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ARTICLE

Genetic parameters and correlations of forage yield and nutritional quality in ruzigrass (*Urochloa ruziziensis*) half-sib families

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Abstract: *This study estimated genetic and phenotypic parameters, heritabilities, correlations, selection accuracy, and expected gain from selection for dry matter yield and nutritional traits in 178 half-sib ruzigrass families. Eight traits were evaluated, with nutritional quality measured via NIRS. Data analysis using mixed models and clustering revealed significant differences between progenies. Narrow-sense heritability ranged from 0.26 to 0.42, and selection accuracy ranged from 0.51 to 0.65. Genetic variation was lower for nutritional quality traits compared to dry matter yield. Dry matter yield had positive genetic correlations with neutral and acid detergent fiber and cellulose, but negative correlations with crude protein and hemicellulose. Clustering identified two groups of families: one correlated with in vitro dry matter digestibility and crude protein, and the other with remaining traits. Selecting for dry matter yield may reduce crude protein content but not affect digestibility; selecting for low fiber or high crude protein may improve forage quality.*

Keywords: *Forages, genetic correlation, spectroscopy, plant breeding*

INTRODUCTION

Grass forages play a significant role in Brazilian livestock production, particularly those belonging to the genus *Urochloa*, as they are present in vast cultivated areas and represent the highest quantity of commercially traded forage seeds (Valle et al. 2022). Ruzigrass (*U. ruziziensis*) has been widely used as a cover crop or forage in integrated crop-livestock-forestry systems in Brazil. It is sensitive to herbicides, allowing for the use of lower doses in pre-seeding desiccation of crops. Its deep root system decreases soil compaction. During rainy seasons, ruzigrass shows good regrowth capacity and rapid establishment. This species' advantages include good ground cover, high nutritional value, concentrated flowering period, high seed yields, and the possibility of being intercropped with legumes (Valle et al. 2022).

The species is allogamous and diploid with 2n=2x=18 chromosomes. *Urochloa* spp. breeding programs use tetraploidized ruzigrass in crosses with other closely related apomictic species to increase genetic variability and select superior apomictic hybrids with good *in vitro* dry matter digestibility, high levels of crude protein, and low levels of lignin and neutral detergent fiber compared Crop Breeding and Applied Biotechnology 24(4): e486124411, 2024 Brazilian Society of Plant Breeding. Printed in Brazil http://dx.doi.org/10.1590/1984- 70332024v24n4a48

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to other *Urochloa* species (Valle et al. 2022). Genetic improvement programs for ruzigrass usually target plants with higher biomass yield, forage quality, and persistence.

Knowledge about the genetic variability for breeding target traits in *U. ruziziensis*, and estimation of their genetic parameters, heritability, and correlations is crucial for the definition of efficient breeding strategies for this species (Simeão et al. 2016a). Estimating the heritability of target traits is valuable in anticipating selection gains, allowing for a more efficient choice of selection methods. Planning genomic selection efforts also depends on understanding the heritability and inheritance of target traits (Simeão et al. 2021). Assessment of the correlation between traits guides selection strategies for more effective gains in breeding programs (Figueiredo et al. 2012; Santos et al. 2023). Reports on the genetic variability, genetic parameters, and correlations for breeding target traits have been published for tetraploid *U. ruziziensis* half-sib families (Simeão et al. 2016a; Simeão et al. 2016b; Simeão et al. 2017). The genetic diversity of diploid ruzigrass germplasm accessions and populations collected in Brazil was assessed with microsatellite markers (Pessoa-Filho et al. 2015). Reports on genetic parameters for diploid *U. ruziziensis* for traits such as green biomass yield, plant vigor, and spittlebug resistance are also available (Teixeira et al. 2019, Dias et al. 2022, Resende et al. 2024). The breeding populations of these works have gone through several cycles of selection or severe bottlenecks during their initial establishment, due to a limited number of parents that were intercrossed. Reports on the genetic variability, genetic parameters, heritabilities and correlations for breeding target traits on a population of ruzigrass with broad genetic diversity are not available in the literature, to the best of our knowledge.

The objective of this study was to estimate genetic and phenotypic parameters, trait heritabilities and their correlations, selection accuracy and expected gain from selection for forage yield and nutritional quality traits in a population of half-sib families of ruzigrass, representative of the available genetic diversity for the species.

MATERIAL AND METHODS

The study was carried out in the experimental fields of Embrapa Cerrados, in Planaltina, Brasília, DF, Brazil (lat 15° 35' S, long 47° 42' W, alt 1,007 m asl), in an area of dystrophic clayey Oxisol and Aw climate according to the Köppen-Geiger classification. An initial population was established from the crossing of plants originating from the physical mixture of seeds from 13 ruzigrass accessions conserved in the Embrapa's Base Collection, as well as commercially available seeds and plants collected from pastures established for decades in the nearby state of Goiás (Pessoa-Filho et al. 2015). The seed mixture was sown in the greenhouse, and 2,000 seedlings were transplanted to the field in January 2019, with a spacing of 1 m between individual plants. Seeds were harvested in bulk to compose a diverse source population and were then sown in the greenhouse to obtain two hundred random mother plants without selection. The mother plants were transplanted in November 2019, with individual plant plots, without replicates, spaced 1.5 m apart. Open-pollinated seeds from each parent were individually harvested in May 2020 from matured inflorescences, which were processed in the Seed Analysis Laboratory of Embrapa Cerrados.

The evaluation of half-sibling families followed a randomized complete block design, with three replicates of 200 families in plots of 2 m² per family, totaling 600 plots. Each plot consisted of eight plants, spaced 25 cm apart, with 1 m spacing between plots. Two cuts of the aboveground biomass were performed with a hedge trimmer to assess forage yield at a height of 15 cm from the soil during the rainy season on February 21 and May 16, 2022.

Green matter (GM) was weighed in the field (kg/plot), and the samples were dried out at 65 °C for 72 hours for dry matter yield (DMY) estimation. For bromatological and chemical analyses, dried samples were ground in a Wiley mill (A.H. Thomas Co., Philadelphia, PA) with a 1-mm mesh. Spectra were obtained using a FOSS NIR 5000 System II type 461006 equipment (FOSS Analytical SA, DK 3400 Hilleroed, Denmark) with ISIScan v.2.85.3 software (ISI Software, FOSS Analytical AB, Höganäs, Sweden), with approximately 2 g of homogenized samples placed in ring cup cells with a quartz window and closed with foam cardboard rings. The equipment captures spectra in a wavelength range of 1100 to 2500 nm with 2-nm resolution. Calibrated models in The Unscrambler® X v.10.5.1 program (CAMO Software AS, Oslo, Norway) were used for estimating neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD), lignin (LIG), cellulose (CEL), and hemicellulose (HEMICEL) from the obtained spectra.

Twenty-two families were lost after transplanting, and 178 were used for statistical analyses without missing data. Observations for each plot were averaged across the two cuts to obtain a single data matrix, considering that, on balanced data, this approach is suitable for estimating genetic parameters (Resende 2007). Normality of residuals, variance homogeneity, and autocorrelation of residuals were verified with Shapiro-Wilk, Levene's and Fligner-Killeen, and Durbin-Watson tests, respectively. Estimation of variance components for each trait was performed with Selegen-REML/BLUP (Resende 2016), using a model for the evaluation of half-sib progenies (model 19), with one observation per plot, as follows:

Y = *X_r* + *Z_a* + *e*, where *Y* is the vector of observed traits, *r* is the vector of the fixed effects of blocks added to the overall mean, *α* is the vector of random additive genetic effects, *a* ~ $N(0, I\sigma_q^2)$, and *e* is the vector of random residuals, *e* ~ *N*(0,/σ_e); *X* and *Z* are incidence matrices of effects; σ² and σ² are additive and residual variances, respectively.

Narrow-sense heritability (*h*²) was calculated for each trait based on the phenotypic means through the formula $h_{PFM}^2 = \frac{\sigma_F^2}{2}$ $\sigma_F^2 + \frac{\sigma_e^2}{r}$, where $\sigma^2_{\rm F}$ is the variance component of families, $\sigma^2_{\rm e}$ is the experimental error variance, and *r* is the number

of blocks (Ńguyen and Sleper 1983). Selective accuracy between progenies was estimated as $\hat{r}_{\rho\rho} = \left(1 - \frac{PEV}{\sigma_o^2}\right)^{1/2}$, where PEV

is the predictive error variance (Resende 2007) and σ_q^2 is the genetic variation among progenies. The genetic coefficient *a* and productive enter variance (necessare ECC), and σ_g is the galletic random allocated the genetic variation, and the relative of variation between progenies was estimated as $CV_{gp} = 100 \sqrt{\frac{0.25 \cdot \sigma_g^2}{m}}$, where coefficient of variation was calculated as $CV_r = \frac{CV_{gp}}{CV_e}$, where CV_e is the environmental coefficient of variation. Expected

gains from direct selection for each specific trait on a plot mean basis were calculated using the formula ΔG = ck $\frac{\sigma_F^2}{2}$

σ*PFM* , where *c* = parental control factor (c = 2 for half-sib progeny test selection, where parents are selected based on the mean performance of their half-sib progenies and then crossed in isolation to obtain a new improved population), *k* = standardized selection differential (3.51 for 5% selection pressure), σ² is the variance component of families, and σ_{ρεΜ} is phenotypic standard deviation (Nguyen and Sleper 1983, Carena et al. 2010). Phenotypic correlations between traits were estimated from observed phenotypic values. Genetic correlations between traits were estimated as $r_{a(x,y)} = \frac{cov_{a(x,y)}}{g}$ σ*ax* σ*ay* , where *cova*(*x*,*y*) is the additive genetic covariance between traits *x* and *y*; and σ*ax* and σ*ay* are the additive

genetic standard deviations for traits *x* and *y* (Falconer and Mackay 1996). The significance of correlations was evaluated with Student's t-tests.

Principal component analysis (PCA) was performed to visualize the correlations between measured traits and the distribution of half-sib families on the dimensional space. Families were grouped using k-means clustering. The optimal number of clusters was estimated with the R package *NbClust* (Charrad et al. 2014) using the Kmeans and Euclidean distance methods. The PCAs were performed and visualized in R using packages *tidyverse* (Wickham et al. 2019), *factoextra* (Kassambara and Mundt 2020), *FactoMineR* (Lê et al. 2008), *corrplot* (Wei and Simko 2021), and *ggcorrplot* (Kassambara 2023).

RESULTS AND DISCUSSION

Genetic variability for yield and quality traits

Significant differences were observed among families for all measured traits (Table 1). Average DMY in this experiment $(0.51 \text{ kg plot} \cdot 10^{-1} \text{ or } 2.6 \text{ tons} \text{ ha} \cdot 1)$ was lower than DMY previously reported for ruzigrass breeding populations and genotypes. For instance, the observed DMY for the commercially available ruzigrass cultivar known as Kennedy was higher than the DMY for the half-sib families evaluated in this work (Souza Sobrinho et al. 2011b). In an integrated system, the average biomass yield for ruzigrass 30 days after maize sowing was reported as 3.6 tons ha⁻¹ (Silva et al. 2010). Different experiments in Southeastern Brazil have reported DMY values of 3.6 tons ha⁻¹ (Souza Sobrinho et al. 2011a), and 3.51 tons ha-1 (Souza Sobrinho et al. 2011b). The recently released BRS Integra cultivar has shown dry matter yields around 4.5 tons ha⁻¹ 45 to 50 days after winter/spring grazing (Paciullo et al. 2022, Rodrigues et al. 2023). The lower average DMY observed in our work likely reflects the broad genetic pool of the half-sib families evaluated in our experiment, without prior selection for any breeding targets.

In this work, the values for nutritional traits were higher than those previously observed for whole *U. ruziziensis* plants

Table 1. Means, standard deviations, narrow-sense heritability ($h^2_{\rho_{FM}}$), selection accuracy and coefficients of variation of eight phe-
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F-test between families: *** p<0.001; ** p<0.01; * p < 0.05. All heritability estimates greater than 2 SE are significant. ¹ DMY = dry matter yield, CP = crude protein, IVDMD = in vitro dry matter digestibility, NDF = neutral detergent fiber, ADF = acid detergent fiber, LIG = lignin, CEL = cellulose, and HEMICEL = hemicellulose.

after 52 days of growth, for which 57.82% for IVDMD and 6.60% for CP were reported (Souza Sobrinho et al. 2011b). Our study showed values for IVDMD and CP that were lower than those reported for cv. Kennedy (Mauri et al. 2015) and for a tetraploid ruzigrass breeding population (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017). The mean for CP observed in this study is consistent with results reported in a meta-analysis covering species of tropical pastures in various environments. The *Urochloa* genus showed CP ranging from 7.36 to 16.4%, depending on the environment type (Jayasinghe et al. 2022). Improved populations of ruzigrass evaluated in Minas Gerais, Brazil, had mean CP values of 14.23% (Marcelino et al. 2020). The BRS Integra cultivar showed an average CP content of 13.85% (Souza Sobrinho et al. 2022). For IVDMD, the overall mean reached 63.21%, higher than previously reported for tetraploid ruzigrass (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017). Improved populations of ruzigrass and BRS Integra showed means for IVDMD of 68.8% (Marcelino et al. 2020), and 69.07% (Souza Sobrinho et al. 2022).

For fibrous components, the NDF mean for the studied population (62.82%) is similar to the means of improved populations of *U. ruziziensis* (62.69%), Kennedy (61.86 %) and BRS Integra (62.56%) and lower than the averages of 66.15% for *U. brizantha* cv. Marandu (Marcelino et al. 2020, Souza Sobrinho et al. 2022), and 65.36% for tetraploid ruzigrass (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017). The ADF mean (33.84%) is close to the levels found in improved populations of *U. ruziziensis* (31.57%), cv. Kennedy (30.26%), BRS Integra (32%), and *U. brizantha* cv. Marandu (34.81%) (Marcelino et al. 2020, Souza Sobrinho et al. 2022), and in tetraploid ruzigrass (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017). Average lignin and cellulose were lower than mean values previously reported for ruzigrass clones (Marcelino et al. 2020) and tetraploid ruzigrass (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017).

The coefficients of genetic variation among progenies ranged between 0.77 for NDF and 12.83 for DMY. Low levels of CVgp for nutritional quality traits were previously reported for a tetraploid ruzigrass breeding population (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017), and values for CP and NDF were higher in this study. The genetic variation among progenies for DMY was lower than previously reported for tetraploid ruzigrass (Simeão et al. 2016a, Simeão et al. 2016b, Simeão et al. 2017). The low values of CVgp reported in this study for yield, nutritional traits and fibrous components were likely affected by the timing of the cuts of our experiment, which were both carried out during the wet season, not fully capturing the phenotypic plasticity of the species and the differentiation among progenies. Future experiments with a higher number of harvests in different seasons will allow a better assessment of the available genetic variability for target traits in this population.

Heritability, selection accuracy and expected gains from selection

Estimates of narrow-sense heritability ranged between 0.26 for DMY and LIG and 0.42 for IVDMD, suggesting a strong environmental influence on the evaluated traits (Table 1). The narrow-sense heritability for DMY was 0.30 for tetraploid *U. ruziziensis* half-sib progenies (Simeão et al. 2017), higher than the estimate of 0.26 observed in our study. Low heritability (0.11) was also reported for dry matter yield in progenies of the second cycle of intrapopulation selection in autotetraploid *U. ruziziensis* (Simeão et al. 2022). Narrow-sense heritability estimates for nutritional quality traits in tetraploid *U. ruziziensis* ranged between 0.04 for IVDMD and NDF to 0.24 for LIG (Simeão et al. 2016a), and were lower

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than the estimates reported in this study. Traits with low heritability call for the efficient use of all genetic information available in experiments and more efficient and accurate breeding methods (Resende et al. 2013). Selection methods targeted at perennial forages make use of between-family and within-family effects for higher selection gains in traits with low narrow-sense heritability (Simeão et al. 2021). Selection accuracies ranged between 0.51 and 0.65 (Table 1) and indicate the need of increasing the number of replications or the number of repeated measurements (harvests) in future experiments.

Results for the expected gains from selection are shown in Table 2. Selection with a 5% intensity resulted in estimated increases for DMY of 0.23 kg plot⁻¹, 1.24 percentage points for CP, and 3.75 percentage points for IVDMD. For fiber components, decreases of 1.91 percentage points for NDF, 1.86 percentage points for ADF, 0.20 percentage points for LIG, 1.79 percentage points for CEL and 1.04 percentage points for HEMICEL were estimated.

Principal Component Analysis and trait correlations

The first two dimensions of the PCA explained approximately 75% of the cumulative variance among families, which were distributed into two clusters. This distribution suggests differences among families, with each grouping possibly related to variations within the studied components, enabling a more detailed analysis between them. A positive correlation was observed between DMY, CEL, ADF, NDF, LIG, HEMICEL, and families included in Cluster 1. A positive correlation was observed between CP, IVDMD, and families included in Cluster 2. Additionally, CP and IVDMD were negatively correlated with DMY and the fibrous components CEL, ADF, NDF, LIG, and HEMICEL (Figure 1).

The values of phenotypic and genetic correlations between the studied traits based on the mean of the two harvests are presented in Table 3. Phenotypic and genetic correlations were mostly congruent with a few exceptions. For instance, DMY and ADF showed no phenotypic correlation, but had a positive and weak genetic correlation. The phenotypic correlation between ADF and HEMICEL was positive and weak, but their genetic correlation was not significant. Dry matter yield (DMY) showed positive but weak genetic correlations with fibrous components. Nonsignificant genetic correlations were observed between DMY and IVDMD and LIG. The results in Figure 1 and Table 3 confirm a negative but weak genetic correlation between DMY and CP. We found no genetic correlation between DMY and IVDMD, indicating that selecting for higher forage yield will not impact forage digestibility. Crude Protein showed a weak positive genetic correlation with IVDMD and negative correlations with the fibrous components, being strong with NDF (-0.87), ADF (-0.76), and CEL (-0.76), and weak with LIG (-0.29) and HEMICEL (-0.42), which indicates that, in this population, an increase in protein content will contribute to an increase in digestibility and a decrease in fiber content. The IVDMD showed a strong and negative genetic correlation with LIG (-0.76), moderate and negative correlation with NDF (-0.49) and HEMICEL (-0.57), weak negative correlation with ADF (-0.24) and with CEL (-0.14). A strong and positive genetic correlation between CP and IVDMD was identified in *Urochloa brizantha* (Torres et al. 2016), which led the authors to suggest IVDMD as an indirect selection criterion for CP. The correlation between these traits was not significant for tetraploid *U. ruziziensis* half-sib progenies (Simeão et al. 2016a), but was positive and significant, although weak, in this work.

Table 2. Mean, mean of selected families with 5% selection pressure (MSF 5%), selection gain (∆G), and expected mean in the next generation (EMNG 5%) for eight traits in half-sib progenies of *Urochloa ruziziensis*

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Phenotypic correlations ranged between -0.89 (NDF and CP) and 0.99 (ADF and CEL), with the lowest values being -0.89, -0.78, and -0.77 between CP and the fibrous components NDF, CEL, and ADF. Among the positive values, the correlations between ADF and CEL (0.99), ADF and NDF (0.87), and NDF and CEL (0.85) stood out. A study on *U. decumbens* or *U. brizantha* x *U. ruziziensis* hybrids showed a negative simple correlation between DMY and CP (-0.24), a moderate negative correlation between CP and NDF (-0.67), and positive correlations between NDF and LIG (0.53) and between CP and IVDMD (0.51) (Gouveia et al. 2020). Their results indicate that an increase in total DMY may be positively correlated with yields of leaves with high nutritional value (Gouveia et al. 2020). That study also showed that leaf dry matter, total dry matter weight, and regrowth capacity are interconnected and may be considered in selecting individuals of higher quality (Gouveia et al. 2022).

Figure 1. PCA correlation of the variables under study and their respective clusters, consisting of 178 half-sib families of *Urochloa ruziziensis*. Where DMY = dry matter yield, CP = crude protein, IVDMD = *in vitro* dry matter digestibility, NDF = neutral detergent fiber, ADF = acid detergent fiber, LIG = lignin, CEL = cellulose, and HEMICEL = hemicellulose.

*** p<0.001; ** p<0.01; *p<0.05; 1 See Table 1

Our results suggest that selection for DMY may decrease forage quality in *U. ruziziensis*, reducing CP values and increasing indigestible fiber content. On the other hand, as cover crops, the increase in DMY at the expense of quality may be positive in interfering with weed germination, emergence, and establishment. Studies show that approximately 4,000 kg ha⁻¹ of dry matter from *U. ruziziensis* are sufficient to suppress weeds when they interfere the most in corn cultivation, thus reducing herbicide application during this phase (Paciullo et al. 2022).

In the genotypes studied, our results show the possibility of selection aiming at plants with higher bromatological quality for animal feed, emphasizing materials with high CP and IVDMD levels and lower quantities of fibrous components. It also highlights genotypes that are more adapted to producing higher amounts of dry matter and are suitable for use as cover crops for mulching.

Ruzigrass families evaluated in this study vary considerably in forage yield and nutritional quality. Therefore, there is sufficient variability to make progress in breeding programs for both biomass yield and forage quality. Negative correlations between forage yield and nutritional quality in ruzigrass highlight the feasibility of directing genetic improvement for both forage and cover crops in the future. Alternatively, an increase in total dry matter yield may be associated with higher leaf productivity, resulting in more productive selections with high nutritional value.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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