

## A diallel model with repeatability information applied in an elephant grass breeding program

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**ABSTRACT:** Elephant grass is an allogamous perennial forage crop with asexual propagation, allowing plant breeders to explore heterosis and develop hybrids. However, selecting parents for diallel crossing schemes is a major hurdle in the development of hybrids. In addition, this perennial crop has several harvests, which increases the dataset size and the complexity of the statistical analyses. Here, we propose a diallel analysis based on linear mixed models with repeatability information to identify parents and hybrids, and the optimum number of harvests. We performed a complete diallel crossing of 11 parents with data from five harvests for morphological traits and two harvests for nutritive value traits. The diallel model with repeatability information aids in the estimation of the genetic effect of diallel analysis to infer the ideal number of harvests and genotype × harvest interaction. We observed that the specific combining ability (SCA) is more critical for morphoagronomic traits, while the general combining ability (GCA) has greater importance for traits related to nutritive value. Furthermore, we detected that five harvests were sufficient for the morphoagronomic traits with a coefficient of determination ( $R^2$ ) of 0.8; however, ten harvests were required for an  $R^2$  of 0.9. The model is a promising single-analysis alternative for plant breeders to analyze a diallel with repeated measures and to estimate important parameters without incurring in additional financial costs.

**Keywords:** forage, combining ability, heterosis, genetic selection, repeated measures

### Introduction

Elephant grass [*Pennisetum purpureum* Schumach. Syn. *Cenchrus purpureus* (Schumach.) Morrone] is an allogamous perennial forage grass native to Africa and has become an essential and widespread grass throughout tropical and subtropical regions in the world (Pereira et al., 2017). Breeding programs of allogamous species, such as elephant grass, aim to develop hybrids with high heterosis. The use of the diallel analysis allows estimating genetic parameters (i.e., general combining ability (GCA), specific combining ability (SCA), heritability, genetic variance) that can aid in identifying the most efficient selection method and provide information to select the best parents for hybridization (Cruz et al., 2012). However, few studies have explored hybrid vigor or the GCA and SCA of elephant grass (Sinche et al., 2021). These authors revealed promising results, with an increase of up to 52 % in hybrid annual biomass production compared to the highest-yielding parent.

Nevertheless, one of the difficulties for selection and evaluation programs is accurately determining the number of evaluations (harvests or grazing seasons) necessary to estimate differences among the genotypes evaluated. The repeatability model, also known as the compound symmetry model, offers an alternative to analyze datasets with various harvests. This model aids to estimate the capacity of organisms to repeat the expression of traits throughout time. High values of the

repeatability ( $r$ ) for any trait indicate that it is possible to predict the real value of individuals based on a certain number of measurements (Ferreira et al., 2021).

Therefore, estimating the repeatability coefficient allows for determining the number of measurements for each individual, providing an accurate assessment and/or a phenotypic characterization (Ferreira et al., 2021). Thus, estimating the repeatability coefficient of a trait is crucial, as repeated evaluations facilitate the quantification of phenotypic variance, which can be split into genetic variance, permanent environmental (plot) effects, and residual effects. Repeated measurements add precision and clarify the nature of the variation caused by the environment; therefore, repeatability is a valuable tool for forage breeders in the selection process.

However, the integration of a repeatability model with the diallel analysis via linear mixed model is little reported in the literature. A precedent for a repeatability model in a half diallel of cashew via a linear mixed model was proposed by Cavalcanti et al. (2007). Here, we extend this methodology by combining the diallel analyses with the repeatability model using the linear mixed model and considering residual heterogeneity across multiple harvests in a complete diallel design. In addition, we describe an application of the methodology to estimate the combining ability and the ideal number of harvests for nutritive value and morphoagronomic traits in elephant grass breeding programs.

## Materials and Methods

Field trials were evaluated in 2013 and 2014 at the Embrapa Gado de Leite facility in Coronel Pacheco, Minas Gerais State, Brazil (21°33'33" S, 43°15'45" W, 418 m). We selected 11 parent plants: four cultivars (Pioneiro, BRS Kurumi, BRS Canará, and BRS Capiaçú) and three clones (CNPGL 91-06-3, CNPGL 96-27-3, and CNPGL 92-38-2) from the Embrapa breeding program, in addition to four accessions from the Elephant grass Active Germplasm Bank of Embrapa (BAGCE 21, BAGCE 30, BAGCE 37, and BAGCE 38).

Hybridizations were performed by controlled crossings among the 11 parents under the scheme of a complete 11 × 11 diallel without parents and reciprocals. Seeds were sown in a polystyrene tray with 128 cells, each filled with a forest substrate, and seedlings were transplanted into the field 21 days after emergence. An experiment was performed using a randomized complete block design with three replications to assess the 55 hybrids. Each plot consisted of a 5-m long row with 0.5 m spacing between individual plants and 1.5 m between plots. Five harvests were taken from each plot, three during the rainy and two during the dry seasons.

The following morphoagronomic traits were measured: phenotypic vigor (VIG), obtained from a grading scale, which ranged from 1 to 5 (5 = high vigor; 1 = low vigor); height (HGT), obtained from the arithmetic mean of the height of three randomly selected plants in each plot, measured from ground level to the curve of the last completely expanded leaf; green mass production (GM), obtained from harvesting at 7.5 cm stubble height at 5 m of the rows using a gasoline-powered trimmer and that was collected by hand. The plot was immediately weighed in the field to estimate green biomass. Dry mass (DM) production was quantified by multiplying the green biomass by the DM concentration (%).

Two harvests were sampled, one each season, to evaluate the nutritive value. The *in vitro* digestibility of dry mass (IVDDM) was obtained based on the methodology described by Tilley and Terry (1963), while crude protein (CP) percentage, lignin (LIG) percentage, and neutral detergent fiber (NDF) were obtained based on the methodology proposed by Goering and Van Soest (1970).

The analysis for each harvest was performed using the following model:

$$y = \mu + X_1 b + Z_1 g + Z_2 s + \varepsilon \quad (1)$$

where:  $y$  is the phenotypic observations vector;  $\mu$  is the intercept;  $b$  is the blocks fixed effects vector;  $g$  is the GCA random effects vector, with  $GCA \sim MVN(0, \sigma_g^2 I_g)$ ;  $s$  is the SCA random effects vector, with  $SCA \sim MVN(0, \sigma_s^2 I_s)$ ;  $\varepsilon$  is the residuals random effects vector, with  $\varepsilon \sim MVN(0, \sigma_e^2 I_e)$ ;  $MVN$  is Multivariate Normal Distribution and  $X_1$ ,  $Z_1$ , and  $Z_2$  represent the incidence matrices for the respective effects.

The joint analysis of the harvests was also performed, assuming the following model:

$$y = \mu + X_1 c + X_2 b + X_3 c \times b + Z_1 g + Z_2 s + Z_3 g \times c + Z_4 s \times c + Z_5 perm + \varepsilon \quad (2)$$

where  $y$  is the phenotypic observations vector;  $\mu$  is the intercept;  $c$  is the harvest fixed effects vector;  $b$  is the blocks fixed effects vector;  $c \times b$  is the harvest × blocks interaction fixed effects vector;  $g$  is the GCA random effects vector, with  $g \sim MVN(0, \sigma_g^2 I_g)$ ;  $s$  is the SCA random effects vector, with  $s \sim MVN(0, \sigma_s^2 I_s)$ ;  $g \times c$  is the GCA × harvest interaction random effects vector, with  $g \times c \sim MVN(0, \sigma_{gxc}^2 I_{gxc})$ ;  $s \times c$  is the SCA × harvest interaction random effects vector, with  $s \times c \sim MVN(0, \sigma_{sxc}^2 I_{sxc})$ ;  $perm$  is the permanent effect random effects vector, with  $perm \sim MVN(0, \sigma_{perm}^2 I_{perm})$ ;  $\varepsilon$  is the residuals random effects vector, with  $\varepsilon \sim MVN(0, \oplus_{i=1}^c D_{e_i} \otimes I_{n_i})$ , where  $MVN$  is Multivariate Normal Distribution,  $D_{e_i}$  is a  $c \times c$  diagonal variance-covariance (VCOV) matrix, in which each harvest ( $c_i$ ) has a specific and independent variance component for the effect of replicates within sets and for the residuals, respectively; and  $X_1$ ,  $X_2$ ,  $X_3$ ,  $Z_1$ ,  $Z_2$ ,  $Z_3$ ,  $Z_4$ , and  $Z_5$  represent the incidence matrices for the respective effects.

For the random effects of the model, the significance of the likelihood ratio test (LRT) was tested using the chi-square test with one degree of freedom and alpha of 0.05.

The parameters of repeatability and ideal harvest number were estimated from the analysis. Repeatability ( $r$ ) was estimated using the following equation:

$$r = \frac{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_{perm}^2}{\hat{\sigma}_{phenotypic}^2}$$

where  $\hat{\sigma}_{phenotypic}^2$  is phenotypic variance at the individual level,  $\hat{\sigma}_g^2$  is the GCA variance,  $\hat{\sigma}_s^2$  is the SCA variance, and  $\hat{\sigma}_{perm}^2$  is the permanent variance. The  $\hat{\sigma}_{phenotypic}^2$  was estimated as suggested by Holland et al. (2003):

$$\hat{\sigma}_{phenotypic}^2 = 2x\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \hat{\sigma}_{perm}^2 + 2x\hat{\sigma}_{gxc}^2 + \hat{\sigma}_{sxc}^2 + \frac{\sum_{i=1}^c \hat{\sigma}_{ei}^2}{\sum_{i=1}^c C_i}$$

where  $\hat{\sigma}_{gxc}^2$  is the GCA × harvest interaction variance,  $\hat{\sigma}_{sxc}^2$  is the SCA × harvest interaction variance,  $\hat{\sigma}_{ei}^2$  is the residual variance of harvest  $i$ , and  $C_i$  is the number of harvests for which a trait was evaluated.

The ideal number of harvests ( $n_0$ ) was estimated for the coefficient of determination ( $R^2$ ) of 0.5, 0.6, 0.7, 0.8, and 0.9, using the following equation:

$$n_0 = \frac{R^2(1-r)}{(1-R^2)r}$$

where  $R^2$  is the coefficient of determination and  $r$  is the repeatability.

The heritability coefficient was also estimated from the analysis. Heritability by harvest ( $h^2$ ) was estimated using the following equation:

$$h^2 = \frac{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2}{2\hat{\sigma}_g^2 + \hat{\sigma}_s^2 + \frac{\hat{\sigma}_e^2}{b}}$$

where  $\hat{\sigma}_g^2$  is the GCA variance,  $\hat{\sigma}_s^2$  is the SCA variance,  $b$  is the number of blocks, and  $\hat{\sigma}_e^2$  is the error variance. All analyses were performed using the ASReml-R program (Butler et al., 2017).

### Results

The diallel analysis by harvest revealed that GCA was significant for VIG in harvests two and three, GM in harvest two, DM in harvest three, and HGT in all harvests, except for harvest five (Table 1). SCA was significant for all morphoagronomic traits, except for VIG in harvests one and HGT in harvests one, three, and five (Table 1). Since SCA was significant for all traits and almost all harvests, while GCA was only significant for a few harvests, suggesting a predominance of SCA for morphoagronomic traits. The diallel analysis for traits related to nutritive value, GCA was significant for all traits in all harvests, except for CP in harvest four. In contrast, SCA was not significant for any trait in any harvests (Table 2), suggesting GCA predominance for traits related to nutritive value.

The joint diallel analysis of the harvests was conducted using the repeatability model to estimate repeatability and to confirm SCA predominance in

morphoagronomic traits and GCA in nutritive value traits (Table 3). The joint analysis of morphoagronomic traits revealed GCA significance for HGT and SCA significance for DM (Table 3). However, the joint analysis of nutritive value traits showed that GCA was significant for NDF and CP, while SCA was only significant for NDF. In agreement with the individual analysis, GCA predominance was observed for the nutritive value traits.

The GCA × harvest interaction was significant for HGT, while the SCA × harvest interaction was significant for all morphoagronomic traits (Table 3). For the nutritive value traits, the GCA × harvest interaction was significant for NDF, as expected, as none of the traits was significant for the SCA × harvest interaction based on the SCA results from the individual analysis (Table 4). The permanent effect of the environment was significant for all morphoagronomic traits and was non-significant for all nutritive value traits (Tables 3 and 4).

Estimates of the GCA average effects ( $g_i$ ) (Table 5) were only calculated for traits in which the GCA was statistically significant using the LRT at the probability level of 5 % (Tables 3 and 4). Two parents obtained high positive values of  $g_i$  for HGT and VIG, namely CNPGL 91-06-3 and CNPGL 96-27-3. CNPGL 92-38-2 and BAGCE 38 obtained negative values for HGT and positive values for VIG (Table 5).

**Table 1** – Variance components for the individual diallel analysis of the five harvests (C1 to C5) for the morphoagronomic traits in the evaluation of elephant grass for forage production.

Variance components	VIG					HGT				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
GCA	0.016	0.0766*	0.0447*	0.0069	0.00001	0.0024*	0.0047*	0.0062*	0.001	0.00001
SCA	0.0234	0.0547*	0.0655	0.0993*	0.0442	0.0039	0.0032*	0.0029	0.003*	0.0029
Error	0.2167	0.2559	0.4178	0.2700	0.2559	0.0237	0.0112	0.019	0.0134	0.0187
$h^2$	0.43	0.71	0.53	0.56	0.34	0.52	0.77	0.71	0.53	0.32
Mean	3.62	3.05	3.56	3.72	3.05	1.61	1.61	1.70	1.80	1.61
Variance components	GM					DM				
	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
GCA	6.9664	6.7131*	3.2479	0.0000	0.0000	0.00001	0.0661	0.2383	0.00001	0.00001
SCA	24.2166*	15.4757*	23.6378*	38.7416*	32.6704*	0.5674*	0.6461*	0.9595*	1.2355*	0.7539*
Error	118.6615	26.7496	46.2391	91.8536	65.5643	2.3162	0.7744	1.7153	2.4012	1.8876
$h^2$	0.49	0.76	0.66	0.56	0.60	0.42	0.75	0.72	0.61	0.55
Mean	57.92	30.49	38.70	52.45	44.08	8.22	5.18	6.72	9.06	7.55

\*Significant by the likelihood ratio test at the level of 5 %. VIG = phenotypic vigor; HGT = height; GM = green mass production; DM = dry mass production; GCA = general combining ability; SCA = specific combining ability.

**Table 2** – Variance components for the individual diallel analysis of the harvests (C2 and C4) for the traits related to the nutritive value in the evaluation of elephant grass for forage production.

Variance components	CP		IVDDM		NDF		LIG	
	C2	C4	C2	C4	C2	C4	C2	C4
GCA	0.1076*	0.0222	0.5754*	0.6288*	0.6192*	0.3226*	0.0267*	0.0288*
SCA	0.1296	0.0095	0.9480	0.00001	0.00001	0.4108	0.0034	0.0466
Error	0.8709	0.4767	7.6472	8.3171	3.0223	2.3692	0.3415	0.3843
Mean	7.90	5.48	55.54	52.92	71.71	72.80	5.41	5.34

\*Significant by the likelihood ratio test at the level of 5 %. IVDDM = *in vitro* digestibility of dry mass; NDF = neutral detergent fiber; CP = crude protein; LIG = lignin; GCA = general combining ability; SCA = specific combining ability.

Estimates of the SCA average effects ( $S_{ij}$ ) (Table 6) were only calculated for traits that were statistically significant using the LRT in the joint analysis (Tables 3

**Table 3** – Variance components for the joint diallel analysis for the morphoagronomic traits in the evaluation of the elephant grass for forage production.

Variance Components	VIG	HGT	GM	DM
GCA	0.015	0.002*	1.173	0.0001
SCA	0.037	0.003*	21.39*	0.961*
Permanent	0.138*	0.008*	29.18*	0.550*
GCA × Harvest	0.007	0.0004*	0.765	0.029
SCA × Harvest	0.038*	0.001*	7.914*	0.223*
Harvest error 1	0.196	0.012	70.44	1.637
Harvest error 2	0.115	0.009	18.67	0.448
Harvest error 3	0.195	0.009	17.17	1.058
Harvest error 4	0.108	0.004	37.06	1.473
Harvest error 5	0.154	0.011	30.51	1.203

\*Significant by the likelihood ratio test at the level of 5 %. VIG = phenotypic vigor; HGT = height; GM = green mass production; DM = dry mass production; GCA = general combining ability; SCA = specific combining ability

**Table 4** – The joint diallel analysis for the traits related to the nutritive value in the evaluation of elephant grass for forage production.

Variance components	IVDDM	NDF	CP	LIG
GCA	0.330	0.347*	0.037*	0.013
SCA	0.248	0.379*	0.042	0.019
Permanent	0.174	0.0001	0.0001	0.008
GCA × Harvest	0.391	0.187*	0.009	0.011
SCA × Harvest	0.625	0.0001	0.079	0.034
Harvest error 2	7.594	2.798	0.881	0.291
Harvest error 4	7.402	2.331	0.365	0.363

\*Significant by the likelihood ratio test at the level of 5 %. IVDDM = *in vitro* digestibility of dry mass; NDF = neutral detergent fiber; CP = crude protein; LIG = lignin; GCA = general combining ability; and SCA = specific combining ability.

**Table 5** – Estimates of the average effects of the general combining capacity ( $g$ ) of the 11 parents of elephant grass for morphoagronomic traits in 55 hybrid combinations resulting from complete diallel crossings in the evaluation of elephant grass for forage production.

Parents	VIG	HGT	NDF	CP
BAGCE 21	-0.1145	-0.0426	-0.0768	0.0934
BAGCE 30	-0.0964	-0.0171	0.3005	0.1113
BAGCE 37	0.0257	0.0296	0.1013	-0.1154
BAGCE 38	0.0348	-0.0013	0.0844	-0.0672
Pioneiro	-0.1206	-0.0174	0.2236	-0.0869
CNPLG 91-06-3	0.0842	0.0458	0.1645	-0.1442
BRS Kurumi	-0.1094	-0.0772	-0.9749	0.3327
BRS Canará	0.0499	0.0108	0.9253	-0.2382
BRS Capiaçú	0.0781	0.0214	0.1149	0.0585
CNPLG 96-27-3	0.122	0.0485	-0.6748	0.0638
CNPLG 92-38-2	0.0462	-0.0006	-0.188	-0.0072

VIG = phenotypic vigor; HGT = height; NDF = neutral detergent fiber; CP = crude protein.

and 4) at the probability level of 5 %. Only three cases were positive for DM (BAGCE 21 × Pioneiro, BAGCE 38 × Pioneiro, and BAGCE 30 × BRS Capiaçú) (Table 6). These crossings showed segregation for dwarf genes allied to DM segregation.

The ideal number of harvests was estimated based on five levels (0.5 to 0.9) of the coefficient of determination ( $R^2$ ). The repeatability ( $r$ ) for each trait (DM, GM, HGT, and VIG) has the number of harvests on the y-axis and the coefficient of determination ( $R^2$ ) values on the x-axis (Figure 1). Importantly, repeatability was calculated only for traits significantly influenced by the permanent effect of the environment. An adequate number of harvests was found for all traits where  $R^2$  values were equal to 0.8. However, the double number of harvests should be taken for  $R^2$  values equal to 0.9 (Figure 1).

## Discussion

Elephant grass is an allogamous species, and the selection of parents poses one of the difficulties in developing hybrids. A hybrid vigor exploration conducted by Sinche et al. (2021) revealed promising results in elephant grass breeding, with an increase of up to 52 % in annual biomass production compared to the highest-yielding parent plant. To select parents for hybrids, breeders typically use the diallel design information. However, the statistical analyses should consider that several harvests comprise elephant grass, which is a perennial crop.

Thus, a diallel analysis model that facilitates GCA, SCA estimation as well as repeatability would be more appropriate than models without the repeatability component. In this study, we proposed a diallel analysis model with repeatability and applied this model to data from a complete diallel cross of elephant grass. The model accounts for the heterogeneity of residual variances and shows a better fit than the one that violates this assumption (Lower Akaike Information Criterion-AIC values). The results show the advantage of the linear mixed model over the ANOVA-based approaches in which homoscedasticity variance is a required assumption (Piepho et al., 2008; Hu et al., 2013).

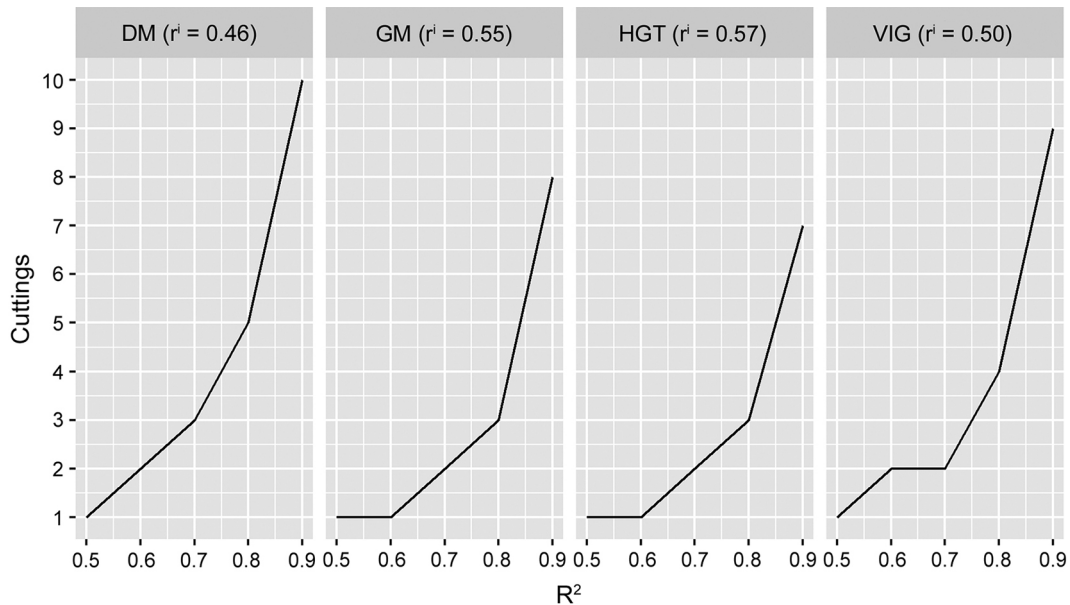
A high presence of the SCA effect was found for morphoagronomic traits but not for nutritive traits (Tables 1 and 2). The divergence and the dominance effects influence the specific combining capacity; therefore, the results presented here may be due to differences in dominance effects (Tables 1 and 2). Thus, since heterosis is positive, the presence of deviations in dominance indicates that the exploitation of the hybrid is favorable (Cruz et al., 2012). Morphoagronomic traits are otherwise directly influenced by natural selection, leading to a high divergence for these traits. However, natural selection is ineffective for nutritive traits, leading to low divergence, which influences the SCA effect.

**Table 6** – Estimates of the average effects of the specific combining capacity ( $S_{ij}$ ) in 55 hybrid combinations resulting from the diallel crossings between 11 parents of elephant grass in the evaluation for forage production.

Hybrids	HGT	GM	DM	NDF
BAGCE 21 × BAGCE 30	-0.0107	-3.4962	-0.6266	0.2581
BAGCE 21 × BAGCE 37	0.0419	0.6584	0.0172	-0.0383
BAGCE 21 × BAGCE 38	0.0231	1.431	0.3836	0.0211
BAGCE 21 × BRS Canará	0.0322	0.7638	-0.0575	0.1199
BAGCE 21 × BRS Capiaçú	-0.0077	-0.699	-0.3361	-0.2562
BAGCE 21 × BRS Kurumi	-0.1564	-9.3879	-2.0036	-0.3002
BAGCE 21 × Pioneiro	0.0003	-2.0408	-0.1938	0.6159
BAGCE 21 × CNPGL 91-06-3	0.0261	2.7867	0.6322	0.0799
BAGCE 21 × CNPGL 92-38-2	0.0165	1.1456	0.3058	-0.6641
BAGCE 21 × CNPGL 96-27-3	-0.0234	-0.4881	-0.1846	0.0798
BAGCE 30 × BAGCE 37	0.0264	1.1008	0.425	0.3878
BAGCE 30 × BAGCE 38	0.0145	5.5611	1.02	0.0628
BAGCE 30 × BRS Canará	-0.0327	-3.6157	-0.393	-0.3202
BAGCE 30 × BRS Capiaçú	-0.0081	2.2565	0.1968	0.1883
BAGCE 30 × BRS Kurumi	0.0081	0.3904	0.1506	0.0313
BAGCE 30 × Pioneiro	-0.0134	-6.2217	-1.2008	-0.8799
BAGCE 30 × CNPGL 91-06-3	0.0181	4.2575	1.0628	0.5574
BAGCE 30 × CNPGL 92-38-2	-0.0046	-3.2648	-0.7496	0.0853
BAGCE 30 × CNPGL 96-27-3	-0.021	-3.2384	-0.7124	-0.0427
BAGCE 37 × BAGCE 38	0.0083	0.1538	0.0664	0.1827
BAGCE 37 × BRS Canará	0.0183	3.1035	0.7003	0.1287
BAGCE 37 × BRS Capiaçú	0.0093	3.5737	0.7892	0.3781
BAGCE 37 × BRS Kurumi	-0.0282	1.3495	-0.0677	-0.7119
BAGCE 37 × Pioneiro	-0.0147	-2.6841	-0.3198	-0.4118
BAGCE 37 × CNPGL 91-06-3	-0.0478	-7.255	-1.4781	0.0067
BAGCE 37 × CNPGL 92-38-2	0.0268	4.7669	0.8949	0.0137
BAGCE 37 × CNPGL 96-27-3	0.0011	0.8824	0.381	0.1749
BAGCE 38 × BRS Canará	-0.0202	-2.334	-0.3661	0.2429
BAGCE 38 × BRS Capiaçú	-0.0421	-6.2637	-1.1539	-0.5498
BAGCE 38 × BRS Kurumi	-0.0026	2.7493	0.6441	0.2028
BAGCE 38 × Pioneiro	-0.0012	-0.7659	0.1451	0.6858
BAGCE 38 × CNPGL 91-06-3	-0.0177	-4.5935	-0.6837	0.2239
BAGCE 38 × CNPGL 92-38-2	0.0075	3.9779	0.4361	-0.2001
BAGCE 38 × CNPGL 96-27-3	0.0287	3.8369	0.6266	-0.7794
BRS Canará × BRS Capiaçú	-0.0102	-0.3358	-0.1252	-0.0569
BRS Canará × CNPGL 92-38-2	-0.0108	-1.2401	-0.2945	0.8872
BRS Canará × CNPGL 96-27-3	0.0137	1.1602	0.3368	0.0552
BRS Capiaçú × CNPGL 92-38-2	-0.0121	-1.8077	-0.6705	0.4474
BRS Capiaçú × CNPGL 96-27-3	0.0053	-1.2975	-0.3891	-0.519
BRS Kurumi × BRS Canará	0.0541	3.0776	0.4532	-0.236
BRS Kurumi × BRS Capiaçú	0.0613	1.3299	0.0349	0.2558
BRS Kurumi × CNPGL 92-38-2	-0.0355	-4.1525	-0.8189	0.3923
BRS Kurumi × CNPGL 96-27-3	0.0131	1.8607	0.1242	-0.2273
CNPGL 91-06-3 × BRS Canará	-0.0164	-2.0349	-0.0725	0.2551
CNPGL 91-06-3 × BRS Capiaçú	0.0042	-0.7403	-0.2214	0.0198
CNPGL 91-06-3 × BRS Kurumi	0.0023	5.4612	0.7452	-0.4832
CNPGL 91-06-3 × CNPGL 92-38-2	0.0121	3.4197	0.7498	-0.4846
CNPGL 91-06-3 × CNPGL 96-27-3	0.0472	6.9462	1.4864	-0.0325
CNPGL 96-27-3 × CNPGL 92-38-2	0.0128	5.7701	0.9095	-0.0801
Pioneiro × BRS Canará	-0.0129	-3.2831	-0.6925	-0.0654
Pioneiro × BRS Capiaçú	0.0297	0.7903	0.3251	0.2181
Pioneiro × BRS Kurumi	-0.0228	-3.8081	-0.7354	0.0118
Pioneiro × CNPGL 91-06-3	0.0351	4.33	1.0091	0.0369
Pioneiro × CNPGL 92-38-2	-0.0134	-1.3619	-0.2995	-0.6017
Pioneiro × CNPGL 96-27-3	-0.0107	-2.4778	-0.2058	0.6343

HGT = height; GM = green mass production; DM = dry mass production; NDF = neutral detergent fiber.





**Figure 1** – Estimates of repeatability ( $r$ ), and of the number of harvests for the coefficient of determination ( $R^2$ ) varying from 0.5 to 0.9 for morphoagronomic traits (VIG = phenotypic vigor; HGT = height; GM = green mass production; DM = dry mass production) in 55 hybrid combinations resulting from the diallel crossings between 11 parents of elephant grass,  $i$  is the target trait.

In both individual and joint analyses of the harvests, there was SCA predominance for morphoagronomic values (Tables 3 and 4). Menezes et al. (2015) and Silva et al. (2014) reported similar results in evaluations of the partial diallel of elephant grass. These studies found the SCA effect for most of the morphoagronomic traits. However, GCA was more important than SCA for traits related to the nutritive value. SCA highlights the importance of the nonadditive interactions resulting from gene complementation among parents, enabling heterosis exploitation (Menezes et al., 2015). Thus, hybridization is essential for the morphoagronomic traits of elephant grass.

This study showed a limitation, as our dataset we had only two harvests for nutritive value. However, our approach could be used for many harvests, as only two harvests for nutritive value could possibly affect the estimation of variance components. Nevertheless, we are convinced that the joint analysis provides valuable information for breeder decisions even with only a few harvests. As previously mentioned, elephant grass is a perennial crop, allowing farmers to conduct several harvests. Therefore, genotypes must perform highly and be stable throughout harvest (Rocha et al., 2018). Thus, for traits related to nutritive value, an absence of interaction is favorable for breeders since the hybrid showed no differences across harvests. In contrast, for morphoagronomic traits, the interaction hinders the selection, and new genotypes are recommended to be used by farmers across several harvests. The effects of the interaction can be mitigated through adjustments of the management strategies, such as changes in the irrigation, fertilization, and harvest scheduling, which

allow the interaction between the genotypes and the environment for better forage quality and yield (Ferreira et al., 2021).

The  $g_i$  provides information on the potential of parents to generate favorable combinations via the additive effects of alleles. It is challenging to select parents with high  $g_i$  values with the same signal and high  $S_{ij}$  values, which are all favorable to several traits (Tables 5 and 6). A few parents, namely BAGCE 21, BRS Kurumi, and CNPGL 96-27-3 stand out for traits related to nutritive value, as they have positive values for CP and negative values for NDF (Table 5). It is desirable in animal feed to have increased protein and digestibility, which is associated to a lower fiber percentage. However, it was impossible to find parents that were  $g_i$  positive for DM and CP (Tables 5 and 6), indicating that the correlation between these traits tends to be negative. Indeed, the mean phenotypic correlation between the evaluated traits showed a negative correlation between DM and CP (Table 7), indicating that one of the alternatives to obtain simultaneous gains in traits is using a selection index (Rocha et al., 2017).

The  $S_{ij}$  shows that 25 crossings were observed with high HGT estimates and positive DM values (Table 6). Combined with the  $g_i$  results, this result can provide breeders that aim to use elephant grass in a harvest schedule with a segregating population that displays a desirable high phenotype. However, a lower height is desired for forage used in pastures.

The statistical model adopted here allowed estimating all diallel analytical components, such as GCA and SCA, and their interactions with the harvests.

**Table 7** – Phenotypic correlation matrix among the traits in 55 hybrid combinations resulting from the diallel crossings between 11 parents of elephant grass in the evaluation for forage production.

Trait/Trait	VIG	HGT	GM	DM	IVDDM	NDF	LIG	CP
VIG	-	0.82	0.78	0.74	0	0.11	0.01	-0.25
HGT		-	0.66	0.68	-0.21	0.27	0.15	-0.37
GM			-	0.95	-0.02	-0.08	0.13	-0.04
DM				-	-0.2	0.07	0.21	-0.16
IVDDM					-	-0.38	-0.46	0.44
NDF		symmetric				-	0.21	-0.77
LIG							-	-0.05
CP								-

VIG = phenotypic vigor; HGT = height; GM = green mass production; DM = dry mass production; IVDDM = *in vitro* digestibility of dry mass; NDF = neutral detergent fiber; LIG = lignin; CP = crude protein.

Moreover, it was possible to estimate the permanent effects of the environment, which are essential for the morphoagronomic traits, affecting the heritability estimations and the number of harvests. For the nutritive value traits, where this parameter was not significant, the fact that we only evaluated two harvests may have affected the estimation of this parameter.

The repeatability ( $r$ ) can be estimated from the permanent effect obtained by the model, and  $r$  values above 0.8 are considered satisfactory when the coefficient of determination is equal to or greater than this value, according to Cruz et al. (2012). This type of model allows for a single analysis that estimates important parameters with no financial cost. In addition, this study shows that the mixed model methodology is flexible in providing breeders with important information on the best breeding strategy.

Repeatability estimates vary according to the trait nature, the population genetic properties, and the conditions under which individuals are maintained. Traits with low repeatability and with strong environmentally induced variation generally require many measurements to predict their real genotypic value (Cruz et al., 2012).

The values for repeatability ( $r$ ) and for the ideal number of harvests for a given coefficient of determination (Figure 1) obtained for the morphoagronomic traits were similar to those observed by Cavalcante et al. (2012), who evaluated different cultivars and non-recurrent crossing populations in a split-plot arrangement with four nitrogen levels. However, in a study to evaluate 16 elephant grass clones in 12 harvest cycles, Daher et al. (2004) found that the mean value of  $r$  was lower than that observed in this present study, clearly indicating the environmental influence on these traits.

For an  $R^2$  value of 0.8, five harvests were sufficient for the morphoagronomic traits, which is routinely practiced in elephant grass breeding programs; however, the required harvests should double for an  $R^2$  of 0.9. A study using the principal components method to determine the forage traits in elephant grass clones and to estimate repeatability obtained an  $r$  value of

0.53 and an  $R^2$  of 0.93 for average plant height. The study also showed that four height measurements were sufficient to obtain a coefficient of determination above 0.8 (Daher et al., 2004).

The number of harvests for the evaluation of traits related to nutritional value was not estimated in our study because the permanent effect of the environment was not significant. This can be explained by the fact that only two harvests were evaluated for these traits, possibly hindering the estimation of this effect. However, this analysis is labor intensive and involves high financial costs, influencing the viability of using it in many harvests.

The analysis used in this study, which combines the diallel model with repeatability information, allowed us to estimate all the necessary parameters for the genetic breeding program of elephant grass. The results presented here highlight that SCA was more important for morphoagronomic traits, while GCA was more important for traits related to nutritive value. The proposed analysis also enables the selection of parents and hybrids. Finally, the proposed model can facilitate the optimization of analytical procedures and provide information to improve the elephant grass breeding program, without incurring in any additional financial costs.

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## Authors' Contributions

**Conceptualization:** Ferreira RADC, Dias KOG, Machado JC. **Data curation:** Machado JC. **Formal analysis:** Ferreira RADC, Cruz CD, Silva FF, Dias KOG. **Funding acquisition:** Machado JC. **Investigation:** Ferreira RADC, Pessoa TV. **Methodology:** Ferreira

RADC, Pessoa TVS. **Project administration:** Machado JC. **Supervision:** Machado JC, Cruz CD. **Writing-original draft:** Ferreira RADC, Pessoa TVS. **Writing-review & editing:** Ferreira RADC, Pessoa TVS, Silva FF, Dias KOG, Carneiro PCS, Cruz CD, Machado JC.

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