$, broad-sense heritability of individual plot; $h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{perm}^2 + \sigma_{gm}^2 + \sigma_e^2}$, mean heritability of genotypes; $\hat{r}gmed = \frac{\sigma_{gm}^2}{\sigma_g^2 + \sigma_{gm}^2} = \frac{h_g^2}{h_g^2 + c_{gm}^2}$, genotypic correlation through measurements (harvests); $\hat{r}gg = \sqrt{h_{mg}^2}$, accuracy of genotype selection; $c_{perm}^2 = \frac{\sigma_{perm}^2}{\sigma_g^2 + \sigma_{perm}^2 + \sigma_e^2}$, coefficient of determination of permanent environmental effects; $c_{gm}^2 = \frac{\sigma_{gm}^2}{\sigma_g^2 + \sigma_{gm}^2 + \sigma_e^2}$, coefficient of determination of the genotype x measurement interaction effects (harvests); $r = \frac{(\sigma_g^2 + \sigma_{perm}^2)}{(\sigma_p^2)}$, repeatability at the plot level; and overall mean of the experiment.

The phenotypic observations at four harvesting times were considered to estimate the adaptability and stability of different grass genotypes. The selection of the superior genotypes was based on the harmonic mean of the relative performance of genetic predicted value (HMRPGV), using the following strategies: selection based on the predicted genetic value, considering the mean performance in all crops (no interaction effect); selection based on the predicted genotypic value, considering the performance of the genotypes at each harvest (with interaction effect); and simultaneous selection for production, stability (HMGV) and adaptability (RPGV).

The stability estimation was obtained by the harmonic mean of the genetic values (HMGV) method using the estimator:

$$HMGV = \frac{n}{\sum_{j=1}^{n}(\frac{1}{Vg_{ij}})} \tag{7}$$

where n represents the number of environments or cutting seasons (n=3 cutting seasons), i is the evaluated genotype, and Vgij is the genotypic value i in environment j.

Adaptability was measured by the relative performance of genetic values (RPGV), using the expression below:

$$RPGV = \frac{1}{n} x \left(\frac{\sum_{j=1}^{n} Vg_{ij}}{M_j}\right) \tag{8}$$

where Mj is the mean of the analyzed variable (dry matter yield), in environment j.

The HMRPGV method was used to select the best individuals within each progeny that stood out, based on three aspects: selection based on the predicted genetic value, considering the mean performance in all harvesting seasons (with no interaction effect); selection based on the predicted genetic value, considering the mean performance in each harvesting season (with the mean interaction effect) and without the interaction effect; and simultaneous selection for production, stability (HMGV), and adaptability (RPGV). This joint selection is given by:

$$HMRPGV = \frac{{}^{n}/\left(\sum_{j=1}^{n} x\ 1\right)}{Vg_{ij}} \quad (9)$$

where n represents the number of environments or harvesting seasons (n=3 harvesting seasons) and Vgij is the value of genotype i in environment j, expressed as a proportion of the mean in that environment (Viana and Resende 2014).

SELEGEN software was used for the REML/BLUP approach as well as for adaptability and stability (Resende 2016). Individuals were ranked according to the genotypic values found. From these values, the selection was applied for the most promising genotypes for each trait at the four harvests.

RESULTS AND DISCUSSION

Analysis of deviance revealed that the genotypes had significant effects on dry matter yield (Table 2), indicating variability between the evaluated genotypes. This suggests the potential for genetic gain through the selection of superior individuals with respect to this trait.

Table 2. Analysis of deviance for the dry matter yield trait in elephant grass genotypes evaluated at four harvests (Universidade Estadual do Norte Fluminense Darcy Ribeiro, municipality of Campos dos Goytacazes, RJ, Brazil, 2022).

Effect	Dry matter yield ($kg \cdot ha^{-1}$)	
	Dev	LRT
Genotype	4379.91	7.14**
Plot	4372.92	0.15ns
Genotype × Measurement	4394.46	21.69**
Full model	4372.77	

LTR Likelihood ratio test; *Signifcant at the 1% (6.63) probability level by the Chi-square test with one degree of freedom. Source: Elaborated by the authors.

The genotype x measurement interaction effects were highly significant. This result can be attributed to environmental factors, which confirmed the significance of the genotype x measurement interaction. Since these traits are quantitative and governed by multiple genes and environmental conditions, such an interaction was expected (Ambrósio et al. 2021; Vidal et al. 2022). The variation in precipitation between harvests resulted in varying yields at each harvest, exposing the plants to different environmental conditions. Consequently, the phenotypic expression of the traits varied across the different harvests, leading to a significant genotype x harvest interaction (Pereira et al. 2013).

This presents challenges in selection, as there is limited consistency among the best-performing genotypes in the evaluated harvests. To address this issue, a model that considers the genotype x harvest interaction is required to accurately recommend promising genotypes. The REML/BLUP methodology used in this study offers several advantages in this regard. It allows for the comparison of individuals or varieties across time (generations, years) and space (locations, blocks), simultaneous correction for environmental effects, estimation of variance components, and prediction of genetic values. Additionally, it can handle complex data structures, such as repeated measurements, different years, locations, and non-orthogonal designs and is particularly useful when dealing with unbalanced data (Viana and Resende 2014).

The analysis of variance components revealed the breakdown of individual phenotypic variance into genotypic variance, variance of genotype x measurement interaction, variance of permanent effects, and temporary residual variance. Notably, for the dry matter yield variable, genetic variance made a relatively small contribution (8.9421), while environmental effects, particularly temporary residual variance (27.7699), were predominant. This suggests a strong influence of environmental conditions on the trait, supported by the low broad-sense heritability at the individual level (Table 3). Nevertheless, the identified genetic variance (Vg) signifies a considerable genetic variability that can be leveraged for selection purposes. As elucidated by Cruz et al. (2012), lower genetic variance coupled with greater environmental effects leads to reduced trait heritability, as demonstrated by our results. Consequently, the expression of the trait is complex due to the involvement of numerous segregating loci that control it, while simultaneously being influenced by environmental effects. Therefore, comprehending the inheritance patterns and determinant components of trait variation is pivotal in the study of quantitative traits.

Table 3. Variance components as obtained by individual REML for the dry matter yield trait in elephant grass genotypes evaluated at four harvests (Universidade Estadual do Norte Fluminense Darcy Ribeiro, municipality of Campos dos Goytacazes, RJ, Brazil, 2023).

Variance components (Individual REML)	Dry matter yield (kg·ha^{-1})
Genotypic variance	8.9421
Permanent environmental variance	6.2669
Genotype x measurement interaction variance	16.5394
Temporary residual variance	27.7699
Individual phenotypic variance	59.5184
Broad-sense heritability at the individual level	0.1502
Repeatability at plot level	0.2555
Coefficient of determination of permanent effects	0.1052
Coefficient of determination of genotype x measurement interaction effects	0.2778
Genotypic correlation across measurements	0.3509
Heritability at the genotype-mean level	0.45433
Mean	22.0981

Source: Elaborated by the authors.

The obtained broad-sense heritability at the individual level for the dry matter yield variable was 0.15. At the genotype-mean level, a heritability value of 0.45 was observed. According to Resende (2016), heritability can be classified into low magnitude ($h < 0.15$), medium magnitude ($0.15 < h < 0.50$), and high magnitude ($h > 0.50$). In this study, the identified heritability values were considered to be of low and medium magnitude for the individual level and genotype-mean level, respectively. However, it is important to note that low and medium magnitude heritability values are expected, particularly for quantitative traits in perennial species that are susceptible to climatic variations over time. Resende (2016) highlights that low magnitude individual heritability is common for quantitative traits. Moreover, the utilization of mixed models for selection procedures in this study is justified since favorable genetic gains can be predicted even for traits with low heritability, and the genotypes under investigation possess selection potential.

The repeatability coefficient of the trait of interest enables the assessment of the time and labor required for the selection of genetically superior individuals. In this study, the repeatability at the plot level yielded low results (0.255), indicating that multiple repetitions will be necessary to achieve a satisfactory determination value. Additionally, the coefficient of determination of permanent effects (0.1052) suggests reduced environmental variability between plots.

For the purpose of selection, the 40 individuals were ranked and selected based on the evaluated trait (Table 4). The predicted genetic gains and the new estimated mean varied depending on the type of gain targeted in relation to the overall mean of the trait. Notably, the selection of genotypes for the agronomic trait yielded significant gains through individual BLUP estimates.

Table 4. Predicted genetic gain for dry matter yield considering the mean performance at four harvests (Universidade Estadual do Norte Fluminense Darcy Ribeiro, municipality of Campos dos Goytacazes, RJ, Brazil, 2023).

Rank no.	Genotype	Gain	New mean
1	17	3.8684	25.9665
2	18	3.8126	25.9107
3	36	3.6607	25.7589
4	32	3.5248	25.6229
5	16	3.3824	25.4806
6	31	3.2556	25.3538
7	15	3.0953	25.1932
8	6	2.9742	25.0723
9	10	2.7828	24.8809
10	27	2.6295	24.7276
11	35	2.4897	24.5879
12	21	2.3679	24.4660
13	19	2.2611	24.3593
14	34	2.1669	24.2651
15	40	2.0789	24.1771
16	1	1.9990	24.0972
17	3	1.9094	24.0075
18	11	1.8183	23.9164
19	24	1.7262	23.8244
20	14	1.6320	23.7302
21	5	1.5461	23.6443
22	38	1.4645	23.5627
23	4	1.3844	23.4825
24	20	1.3040	23.4022
25	8	1.2181	23.3162
26	28	1.1379	23.2361
27	37	1.0536	23.1518
28	23	0.9740	23.0722
29	22	0.8998	22.9979
30	30	0.8223	22.9205
31	25	0.7428	22.8409
32	39	0.6603	22.7585
33	26	0.5826	22.6808
34	7	0.5081	22.6063
35	33	0.4372	22.5353
36	29	0.3690	22.4671
37	2	0.2924	22.3906
38	13	0.2031	22.3013
39	12	0.1074	22.2055
40	9	0	22.0982

OM overall mean. Source: Elaborated by the authors.

In previous studies on sugarcane, the mixed models methodology has been employed to select superior genotypes for biomass production (Lucius 2014; Oliveira et al. 2008; 2011; Xavier et al. 2014). These studies have shown that selecting genotypes with genotypic values above the experimental mean can lead to substantial gains in sugarcane yield per hectare. Furthermore, using the REML/BLUP mixed models approach enables the identification of genotypes with high genotypic values, increasing the likelihood of selecting potential clones.

Although studies on elephant grass are limited, it is worth mentioning the work of Silva et al. (2020), who selected segregating plants for cloning elephant grass for energy production using REML/BLUP. The results identified 18 potential plants with the highest gain in dry matter yield, particularly progenies from the IJ7139 x Cameroon family showing notable gains in dry matter and neutral detergent fiber production.

Regarding the most promising genotypes, satisfactory predicted genetic gains were observed for dry matter yield (ranging from 3.8% to 0.1%). All individuals evaluated (100%) exhibited new means that surpassed the overall mean (22.0981) for the evaluated trait, indicating a high probability of finding promising new genotypes. Consequently, successful selection can be achieved when focusing on this trait.

Genotypes 17, 18, 36, 32, 16, 31, 15, 6, and 10 displayed the highest values of genetic gain, indicating their potential for selection. Notably, the production of dry biomass is a crucial trait when aiming to increase bioenergy production. Therefore, selecting a larger number of superior genotypes for biomass production is important as it enhances the probability of identifying individuals with superior bioenergy production potential.

The individual selection of these promising varieties led to the acquisition of significant gains. Consequently, the selected individuals are suitable for advancing elephant grass breeding to develop superior cultivars specifically for macro-regions with environmental conditions similar to those of the north and northwest regions of the state of Rio de Janeiro, Brazil. These selected varieties can serve as parents in new crosses or self-pollinations, contributing to ongoing breeding programs. Additionally, they can be cloned for VCU trials with the goal of releasing a new elephant grass cultivar for energy purposes.

It is worth emphasizing that these selected genotypes exhibited superior performance consistently across assessment harvests. This stability is advantageous considering that elephant grass, like other forage plants, is subject to seasonal variations (Cunha et al. 2011), resulting in fluctuations in yield throughout the year. With the possibility of harvesting twice per year, it is desirable to select genotypes that exhibit stable dry biomass production across the harvests.

Regarding the difference between the highest (25.9665) and the lowest (22.0982) new mean in the genotype ranking, there is a small amplitude for the trait. This narrow range is due to the compression of predicted means caused by REML/BLUP, which reduces the differences between genotypes, making them primarily attributable to genetic rather than environmental effects (Resende 2016).

Elephant grass undergoes multiple harvests during periodic evaluations, allowing for the identification of clones with high stability, adaptability, and suitability for energy production. Therefore, in terms of stability and phenotypic adaptability analysis, there is a consensus in the ranking of the most productive genotypes based on adaptability (RPGV), stability (HMGV), and both criteria simultaneously (HMRPGV) (Resende 2016; Neto et al. 2021).

In the stability analysis, genotypes 18, 17, 32, 36, 16, 6, 34, 15, 40, and 31 were found to be the most stable (Table 5). The harmonic mean of genotypic values (HMGV) simultaneously evaluates stability and productivity. Therefore, selection based on HMGV takes into account both attributes. By penalizing instability when genotypes are evaluated in different locations, the resulting new mean is adjusted accordingly. This approach ensures greater precision and accuracy in ranking genotypes within and between locations. Moreover, HMGV values represent the productivity values themselves, penalized for instability, which facilitates the selection of productive and stable genotypes. Considering the greater climatic instability and soil heterogeneity in tropical conditions, recommended cultivars should combine productivity and stability. Thus, the HMGV criterion fulfills these two premises of an ideal cultivar (Borges et al. 2010).

Adaptability refers to the ability of genotypes to respond advantageously to improved environmental conditions (Mariotti et al. 1976), making it a highly valuable trait sought after by breeders for new cultivars. In this context, genotypes 18, 17, 36, 32, 16, 6, 15, 31, 34, and 27 demonstrated greater phenotypic stability, indicating a reduced contribution to genotype × harvest interaction. These genotypes displayed higher genotypic adaptability associated with productivity, showing a favorable response to improved environments.

Table 5. Genotype x mean environment interaction (u+g+gem), stability of genotypic values (HMGV), adaptability of genotypic values (RPGV), and stability and adaptability of genotypic values (HMRPGV) for dry matter yield in elephant grass from the evaluation of 40 genotypes cultivated in four harvesting seasons (Universidade Estadual do Norte Fluminense Darcy Ribeiro, municipality of Campos dos Goytacazes, RJ, Brazil, 2023).

Genotype	u+g+gem	Genotype	HMGV	Genotype	RPGV *OM	Genotype	HMRPGV *OM
17	27.7553	18	23.2880	18	28.5183	17	27.5373
18	27.5921	17	23.1529	17	27.6594	18	27.4172
36	27.0075	32	21.6761	36	26.7125	32	26.2334
32	26.6562	36	21.6228	32	26.3586	16	25.8126
16	26.2119	16	21.4689	16	25.9740	36	25.7616
31	25.9322	6	21.4141	6	25.2724	6	25.0240
15	25.2150	34	20.6682	15	24.8703	15	24.7951
6	25.2105	15	20.4901	31	24.8345	31	24.3505
10	23.9285	40	20.4237	34	24.1075	34	23.8484
27	23.9260	31	20.0351	27	23.8802	27	23.8395
35	23.6955	1	19.9907	10	23.8719	10	23.6740
21	23.6008	27	19.9228	40	23.8295	40	23.4959
19	23.5315	10	19.9110	35	23.6709	21	23.4826
34	23.4758	21	19.8797	21	23.5390	35	23.4528
40	23.3363	19	19.7991	1	23.4866	1	23.4418
1	23.2690	35	19.5180	19	23.4762	19	23.4326
3	22.7935	3	19.3949	3	22.9477	3	22.9334
11	22.4925	24	18.7276	11	22.4338	24	22.0455
24	22.1998	11	18.6092	24	22.1513	11	21.7177
14	21.8674	38	18.2192	14	21.6875	5	21.5895
5	21.8454	28	18.1560	5	21.6820	38	21.5719
38	21.7350	5	18.0944	38	21.6684	14	21.4719
4	21.5431	4	17.9641	4	21.4260	4	21.4213
20	21.3025	14	17.9352	28	21.1993	28	20.9768
8	20.8631	8	17.3086	8	20.6308	8	20.5358
28	20.8312	7	17.2492	20	20.6246	20	20.4320
37	20.4358	25	17.2384	37	20.2653	37	20.2553
23	20.3785	33	17.1467	23	20.2135	23	20.1156
22	20.3740	23	17.0017	25	20.1715	25	20.0718
30	20.0159	37	16.9228	33	19.9975	33	19.7132
25	19.6935	20	16.8975	7	19.9952	22	19.6692
39	19.3278	26	16.6229	22	19.9808	7	19.5781
26	19.3139	22	16.0012	26	19.6795	26	19.5368
7	19.2437	29	15.8814	39	19.5110	30	19.2425
33	19.2117	39	15.8658	30	19.4756	39	19.1115
29	19.1471	30	15.5607	29	19.2130	29	19.0646
2	18.4956	2	15.4867	2	18.4963	2	18.4653
13	17.5630	12	14.0955	13	17.2726	13	17.0287
12	16.9338	13	13.9901	12	17.0038	12	16.8867
9	15.9745	9	13.7479	9	16.1376	9	16.1019

Source: Elaborated by the authors.

Genotypes 17, 18, 32, 16, 36, 6, 15, 31, and 34 exhibited both adaptability and stability, along with high dry matter yield, consistently across different harvests. These results suggest that the most productive genotypes also demonstrate stable responses and greater adaptability, particularly among the first nine genotypes selected. Therefore, the harmonic mean of the relative performance of predicted genotypic values (HMRPGV) method, based on predicted genotypic values using mixed models, integrates stability, adaptability, and productivity into a single statistic, facilitating the selection of superior genotypes (Borges et al. 2010; Regitano Neto et al. 2013).

It is worth highlighting that the HMGV, RPGV, and HMRPGV methods, as noted by Pinto Júnior et al. (2006), Resende (2007), and Resende (2016), are consistent in ranking genetic materials. These selection criteria contribute to the refinement of selection processes and provide reliable predictions of genetic values while considering productivity, stability, and adaptability (Streck et al. 2019). The corresponding HMRPGV values indicate the superiority of genotypes in relation to the mean of the environment in which they are grown, offering an estimate of expected productivity (Resende 2009). Such estimates are useful for planting in multiple locations with varying genotype-environment interaction patterns.

In a study involving full-sib families of elephant grass, Vidal et al. (2022) observed agreement between the HMGV, RPGV, and HMRPGV statistics in selecting the most productive, adaptable, and stable genotypes. The selected families were considered genetically superior due to their high productive potential, adaptability, and genotypic stability. These selected individuals can contribute to the advancement of elephant grass breeding, specifically targeting the development of superior cultivars for the north and northwest regions of the state of Rio de Janeiro.

Atroch et al. (2013), Ambrósio et al. (2021), Carvalho et al. (2020), Ambrósio et al. (2023) and Vidal et al. (2022; 2023a) emphasized that stability, adaptability, and yield (HMRPGV) should be the primary criteria for selecting the best genotypes/varieties/progenies. Therefore, characterizing elephant grass genotypes based on their patterns of adaptability and stability after the selection process for dry matter yield capacity is crucial for selecting genotypes to continue the breeding program. Based on the patterns of adaptability, stability, and productivity, superior genotypes can be identified for the development of full-sib, half-sib, and inbred families, thus ensuring the progress of the ongoing breeding program.

Furthermore, the results obtained in this study indicate that it is possible to utilize elephant grass as a bioenergetic plant. The varieties of elephant grass showed high dry matter production, which can be exploited for direct biomass combustion. Consequently, this biomass can be employed for direct combustion, generating energy in a more sustainable manner compared to fossil fuels. Moreover, cultivating elephant grass for this purpose presents environmental benefits, as its combustion releases carbon neutrally and contributes to efficient land use, as it can thrive in diverse conditions without competing with food crops. This approach holds significant potential to drive the global transition to cleaner and renewable energy sources.

CONCLUSION

The implementation of mixed models to estimate the harmonic mean of genotypic values successfully enabled the identification of genotypes that exhibited both stability and adaptability, while also displaying higher yield.

Specifically, genotypes/varieties 17, 18, 32, 16, 36, 6, 15, 31, and 34 demonstrated remarkable adaptability, stability, and notably high dry matter yield across multiple harvests. These findings highlight the prevalence of these desirable attributes in these genotypes/varieties.

The superior genotypes/varieties can be used to obtain full-sib, half-sib, and inbred families, allowing the continuity of the program under development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: Ambrósio M.; Daher R.F.; Methodology: Ambrósio M.; Gonçalves Junior D.H.; **Investigation:** Ambrósio M.; Gonçalves Junior D.H.; Leite C. L.; Freitas R.S.; Souza A.G.; Stida W.F.; Santos R.M.; **Data curation:** Daher R.F.; **Formal analysis:** Santana J.G.S.; Vidal A.K.; **Project administration:** Daher R.F.; Supervision: Freitas R.S.; **Writing - Review & Editing:** Ambrósio M.; Santana J.G.S.; Leite C. L.; Souza A.G.; Stida W.F.; Santos R.M.; Nascimento M.R.; Farias J.E.C.

DATA AVAILABILITY STATEMENT

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

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