

Applications of linear mixed models in *Cynodon* spp. breeding

Arthur Mayrink Elizeu¹, Marco Antônio Peixoto¹, Jeniffer Santana Pinto Coelho Evangelista¹, Saulo Fabrício da Silva Chaves¹, Emanuel Ferrari Nascimento¹, Flávio Benites², Paulo Eduardo Teodoro³, Kaio Olimpio das Gracas Dias¹ and Leonardo Lopes Bhering^{1*}

Abstract: Species of the genus *Cynodon* are among the most cultivated forage crops in the world due to their high yield and nutritional quality, and its use in cattle feeding has been associated with gains in animal weight and increased milk production. The objective of this study was to model covariance structures in *Cynodon* spp. clones and study the changes in ranking of the selected genotypes, since it is believed that affects the ranking of genotypes. A total of 202 genotypes were evaluated in an experiment conducted in an augmented block design with four replications and four harvests. The genotypes were assessed for plant height, green weight, percentage of dry matter, and plant vigor. Nineteen repeated measures models with different covariance structures were tested. The best-fitted model adopts the CORH covariance structure for the genetic effects. Correct modeling of the covariance structure affected the ranking of genotypes in all variables evaluated.

Keywords: Covariance structure, repeated measures, forage breeding, REML/BLUP

INTRODUCTION

Characterized by its rich genetic diversity and broad adaptation to different soil types, *Cynodon* is a genus of warm-season grasses globally distributed in tropical, subtropical and temperate regions (Singh et al. 2023, Soares et al. 2023). In Brazil, *Cynodon* spp. have been widely used as pasture forage for dairy cattle. Low production cost, ease of handling, good digestibility and palatability combined with grazing tolerance and high response to fertilization make *Cynodon* spp. a compelling forage choice for livestock (Araújo et al. 2018, Baxter et al. 2022).

In *Cynodon* breeding programs, the phenotype of a given genotype is assessed through repeated measurements over the crop cycle. However, harvesting the same individual over time shows the existence of correlations between measures (Kozak and Piepho 2018, Evangelista et al. 2023).


Mixed model provides a unified longitudinal data analysis strategy that facilitates the treatment of correlated experimental information, heterogeneous variances, and unbalanced databases (Chaves et al. 2021, Evangelista et al. 2023). Given these broad advantages, the mixed model methodology has been

Crop Breeding and Applied Biotechnology
24(3): e48592432, 2024
Brazilian Society of Plant Breeding.
Printed in Brazil
<http://dx.doi.org/10.1590/1984-70332024v24n3a27>



*Corresponding author:

E-mail: leonardo.bhering@ufv.br

 ORCID: 0000-0001-8820-0434

Received: 22 February 2024

Accepted: 10 April 2024

Published: 01 May 2024

¹ Universidade Federal de Viçosa, Avenida P H Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, MG, Brazil

² Embrapa Gado de Leite, Avenida Eugênio do Nascimento, 610, Aeroporto, 36038-330, Juiz de Fora, MG, Brazil

³ Universidade Federal de Mato Grosso do Sul, Agronomia, Rodovia MS 306, km 105, 79560-000, Chapadão do Sul, MS, Brazil

widely applied in perennial crop breeding programs (Faveri et al. 2015, Rocha et al. 2018, Shalizi and Isik 2019, Brito da Silva et al. 2020, Ferreira et al. 2020, Ferreira et al. 2021, Malikuski et al. 2021, Evangelista et al. 2023).

Multiple statistical approaches are adopted to predict effects and model the covariance structure and correlation between measurements. However, intermediate covariance structures may be more efficient for evaluating perennial crops. These structures can assume homogeneity or heterogeneity of variances and covariances between measures and can be applied to the several random factors in the statistical model (Marigulele et al. 2011, Faveri et al. 2015, Lara et al. 2019, Shalizi and Isik 2019, Evangelista et al. 2023).

Multivariate models are the most robust for analyzing repeated measurements, considering the correlation between crop seasons or measurements (Marigulele et al. 2011, Faveri et al. 2015, Shalizi and Isik 2019). However, fitting the model becomes challenging when considering more than three measurements due to the large number of parameters to be estimated and the high correlations between repeated measures, resulting in non-convergence in the variance component estimation process (Resende 2007, Resende et al. 2014).

The appropriate modeling of variance and covariance structures and temporal correlations between repeated measurements is essential for obtaining more accurate predictions of genetic and non-genetic effects (Faveri et al. 2015, Evangelista et al. 2023). Despite the advantages of using mixed model methodologies in perennial crop breeding, there are few studies involving the estimation of genetic parameters and longitudinal data analysis in forage species (Rocha et al. 2018, Lara et al. 2019, Ferreira et al. 2020, Ferreira et al. 2021), and none with *Cynodon*. Since it is believed that the correct modeling of the covariance structure affects the ranking of elite genotypes, the objectives of this study were to (i) model covariance structures in *Cynodon* spp. clones to find the structure that best represents the data evaluated and (ii) study the changes in the ranking of the selected genotypes when using the simplest and the best-fitted models.

MATERIAL AND METHODS

The experiment was conducted in the experimental field of Embrapa Dairy Cattle, Valença, Rio de Janeiro, Brazil (lat 22° 14 '44" S, long 43° 42' 01" W, alt 560 m asl) in 2012 using an augmented block design with four replications. Spaced 0.5 m meters apart, each experimental plot consisted of one plant. A total of 197 progenies from self-fertilization of the cultivar Grama Estrela Roxa and five commercial checks were evaluated. The five checks Florona, Porto Rico, Roxa, Tifton 68 and Tifton 85 were referred to as T1, T2, T3, T4 and T5, respectively. Four harvests were made to evaluate plant height (PH), green weight (GW), percentage of dry matter (DM) and plant vigor (PV).

Plant height (cm) was obtained from the arithmetic mean of three random measurements in each plot, measured from the ground level to the curve of the last fully expanded leaf. Plant green weight (kg plot⁻¹) was assessed by cutting 10 cm stubble height in the plots using a gasoline-powered trimmer and then the hand-harvested green biomass was weighed. The percentage of dry matter (%) was obtained by sampling plants from each plot, which were dried in a forced ventilation oven at 65 °C for 72 hours. The samples were weighed again (dry weight) and the dry matter percentage was determined by the ratio between the dry weight and the fresh green weight. Phenotypic plant vigor was rated on a visual scale given by three evaluators, ranging from 1 to 5, where 1 indicates low plant vigor and 5 indicates high plant vigor.

The mixed model methodology (restricted maximum likelihood/best linear unbiased prediction - REML/BLUP) was applied to estimate the variance components and to predict genotypic values, according to Patterson and Thompson (1971) and Henderson and Quaas (1976), respectively. The repeatability statistical model was given by:

$$y = Xm + Zg + Ti + Qp + e,$$

where $y^{(n \times 1)}$ is the vector of phenotypic data; $m^{(j \times 1)}$ is the vector of the measurement-repeat combinations effects (assumed as fixed), added to the overall mean; $g^{(ij \times 1)}$ is the vector of genotypic effects within measurement (assumed as random), $g \sim \text{NID}(0, \sigma_g^2)$; $i^{(ij \times 1)}$ is the vector of the genotypes x measurements interactions (random), $i \sim \text{NID}(0, \sigma_{gm}^2)$; $p^{(k \times 1)}$ is the vector of permanent plot effects (random), $p \sim \text{NID}(0, \sigma_p^2)$; and $e^{(n \times 1)}$ is the vector of residuals (random), $e \sim \text{NID}(0, \sigma_e^2)$. σ_g^2 is the genetic variance, σ_{gm}^2 is the variance of the genotypes x measurements interactions, σ_p^2 is the variance of the permanent plot effects and σ_e^2 is the residual variance. The capital letters $X^{(n \times j)}$, $Z^{(n \times ij)}$, $T^{(n \times ij)}$ and $Q^{(n \times k)}$ represent the incidence matrix for m , g , i and p effects, respectively.

The covariance structures tested to model the residual effects were Compound Symmetry (CS), Heterogeneous diagonal (DIAGH), First-order autoregressive heterogeneous structure (AR1H), Second-order autoregressive heterogeneous structure (AR2H), Third-order autoregressive heterogeneous structure (AR3H), Power structure (PWR), Heterogeneous power structure (PWRH) and unstructured (US). After modeling residual effects, the permanent plot effects were modeled by the covariance structures of identity (IDV), DIAGH, heterogeneous correlation (CORH), AR1H, AR2H and US. Lastly, the genotypic effect within measurement was modeled considering DIAGH, CORH, first-order analytical factor (FA1) and US covariance structures. A total of nineteen repeated measures models with different covariance structures were tested (Table 1).

For models with the same number of fixed effects, the residual likelihoods are comparable and therefore information criteria based on the residual likelihood can be used (Verbyla 2019). The goodness-of-fit of the models to the data was tested by the Akaike information criterion (AIC) (Akaike 1974), with the lowest AIC value indicating the best-fitted model. To model the block effect, considering the nature of this effect as fixed or random, a modification of the AIC, proposed by Verbyla (2019), was adopted.

The significance of the random effects of the best-fitted model was tested using the likelihood ratio test (LRT) (Rao 1973). This test evaluates the contribution of random effects to the model and provides the log of convergence (L) and deviation for the model with and without the effect to be tested. LRT also provides the difference between the deviations and compares the significance of this difference using the chi-square statistic with one degree of freedom and 0.01 of probability level.

Phenotypic variance ($\hat{\sigma}_{phen}^2$) and accuracy ($r_{\hat{g}g}$) were obtained according Resende et al. (2014):

$$\hat{\sigma}_{phen}^2 = \hat{\sigma}_g^2 + \hat{\sigma}_p^2 + \hat{\sigma}_{gp}^2 + \hat{\sigma}_e^2,$$

$$r_{\hat{g}g} = \sqrt{1 - \frac{PEV}{\hat{\sigma}_g^2}}$$

where $\hat{\sigma}_g^2$ is the genetic variance; $\hat{\sigma}_p^2$ is the variance of the permanent plot effect; $\hat{\sigma}_{gp}^2$ is the variance of the genotypes x measurements interaction and $\hat{\sigma}_e^2$ is the residual variance. *PEV* is the variance of the prediction error extracted from the diagonal of the generalized inverse matrix of coefficients of the mixed model equations.

The broad-sense heritability (\hat{H}_c^2) was estimated according to Cullis et al. (2006):

$$\hat{H}_c^2 = 1 - \frac{PEV}{2\hat{\sigma}_g^2},$$

The concordance of the selected genotypes in each pair of measurements was calculated using the Kappa coefficient (K) (Cohen 1960), given by:

$$K = \frac{A - C}{D - C}$$

Where *A* is the number of matching selected genotypes, *D* is the number of selected genotypes (20) and *C* is the number of selected genotypes by chance ($C = bD$, and *b* is the selection percentage). Statistical analyses were performed in Rbio (Bhering 2017) and R software (R Core Team 2024) using the ASReml-R 4.1 package (Butler et al. 2017).

RESULTS AND DISCUSSION

Table 1 (models M1 and M2) shows the results obtained by the Akaike information criterion (AIC) with adaptations from Verbyla (2019), when considering the block effect as fixed or random, for the traits plant height (PH), green weight (GW), percentage of dry matter (DM) and plant vigor (PV). The AIC (Akaike 1974) is widely used to select the best-fitted model (Resende and Alves 2020, Evangelista et al. 2023). This criterion makes it possible to compare models with the same number of fixed effects, considering the maximum value of the logarithm of the likelihood function and the number of parameters estimated by the model. The modification of the AIC, proposed by Verbyla (2019), allows the comparison between models with different numbers of fixed effects.

Verbyla (2019) proposes that by partitioning the total log-likelihood into two portions, a marginal (residual) likelihood and a conditional likelihood, the models evaluated become comparable even if they have a different number of fixed

effects. Thus, this modification made it possible to test whether the best-fitted model considers the block effect as fixed (M2) or as random (M1). For all the traits evaluated, the best-fitted model, according to the AIC modified by Verbyla (2019), was the one that considered block effect as a fixed. This choice corroborates Resende and Duarte (2007), who propose that if the number of blocks is less than or equal to five, it is preferable to treat this effect as fixed. Once the nature of the block effect had been defined as fixed, other covariance structures were tested for the other effects of the model, for the four traits evaluated (Table 1).

For the residual effects, the covariance structures that had the best fit were AR2H (M5) for plant height, DIAGH (M3) for dry matter percentage, AR1H for green weight, and AR3H for plant vigor. All these selected covariance structures suppose heterogeneity of variances, showing the effect of the environmental conditions on the residual effects of the harvests evaluated (Faveri et al. 2015).

Heterogeneity of variances and covariances is often found for perennial crops, however, these heterogeneities are usually not taken into account in studies involving perennial crops (Acharya et al. 2020, Brito da Silva et al. 2020, Rodrigues et al. 2020). Similar results have been observed in *Cynodon* and other perennial forage grasses with the adoption of the compound symmetry structure, which assumes homogeneity of variances (Ferreira et al. 2020, Ferreira et al. 2021).

Three out of the four traits evaluated showed an autoregressive heterogeneous structure (ARH) for the residual modeling (Table 1). This structure adopts a serial correlation as the component of the residual covariances between crop seasons and measurements. Autoregressive structures, especially AR1H, have been used in spatial analysis, considering that, as two individuals move away from each other, the correlation between them decreases (Andrade et al. 2020, Bernardeli et al. 2021). The same concept can be incorporated into studies of repeated measures over time (Faveri et al. 2015, Verbyla et al. 2021) since, as the interval between two measurements increases, the correlation between them tends to decrease, due to the environmental factors that influence the period of each harvest and the differences associated with the plant's maturity stage.

The use of the Akaike information criterion was effective in selecting the model that includes the appropriate covariance structure for the residual effects. The effectiveness of AIC has been proven in several previous studies (Faveri et al. 2015, Pereira et al. 2018, Cavanaugh and Neath 2019, Melo et al. 2020, Resende and Alves 2020, Evangelista et

Table 1. Values obtained by the Akaike information criterion (AIC) for all the models tested with the different covariance matrix structures and accuracy values

Models	Effect	COV	Plant height (PH)		Dry matter (DM)		Green weight (GW)		Plant vigor (PV)	
			AIC	r_{gg}	AIC	r_{gg}	AIC	r_{gg}	AIC	r_{gg}
M1 ⁺		CS	4841.12	-	2739.84	-	12591.73	-	-90.05	-
M2 ⁺⁺		CS	4779.98	0.89	2638.35	0.72	12349.57	0.59	-109.76	0.77
M3		DIAGH	4766.18	0.88	2607.73	0.70	12342.49	0.59	-155.11	0.80
M4	Res	AR1H	4742.53	0.80	2609.46	0.70	12296.27	0.58	-281.91	0.68
M5		AR2H	4736.32	0.88	2611.45	0.71	12301.88	0.48	*	-
M6		AR3H	*	-	2612.93	0.67	*	-	-285.14	0.69
M7		PWR	4766.16	0.87	2630.94	0.73	12339.60	0.59	-127.19	0.76
M10		IDV	4736.32	0.88	2607.73	0.70	12296.27	0.58	-285.14	0.69
M11	Plot	DIAGH	4740.68	0.88	*	-	12236.83	0.52	*	-
M12		CORH	*	-	*	-	*	-	*	-
M13		AR1H	*	-	2613.22	0.68	*	-	*	-
M16		DIAGH	4762.80	0.31	2616.94	0.60	12246.50	0.41	-261.62	0.45
M17	Gen	CORH	4734.85	0.86	2607.62	0.68	12220.71	0.63	-289.50	0.73
M18		FA1	4739.04	0.88	2612.15	0.73	*	-	*	-
M19		US	4741.74	0.83	2615.03	0.49	*	-	-286.13	0.76

Numbers in bold indicate the best-fitted model for each effect. COV: covariance structures; Res: residual effect; Plot: permanent plot effects; Gen: clone effect. CS: Compound Symmetry; DIAGH: Heterogeneous diagonal; AR1H: First-order autoregressive heterogeneous structure; AR2H: Second-order autoregressive heterogeneous structure; AR3H: Third-order autoregressive heterogeneous structure; PWR: Power structure; PWRH: Heterogeneous power structure; US: unstructured; IDV: identity covariance structure; CORH: heterogeneous correlation; FA1: first-order analytical factor. * Indicates the AIC of the models that did not converge. - Indicates the accuracy of the models that did not converge. M8, M9, M14 and M15 models are not shown in the table as they did not converge on any traits; *: compound symmetry model with block effect assumed as random; **: compound symmetry model with block effect assumed as fixed.

al. 2023). The best-fitted statistical model for the residual effects, identified by the lowest AIC value, was incorporated into the modeling of the permanent plot effect.

When modeling the permanent plot effect, the DIAGH structure had the best fit only for green weight, while the best-fitted model for the other traits was determined by the IDV covariance structure (Table 1). In fact, for green weight, there is likely to be heterogeneity in the variation of this effect over the crop seasons in *Cynodon* spp. and other perennial crops, due to the changing biotic and abiotic conditions that influence the differential expression of this trait. The better fit of a structure with homogeneous variance for the permanent plot effect in other traits can be explained by the fact that the intensity of this effect did not vary throughout the measurements of such traits.

The modeling of genotypic effects considered the best fit for the previously modeled effects. CORH was found to be the covariance structure that best fitted the model for all the traits evaluated. (Table 1). This structure indicates heterogeneity of variance between the measures but assumes that there is a correlation between them. The most complete model is the Unstructured as it allows particular predictions for each measurement by considering each of them as a separate variable (Piepho 1997). However, this approach becomes prohibitive when the number of measurements is high (five or more), leading to the model not converging (Mariguele et al. 2011). As multiple measurements are taken on the same genotype over time, adopting this structure to explain the genotypic effect makes biological sense as it is expected to find a correlation between measures from the same individual.

Selecting the best-fitted model also resulted in better estimates of selective accuracy for GW when compared to the simplest model, which assumes homogeneous variances (Table 1). The compound symmetry model had an accuracy of 0.59 and, when fitted, 0.63. In contrast, the other traits showed that selective accuracy does not increase when the best-fitted model is selected. PH had an accuracy of 0.89 for the simplest model and 0.86 after adjustment. DM showed accuracy values of 0.72 and 0.68 for the compound symmetry model and the heterogeneous correlation model, respectively. Finally, the selective accuracy of PV was 0.77 for M2 and 0.73 for the best-fitted model.

According to Resende and Alves (2020), the accuracy parameter has the property of informing the correct arrangement of genotypes for selection purposes, as well as inferring the reliability of genotypic values. For PH, DM and PV, accuracy did not increase when the best-fitted model was adopted (Table 1), but the accuracy value was classified as high for the simplest model (M2), which is normally used in repeatability analyses. However, it is important to consider that the accuracy values found for the compound symmetry model (M2) may be overestimated as the most appropriate structure was not used to represent the data studied, which influences the estimation of genetic and non-genetic parameters used in the calculation of selective accuracy.

For GW, which had the lowest values among the traits evaluated, the selective accuracy increased from 0.59 (M2) to 0.63 (M17) with the adoption of the best-fitted model (Table 1). For greater reliability in selecting promising genotypes, accuracy values above 0.70 are recommended (Resende and Alves 2020). Therefore, modeling the genetic and non-genetic effects for GW allowed reaching values closer to the recommended, and thus, together with other parameters that reflect reliability, selection can be conducted.

The significance of the genotypic variance was found in both the simplest model (M2) and the best-fitted model, which makes the selection practice valid. Considering the model selected by AIC, the best-fitted model assumes heterogeneous variances for almost all the effects (Table 2). The permanent plot effect was the only one that, after modeling, continued to adopt homogeneous variances for the PH, DM and PV traits. The fact that the permanent plot effect does not show heterogeneity of variances for most of the traits may be associated with the nature of this effect. As a genotype has constant behavior over multiple measurements, it makes sense for this variance to be constant throughout the measurements. GW showed heterogeneous variance for this same effect, with DIAGH as the best-fitting structure.

In general, the genotypic variance increased in most of the harvests when comparing the best-fitted model with the simplest model (Table 2). The genotypic variances differed across the measurements, showing that a model that adopts homogeneous variances, such as M2, does not represent the real nature of the data (Melo et al. 2020). In addition, modeling these matrices made it possible to identify the differential contribution of each effect to the phenotypic variance in the different harvests, which would not have been possible if only the simplest model had been considered.

The modeling of the genotypic effect was also reflected in the calculation of the average heritability, which increased

Table 2. Variance components and coefficients of determination of the simplest model and the best model selected by AIC

Trait	Model	Measure	Variance components					Genetic parameters		
			σ^2_g	σ^2_p	σ^2_{gp}	σ^2_e	σ^2_{phen}	h^2_g	ρ	r_{gg}
PH	M2	All	61.63	2.73	12.37	50.67	127.40	0.48	-	0.89
		1	57.74		-	78.65	142.16			
	M17	2	66.69		-	63.57	130.25			
		3	86.30	5.77	-	28.09	114.39	0.75	0.92	0.86
		4	52.10		-	49.73	101.84			
DM	M2	All	1.78	0.16	1.03	5.15	8.12	0.22	-	0.70
		1	2.31		-	6.50	9.05			
	M17	2	1.70		-	2.74	4.68			
		3	5.50	0.24	-	4.76	10.50	0.47	0.67	0.68
		4	1.73		-	6.29	8.26			
GW	M2	All	315178.4	461745.32	80290.1	308302.94	1165516.7	0.27	-	0.59
		1	552951.8	0.073 ^{ns}	-	965238.0	1518189.9			
	M17	2	496226.0	56754.71	-	885961.3	1438942.0			
		3	83988.87	269635.40	-	419604.0	773228.3	0.41	0.94	0.63
		4	272850.3	55429.34	-	518646.8	846926.44			
PV	M2	All	0.2921	0.1690	0.0434	0.1172	0.6217	0.47	-	0.77
		1	0.1389		-	0.5299	0.6688			
	M17	2	0.2760		-	0.5483	0.8243			
		3	0.3047	0.000 ^{ns}	-	0.2258	0.5305	0.54	0.91	0.73
		4	0.2357		-	0.2247	0.4604			

^{ns}: non-significant variance component.

considerably after the fitted-model strategy (Table 2). According to the classification proposed by Resende and Alves (2020), heritability exhibited high magnitudes ($0.50 < h^2_g < 0.80$) for PH and PV, indicating that the selection will be successful. For the DM and GW traits, even though this parameter remained in the moderate classification ($0.30 < h^2_g < 0.50$), modeling these effects resulted in an important increase in heritability. Another parameter that has already been discussed and that supports experimental precision is high accuracy (Tables 1 and 2). The accuracy values found in this study are classified as high (>0.70) according to Resende and Alves (2020) for most of the traits, showing, along with the heritability, the reliability of selection.

The prediction of genetic values is also influenced by the model adopted and its respective covariance structures (Lara et al. 2019, Verbyla et al. 2021, Evangelista et al. 2023). This influence acts directly on the ranking of the best genotypes as well as the individuals selected in each model (Table 3). It was identified that two of the 20 selected genotypes differed for the PH trait according to the model adopted. This difference increases even more for the other traits evaluated, with four different genotypes among those selected for DM and GW and five for PV (Table 3). The differences observed in the group of selected genotypes are 10%, 20%, 25% and 25% for the PH, DM, GW, and PV traits, respectively.

The selection of inferior genotypes due to the use of an inadequate model can result in reduced genetic gains. Thus, modeling genetic and non-genetic effects represents an efficient strategy for optimizing a breeding program and a powerful tool for selecting the best genotypes. Differences in predicted genetic values and changes in the ranking of selected individuals are concerns in breeding programs due to the risk of genotypes being wrongly selected. The importance of modeling covariance structures is directly associated with obtaining more reliable inferences about the reality of the data, as well as more accurate selection within a breeding program.

Modeling in the evaluation of different measures proved to be efficient in selecting superior individuals, similar to the results found in studies involving the evaluation of different crop seasons (Melo et al. 2020, Evangelista et al. 2023). The combined use of appropriate statistical methods and high-quality phenotyping enables the selection of truly superior genotypes and, consequently, the optimization of a breeding program (Stringer et al. 2017, Melo et al. 2020, Evangelista et al. 2023).

Table 3. Ranking of the 20 *Cynodon* spp. genotypes selected based on the genetic values obtained by the simplest (M2) and best-fitted (M17) models

PH		DM		GW		PV	
M2	M17	M2	M17	M2	M17	M2	M17
96	73	76	76	T4	T4	T4	T4
73	T1	52	52	T5	T5	11	T3
T1	96	152	152	11	T3	T3	T1
171	70	57	57	T3	11	T5	11
85	40	139	139	T2	7	T2	T2
T4	T4	134	45	7	128*	118	T5
131	171	161	54	118	T2	126	193
70	85	54	134	183	35	35	118
117	117	45	161	126	118	96	183
132	132	78	122*	35	145	7	41
40	131	118	118	41*	149	183	89*
119	T2	11	168	145	126	193	123*
12	119	166*	78	159	34*	134*	159
T2	193	133	187	149	161*	41	35
51	183*	155	11	96*	159	6*	30*
67	67	17*	155	12	183	81*	7
193	162*	187	154*	165	147*	165*	117*
72*	12	168	133	9*	12	T1	126
170*	180	130*	148*	193*	81*	149*	107*
180	51	19*	103*	134*	165	159	96

*: indicates genotypes that differ in the group of those selected in the different models.

According to the Kappa coefficient (K) (Cohen 1960), the concordances between the genotypes selected in pairs of measures for all the traits considering the best-fitted model ranged from 0.78 to 0.94. The concordances between the 10% best genotypes for the plant height trait in the four measures were: measure1 x measure2: 0.89; measure1 x measure3: 0.83; measure1 x measure4: 0.89; measure2 x measure3: 0.83; measure2 x measure4: 0.83; measure3 x measure4: 0.94. For dry matter percentage, the concordances of the selected genotypes were measure1 x measure2: 0.78; measure1 x measure3: 0.83; measure1 x measure4: 0.78; measure2 x measure3: 0.78; measure2 x measure4: 0.83; measure3 x measure4: 0.83. For green weight, the concordances were measure1 x measure2: 0.83; measure1 x measure3: 0.83; measure1 x measure4: 0.89; measure2 x measure3: 0.94; measure2 x measure4: 0.89; measure3 x measure4: 0.89. Lastly, for plant vigor, the concordances found were measure1 x measure2: 0.94; measure1 x measure3: 0.78; measure1 x measure4: 0.83; measure2 x measure3: 0.78; measure2 x measure4: 0.78; measure3 x measure4: 0.83.

It can be inferred that selection can be carried out on any crop, as the performance of the best genotypes based on the average of the four measurements reveals a pattern. There were only variations in the values obtained for the traits evaluated in each measure, few changes in the ranking order and a high degree of concordance of the genotypes selected for all pairs of measurements, as evidenced by the Kappa coefficient. As there is high concordance of the genotypes selected in each measure, selection based on the average of the harvests for each trait becomes feasible. As a result, significant gains were obtained in all traits evaluated in this study. The direct gain for PH was 21.19% when selecting the 10% best genotypes. GW, DM and PV showed direct gains of 37.19, 6.52 and 20.92%, respectively.

Modeling covariance structures and identifying the best-fitted model generate more reliable results when estimating variance components, predicting genotypic values, and selecting superior genotypes for the traits evaluated. In addition, it is possible to make reliable selections based on the average of the four harvests, which can facilitate decision-making in *Cynodon* spp. breeding programs.

CONCLUSION

Model 17 with heterogeneous compound symmetry (CORH) covariance structure was the best-fitted model for all the traits evaluated. When using the best-chosen model, the ranking of selected genotypes was changed, showing that this type of analysis should be used in breeding program.

ACKNOWLEDGEMENTS

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Finance Code 001), to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig).

DATA AVAILABILITY

The dataset analyzed during the current research is accessible in the CBAB repository, available at <https://data.scielo.org/dataverse/brcbab>.

REFERENCES

- Acharya JP, Lopez B, Gouveia BT, Oliveira IB, Resende MFR, Muñoz PR and Rios EF (2020) Breeding alfalfa (*Medicago sativa* L.) adapted to subtropical agroecosystems. **Agronomy** **10**: 742.
- Akaike H (1974) A new look at the statistical model identification. **IEEE Trans Autom Control** **19**: 716-723.
- Andrade MHML, Fernandes Filho CC, Fernandes MO, Bastos AJR, Guedes ML, Marçal TS, Gonçalves FMA, Pinto CABP and Zotarelli L (2020) Accounting for spatial trends to increase the selection efficiency in potato breeding. **Crop Science** **60**: 2354-2372.
- Araújo ED, Borges AC, Dias NM and Ribeiro DM (2018) Effects of gibberellic acid on Tifton 85 bermudagrass (*Cynodon* spp.) in constructed wetland systems. **PLoS One** **13**: 1-26.
- Baxter LL, Anderson WF, Gates RN, Rios EF and Hancock DW (2022) Moving warm-season forage bermudagrass (*Cynodon* spp.) into temperate regions of North America. **Grass and Forage Science** **77**: 141-150.
- Bernardeli A, Rocha JRASC, Borém A, Lorenzoni R, Aguiar R, Silva JNB, Bueno RD, Alves RS, Jarquin D, Ribeiro C and Lamas Costa MDB (2021) Modeling spatial trends and enhancing genetic selection: An approach to soybean seed composition breeding. **Crop Science** **61**: 976-988.
- Bhering LL (2017) Rbio: A tool for biometric and statistical analysis using the R platform. **Crop Breeding and Applied Biotechnology** **17**: 187-190.
- Brito da Silva V, Daher RF, Souza YP, Menezes BRS, Santos EA, Freitas RS, Oliveira ES, Stida WF and Cassaro S (2020) Assessment of energy production in full-sibling families of elephant grass by mixed models. **Renewable Energy** **146**: 744-749.
- Butler DG, Cullis BR, Gilmour AR, Gogel BG and Thompson R (2017) **ASReml-R reference manual version 4**. VSN International Ltd, Hemel Hempstead, 188p.
- Cavanaugh JE and Neath AA (2019) The Akaike information criterion: Background, derivation, properties, application, interpretation, and refinements. **Wiley Interdisciplinary Reviews: Computational Statistics** **11**: e1460.
- Chaves SFS, Alves RM, Alves RS, Sebbenn AM, Resende MDV and Dias LAS (2021) *Theobroma grandiflorum* breeding optimization based on repeatability, stability and adaptability information. **Euphytica** **217**: 211.
- Cohen J (1960) A coefficient of agreement for nominal scales. **Educational and Psychological Measurement** **20**: 37-46.
- Cullis BR, Smith AB and Coombes NE (2006) On the design of early generation variety trials with correlated data. **Journal of Agricultural, Biological and Environmental Statistics** **11**: 381-393.
- Evangelista JSPC, Peixoto MA, Coelho IF, Ferreira FM, Marçal TS, Alves RS, Chaves SFS, Rodrigues EV, Laviola BG, Resende MDV, Dias KOG and Bhering LL (2023) Modeling covariance structures and optimizing *Jatropha curcas* breeding. **Tree Genetics & Genomes** **19**: 21.
- Faveri J, Verbyla AP, Pitchford WS, Venkatanagappa S and Cullis BR (2015) Statistical methods for analysis of multi-harvest data from perennial pasture variety selection trials. **Crop and Pasture Science** **66**: 947-962.
- Ferreira FM, Bhering LL, Fernandes FD, Lédo FJS, Rangel JHA, Kopp M, Câmara TMM, Silva VQR and Machado JC (2021) Optimal harvest number and genotypic evaluation of total dry biomass, stability, and adaptability of elephant grass clones for bioenergy purposes. **Biomass and Bioenergy** **149**: 106104.
- Ferreira FM, Rocha JRASC, Alves RS, Elizeu AM, Benites FRG, Resende MDV, Sobrinho FS and Bhering LL (2020) Estimates of repeatability coefficients and optimum number of measures for genetic selection of *Cynodon* spp. **Euphytica** **216**: 70.
- Henderson CR and Quaas RL (1976) Multiple trait evaluation using relatives' records. **Journal of Animal Science** **43**: 1188-1197.
- Kozak M and Piepho HP (2018) What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. **Journal of Agronomy and Crop Science** **204**: 86-98.
- Lara LAC, Santos MF, Jank L, Chiari L, Vilela MM, Amadeu RR, Santos JPR,

Applications of linear mixed models in *Cynodon* spp. breeding

- Pereira GS, Zeng, ZB and Garcia AAF (2019) Genomic selection with allele dosage in *Panicum maximum* Jacq. **G3: Genes, Genomes, Genetics** 9: 2463-2475.
- Malikouski RG, Peixoto MA, Morais AL, Elizeu AM, Rocha JRASC, Zucoloto M and Bhering LL (2021) Repeatability coefficient estimates and optimum number of harvests in graft/rootstock combinations for “tahiti” acid lime. **Acta Scientiarum – Agronomy** 43: 1-10.
- Marigule KH, Resende MDV, Viana JMS, Silva FF, Silva PSL and Knop FC (2011) Métodos de análise de dados longitudinais para o melhoramento genético da pinha. **Pesquisa Agropecuária Brasileira** 46: 1657-1664.
- Melo VL, Marçal TS, Rocha JRASC, Anjos RSR, Carneiro RCS and Carneiro JES (2020) Modeling (co) variance structures for genetic and non-genetic effects in the selection of common bean progenies. **Euphytica** 216: 77.
- Patterson HD and Thompson R (1971) Recovery of inter-block information when block sizes are unequal. **Biometrika** 58: 545-554.
- Pereira FAC, Carvalho SP, Rezende TT, Oliveira LL and Maia DRB (2018) Selection of *coffea arabica* L. hybrids using mixed models with different structures of variance-covariance matrices. **Coffee Science** 13: 304-311.
- Piepho HP (1997) Analyzing genotype-environment data by mixed models with multiplicative terms. **Biometrics** 53: 761-766.
- R Core Team (2024) **R: A language and environment for statistical computing**. R Foundation for statistical computing. Vienna. Available at <<https://www.r-project.org/>>.
- Rao CR (1973) **Linear statistical inference and its applications**. Wiley, Hoboken, 625p.
- Resende MDV (2007) **Matemática e estatística na análise de experimentos e no melhoramento genético**. Embrapa Florestas, Colombo, 561p.
- Resende MDV and Alves RS (2020) Linear, generalized, hierarchical, bayesian and random regression mixed models in genetics/genomics in plant breeding. **Functional Plant Breeding Journal** 2: 1-31.
- Resende MDV and Duarte JB (2007) Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical** 37: 182-194.
- Resende MDV, Silva FF and Azevedo CF (2014) **Estatística matemática, biométrica e computacional: modelos mistos, multivariados, categóricos e generalizados (REML/BLUP), inferência bayesiana, regressão aleatória, seleção genômica, QTL, GWAS, estatística espacial e temporal, competição, sobrevivência**. UFV, Viçosa, 881p.
- Rocha JRASC, Marçal TS, Salvador FV, Silva AC, Machado JC and Carneiro PCS (2018) Genetic insights into elephantgrass persistence for bioenergy purpose. **PLoS One** 13: 1-16.
- Rodrigues EV, Rocha JRASC, Alves RS, Teodoro PE, Laviola BG, Resende MDV, Carneiro PCS and Bhering LL (2020) Selection of jatropha genotypes for bioenergy purpose: An approach with multitrait, multiharvest and effective population size. **Bragantia** 79: 346-355.
- Shalizi MN and Isik F (2019) Genetic parameter estimates and GxE interaction in a large cloned population of *Pinus taeda* L. **Tree Genetics and Genomes** 15: 46.
- Singh L, Wu Y, McCurdy JD, Stewart BR, Warburton ML, Baldwin BS and Dong H (2023) Genetic diversity and population structure of bermudagrass (*Cynodon* spp.) revealed by genotyping-by-sequencing. **Frontiers in Plant Science** 14: 1155721.
- Soares PR, Galhano C and Gabriel R (2023) Alternative methods to synthetic chemical control of *Cynodon dactylon* (L.). A systematic review. **Agronomy for Sustainable Development** 45: 51.
- Stringer JK, Atkin FC and Gezan SA (2017) Statistical approaches in plant breeding: Maximising the use of genetic information. In Campos H and Caligari PDS (eds) **Genetic improvement of tropical crops**. Springer International Publishing, Berlin, p. 1-320.
- Verbyla AP (2019) A note on model selection using information criteria for general linear models estimated using REML. **Australian and New Zealand Journal of Statistics** 61: 39-50.
- Verbyla AP, Faveri J, Deery DM and Rebetzke GJ (2021) Modelling temporal genetic and spatio-temporal residual effects for high-throughput phenotyping data. **Australian and New Zealand Journal of Statistics** 63: 284-308.