



Visual selection of *Urochloa ruziziensis* genotypes for green biomass yield

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ABSTRACT. The breeding program of *Urochloa ruziziensis* evaluates many genotypes in initial phases. Evaluations through grades might make the selection less costly. The aim of this study was to verify the efficiency of visual selection for green biomass yield in relation to different selection strategies, such as mass selection by phenotypic mean, BLUP (Best Linear Unbiased Prediction) and at random. For this purpose, 2,309 regular genotypes were evaluated in an augmented block design in two cuts. The evaluators gave grades for plant vigor, and later, the plots were measured for green biomass yield. The coincidences of the selected genotypes were estimated by different selection strategies. Then, 254 clones of the genotypes selected in different strategies were evaluated in a clonal test in a triple lattice design in four cuts. The statistical analyses were performed in SAS using the Mixed procedure. The regular genotype level and clone-mean basis heritabilities were 31.16 and 62.91%, respectively, for green mass yield. The expected selection gains were 21.09% (visual), 25.43% (phenotypic mean), and 27.5% (BLUP). Moreover, the realized heritabilities for these strategies were 15.58, 11.87, and 15.86%, respectively, which might be associated with genotype by environment interaction. Therefore, the visual selection could be a useful strategy in initial phases of a *U. ruziziensis* breeding program because the efficiency was moderate to high in relation to phenotypic mean and BLUP.

Keywords: mass selection; realized heritability; BLUP; *Brachiaria*.

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Introduction

The feed of Brazilian livestock is based on pasture. Among the forage species grown in Brazil, *Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Crins (sin. *Brachiaria ruziziensis*) has great value for pasture diversification, particularly for milk production (Pessoa-Filho et al., 2015). This species has high nutritional quality when compared with other species of this genus (Lopes et al., 2010) and has shown potential for dry biomass yields that range from 12 to 15 tons/ha (Souza Sobrinho, Lédo, & Kopp, 2011). Moreover, this species shows adaptability to non-acid and well drained soils, tolerance to aluminum (Bitencourt et al., 2011; Martins et al., 2011), and resistance to spittlebug (Souza Sobrinho, Auad, & Lédo, 2010a).

Notably, *U. ruziziensis* is the only crop forage species that is diploid ($2n = 18$) and with a sexual reproduction mode. Therefore, this species has been used in intraspecific and interspecific breeding programs (Souza Sobrinho, Borges, Lédo, & Kopp, 2010b; Timbó et al., 2014).

Many genotypes are evaluated in the initial generations of a forage breeding program. Concerns at this stage are related to the difficulty and cost of phenotyping and therefore to the accuracy of the selection of superior genotypes. In this regard, strategies using advanced phenotyping, i.e., high-throughput methods, have been developed and tested (Walter, Sduder, & Kölliker, 2012), but the costs continue to be a challenge. Thus, visual selection is a low-cost alternative, and breeders have applied this type of selection constantly in plant breeding programs.

The efficiency of visual selection in the discrimination of superior genotypes is questionable (Cutrim, Ramalho, & Carvalho, 1997). When confronted with a selection for productivity, generally, the visual selection is less efficient in generating superior populations. However, based on common sense, this

efficiency is strongly related to high-heritability traits (Abreu, Ramalho, Toledo, & Souza, 2010). Visual selection has been practiced on forage species through assigned grades to the production of green biomass, for example, in *Panicum virgatum* L. (Casler & Vogel, 2014; Casler, 2010). However, the efficiency of this selection strategy has not yet been measured in *U. ruziziensis*.

The aim of this study was to evaluate the efficiency of the visual selection of *U. ruziziensis* genotypes for green biomass yield in relation to different selection strategies, such as mass selection by phenotypic mean, BLUP (Best Linear Unbiased Prediction) and at random.

Material and methods

Description of the location

The data were obtained in experiments conducted by Brazilian Agricultural Research Corporation (Embrapa) Dairy Cattle at the experimental field located in the municipality of Coronel Pacheco, Minas Gerais State, Brazil, situated in the Zona de Mata Mineira region with a 414 m altitude at 21°33' S latitude and 43°06' W longitude. According to the Köppen classification, the climate of the region is a Cwa type, mesothermic, high altitude tropical climate, with an average annual temperature of 19°C, cold and dry winters and rainy summers with moderately high temperatures. The soil of the experimental area is classified as red-yellow Argisol.

Regular genotype test

To evaluate the efficiency of the visual selection, an experiment was established in an augmented block design (Federer, 1956) with planting date in August 2011, with 51 blocks that varied from 28 to 72 genotypes within each block. The regular genotypes consisted of 2,309 genotypes of *U. ruziziensis*, obtained by seeds, resulting from the second cycle of intraspecific recurrent selection conducted by Embrapa Dairy Cattle. The common genotypes consisted of two controls: cultivar Marandu (*U. brizantha* (Hocst. ex. A. Rich.) R. D. Webster (sin. *Brachiaria brizantha* (A. Rich.) Stapf.)) and cultivar Basilisk (*U. decumbens* (Stapf.) R. D. Webster (sin. *Brachiaria decumbens*)). A plot consisted of one plant, with a spacing of 1 m among plots. In the beginning of December 2011, a standardization cut of the plots to 5.0 cm above soil level was conducted. In 2012, two evaluation cuts were conducted, one in January and another in February, with an interval of 42 days.

The following traits were measured for each cut: a) vigor grades of the plant shoot area, attributed by five evaluators; before each cut, the evaluators attributed vigor grades for each plot according to the following scale: 1-Very bad, 2-Bad, 3-Regular, 4-Good, and 5-Very Good; and b) green biomass yield (in grams), determined from the cut of plants at five centimeters from the soil and measured with a portable digital scale.

The four following strategies of mass selection were used: 1) visual selection based on the average of the vigor grades given by the evaluators; 2) selection by phenotypic mean based on the mean of the green biomass yield in the two cuts; 3) selection based on BLUP involving the two cuts; 4) selection at random in which the selection was performed by chance to compose a random sample of the population.

For the selection based on BLUP, the predictions of the genotypic values were obtained by joint analysis of the green biomass yield data, obtained in two cuts, based on the following model:

$$y_{ijk} = \mu + p_i + b_j + pb_{ij} + c_k + bc_{jk} + pc_{ik} + e_{ijk} \quad (1)$$

where: y_{ijk} is the observed value of the genotype i in block j and in cut k ; μ is the overall average; p_i is the effect of genotype i , which can be partitioned in: t_i referring to the fixed effect of the control i ($i = 1$ and 2) and g_i referring to the random effect of regular genotype i ($i = 1, 2, \dots, 2,309$), with $g_i \sim N(0, \sigma_g^2)$ and σ_g^2 is the variance of regular genotypes; b_j is the random effect of block j ($j = 1, 2, \dots, 51$), with $b_j \sim N(0, \sigma_b^2)$ and σ_b^2 is the variance of blocks; pb_{ij} is the random effect of interaction between genotype i and block j , with $pb_{ij} \sim N(0, \sigma_{pb}^2)$ and σ_{pb}^2 is the variance of genotype by block interaction; c_k is the fixed effect of cut k ($k = 1$ and 2); bc_{jk} is the random effect of interaction between block j and cut k , with $bc_{jk} \sim N(0, \sigma_{bc}^2)$ and σ_{bc}^2 is the variance of block by cut interaction; pc_{ik} is the interaction effect between genotype i and cut k , which can be partitioned in: tc_{ik} referring to the fixed effect of interaction between control i and cut k , and gc_{ik} referring to the random

effect of the interaction between regular genotype i and cut k , with $g_{Cik} \sim N(0, \sigma_{gc}^2)$ and σ_{gc}^2 is the variance of regular genotype by cut interaction; and e_{ijk} is the random effect of the error associated with y_{ijk} , with $e_{ijk} \sim N(0, \sigma_e^2)$ and σ_e^2 is the residual variance.

The assumption of normality was verified by a Shapiro-Wilk test using Univariate procedure in SAS 9.4, University Edition (Royston, 1992; SAS, 2015). The homogeneity of variances for genotypes and error effects was assessed using Akaike's Information Criterion (AIC) (Akaike, 1974; Burnham & Anderson, 2003).

The mixed model approach was used to analyze the data in which the estimation of the fixed (Best Linear Unbiased Estimation - BLUE) and random effects (Best Linear Unbiased Prediction - BLUP) was conducted by solving the system of equations of Henderson (Piepho, Mohring, & Melchinger, 2008). The variance components were estimated by the restricted maximum likelihood method (REML). Additionally, the confidence intervals of the variance components at 95%, the heritability at the genotype level and the selective accuracy ($r_{\bar{g}g}$) (Resende, 2002) were estimated. The statistical analyses were performed in SAS 9.4 using the Mixed procedure (SAS, 2015).

The coincidence of genotypes of *U. ruziziensis* selected in each mass selection strategy was estimated by the coincidence index (Hamblin & Zimmermann, 1986):

$$IC = \frac{A - C}{B - C} \times 100$$

where: A is the number of coincident regular genotypes between two mass selection strategies, within the respective selection intensity; B is the number of selected regular genotypes by their respective selection intensity; and C is the number of coincidences due to chance, calculated by B multiplied by the selected percentage, which varied from 4 to 54%, with 5% intervals.

Clonal test

The 100 best regular genotypes of each mass selection strategy were selected, and these were cloned to perform the clonal test. A total of 254 clones were evaluated, excluding the coincidences between the mass selection strategies and plus two controls, cultivars Marandu (*U. brizantha*) and Basilisk (*U. decumbens*). The experiment was installed in September, 2012 at the experimental field of Embrapa Dairy Cattle located in the municipality of Coronel Pacheco, Minas Gerais State, Brazil. The experimental design was a triple lattice 16 x 16, with plots of one plant, spaced at 1 m. In November 2012, a standardization cut was conducted and after 51 days, a first evaluation cut was performed (January, 2013). The other cuts occurred in February, March, and June, 2013, with intervals of 27, 50, and 71 days, respectively, for the plots to reach the ideal stage of cutting.

The joint analysis of the data of the four evaluation cuts followed model 1, because the lattice efficiency was less than 105% (SAS, 2015). In this regard, random clones and fixed controls were assumed. The statistical analyses were performed by the approach of mixed models, as previously mentioned in the selection stage of regular genotypes.

From the clonal test, the realized heritability (h_r^2) for each mass selection strategy was estimated, according to the equation:

$$h_r^2 = \frac{GS_{g'} / \bar{x}_{g'}}{GS_g / \bar{x}_g} \times 100$$

where: $GS_{g'}$ and GS_g are the gains with the obtained selection by the average of BLUP predictions of the clones and selected regular genotypes, respectively, in each mass selection strategy; $\bar{x}_{g'}$ and \bar{x}_g are the average of the clones in the clonal test and of the regular genotypes of the original population, respectively.

Additionally, the t-Student confidence intervals at 95% of probability for gains with the selection (GS) in the assessment of the regular genotypes ($m = g$) or clones ($m = g'$) were obtained with the following equation (Resende, 2002):

$$GS_m \pm 1.96 \sqrt{\frac{1}{n} [(1 - r_{mm}^2) \sigma_m^2]}$$

where: n is the number of selected genotypes.

Results and discussion

According to the AIC, the proposed split-plot-in-time model, similar to Compound Symmetry Covariance Structure, was adequate (Burnham & Anderson, 2003). The accuracy for the selection of regular genotypes was 54.23% for green biomass yield in *U. ruziziensis*, which according to Resende and Duarte (2007) can be considered moderate (Table 1). By definition, the accuracy is the correlation between the true genetic value of the individual and the phenotypic predictor of its estimation (Resende, Ramalho, Guilherme, & Abreu, 2015). Therefore, this estimate allowed us to anticipate success with the selection, whereas the genetic gains were positively proportional to accuracy.

Table 1. Estimates of genetic and phenotypic parameters for green biomass yield (in grams) of genotypes of *U. ruziziensis* evaluated in two cuts.

Parameters	Estimate	Confidence Interval (95%)	
		Lower limit	Upper limit
σ_g^2	54.779	32.069	114.186
σ_{gc}^2	33.485	20.251	65.745
σ_e^2	65.083	50.037	88.145
h^2	0.3116	-	-
r_{gg}	0.5423	-	-
\bar{x}_t	723b	-	-
\bar{x}_g	1.105a	-	-

σ_g^2 : variance of regular genotypes; σ_{gc}^2 : variance of regular genotype by cut interaction; σ_e^2 : residual variance; h^2 : heritability at regular genotype level; r_{gg} : selective accuracy; \bar{x}_t : mean of the controls; \bar{x}_g : mean of the regular genotypes.

The variance of regular genotypes for green biomass yield was significant, when the lower limits of confidence intervals (CI) were positive; however, the heritability was only 31.16% (Table 1). This low heritability showed strong environmental influence on the trait expression but could also be linked to the experimental design employed.

In the original augmented block design, the environmental variance was completely estimated based on the controls, because the regular genotypes were nonreplicated in the trial. Note that the use of this design is common in initial stages of breeding cycles, primarily because of the high number of genotypes and restricted resources (Paternelli, Souza, Barbosa, & Carvalho, 2009; Prado et al., 2013). However, this design might result in selective gains, and the accuracy might be improved with the use of spatial analysis and intereffect recovery (Silva Filho, 2013; Rodriguez-Álvarez, Boer, van Eeuwijk, & Eilers, 2018). In the present study, the analysis was performed with interblock and intergenotypic information recovery.

The variance of the regular genotype by cut interaction was of high magnitude, because it corresponded to 61% of variance of regular genotypes (Table 1). Therefore, the performances of some genotypes were not the same across the evaluated cuts. This interaction effect has been observed in *U. ruziziensis* (Souza Sobrinho et al., 2010b) and in other tropical forage species, such as *Urochloa humidicola* (sin. *Brachiaria humidicola*) (Figueiredo, Nunes, & Valle, 2012) and *U. decumbens* (Mateus et al., 2015).

The mean of green biomass yield of the regular genotypes was superior to that of the controls (Table 1). Note that the controls ('Marandu' and 'Basilisk') are the most commonly used cultivars in Brazil (Euclides et al., 2010). This result showed the potential of this species to provide competitive cultivars for the market. Souza Sobrinho et al. (2011) also found productive genotypes of *U. ruziziensis* and even superior to the other *Brachiaria* species.

The visual selection efficiency was moderate to high in relation to the phenotypic mean and BLUP (Figure 1). The coincidence index of the visual selection was equal or superior to 70% when using a selected proportion equal to or higher than 14% in relation to the phenotypic mean and of 39% in relation to BLUP (Figure 1). In the initial phase of the breeding cycle, applying low or medium selection intensity is reasonable (Resende, 2002). This caution is necessary because, in general, testing the genotypes in replicated trials is not affordable. Therefore, the results achieved for the visual selection, particularly in relation to BLUP, were promising, because BLUP predictions were more accurate because they were obtained by the adjustment for interblock and intergenotypic information recovery (Resende, 2002). In studies with corn, the efficiency of visual selection was also observed across selection cycles (Ordás, Caicedo, Romay, Revilla, & Ordás, 2012). In this way, the visual selection for green biomass yield of *U. ruziziensis* through the vigor grade scale might be considered efficient.

In general, the strategies of visual selection, phenotypic mean and BLUP were efficient, which was demonstrated by low coincidences with selection at random (Figure 1). With the intention to more effectively evaluate visual selection, a subsequent experiment was conducted with 254 clones of the selected regular genotypes under different strategies. The selective accuracy in the clonal test (78.72%) was superior to that of the evaluation trial of the regular genotypes (Tables 1 and 2), i.e., the selected clones were evaluated with increased precision because of the increase in number of replications and cuts. The clones of *U. ruziziensis* presented average performance equal to that of the controls. Variation occurred among the clones, and furthermore, the clones showed relative differential performance on the evaluated cuts (Table 2).

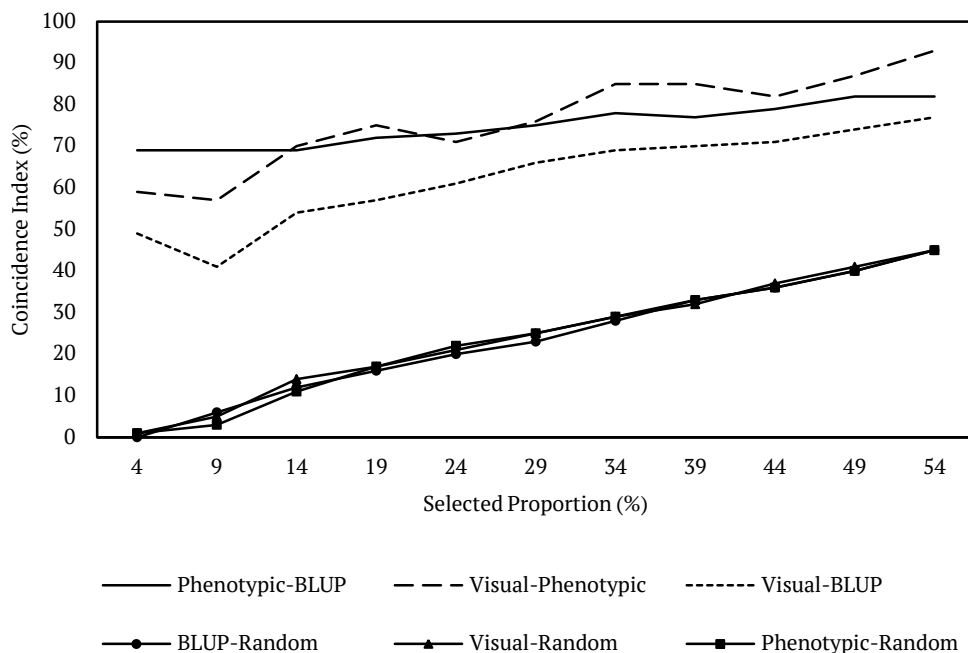


Figure 1. Coincidence index (%) among mass selection strategies of regular genotypes of *Urochloa ruziziensis* based on visual evaluation using a vigor grade scale, phenotypic mean, BLUP and at random across cuts in different selected proportions.

Table 2. Estimates of genetic and phenotypic parameters for green biomass yield (grams) of clones of *U. ruziziensis* evaluated in four cuts.

Parameters	Estimate	Confidence Interval (95%)	
		Lower limit	Upper limit
σ_g^2	77.012	58.659	105.609
$\sigma_{g^c}^2$	26.289	17.175	45.238
σ_e^2	232.179	215.721	250.605
h^2	0.6291	-	-
$r_{\bar{g}^t g^t}$	0.7872	-	-
\bar{x}_t	1.525a	-	-
\bar{x}_{g^t}	1.396a	-	-

σ_g^2 : variance of clones; $\sigma_{g^c}^2$: variance of clone by cut interaction; σ_e^2 : residual variance; h^2 : heritability at clone level; $r_{\bar{g}^t g^t}$: selective accuracy; \bar{x}_t : mean of the controls; \bar{x}_{g^t} : mean of the clones.

The clone-mean basis heritability was 62.91% (Table 2). Few studies report estimates of heritability in *U. ruziziensis*. In interspecific hybrids of *U. ruziziensis* with the species *U. brizantha* and *U. decumbens*, the estimates of heritability oscillated from 0.37 to 0.52 for dry biomass yield (Resende et al., 2007). Values of heritabilities for biomass yield in other forage species are also reported, such as 0.69 for dry biomass yield in *U. humidicola* (Figueiredo et al., 2012), 0.64 to 0.73 dry biomass yield in *U. brizantha* (Basso, Resende, Valle, Gonçalves, & Lempp, 2009) and 0.52 for green biomass yield in *U. decumbens* (Mateus et al., 2015).

The realized heritabilities were of low magnitude for different mass selection strategies (Table 3), with values of 15.86, 15.58, and 11.87% for BLUP, visual selection, and phenotypic mean, respectively. Despite the low estimates, these strategies showed efficiency in relation to the selection at random (Table 3). Some factors might have contributed to explain this result, such as low accuracy in the evaluation trial of the

regular genotypes (Table 1) and the influence of the genotype by environment (GE) interaction. The GE effect has a negative effect on realized heritability and selection gain, which might also cause alterations in the clone ranking (Elias, Robbins, Doerge, & Tuinstra, 2016).

Table 3. Genetic gains for green biomass yield (grams) with the selection of regular genotypes (GS_g) and clones (GS_c) of *U. ruziziensis* and the realized heritability (h^2_r) based on the strategies of visual selection, phenotypic mean, BLUP and at random.

Parameters	Visual	Phenotypic Mean	BLUP	Random
GS_g	233 ± 38	281 ± 38	304 ± 38	21 ± 38
\bar{x}_g	1,338	1,386	1,409	1,126
GS_c	48 ± 33	44 ± 33	63 ± 33	-53 ± 32
$\bar{x}_{g'}$	1,476	1,469	1,494	1,328
h^2_r	15.58	11.87	15.86	0,00

\bar{x}_g : mean of the selected regular genotypes. $\bar{x}_{g'}$: mean of the selected clones.

The expected gain with visual selection and its realized heritability were equivalent to those obtained with the strategies based on phenotypic mean and BLUP (Table 3). These results are favorable for the use of visual selection in a breeding program for *U. ruziziensis*. This strategy has advantages related to low cost and ease in evaluating and selecting genotypes for biomass yield. Ordás et al. (2012) reinforce that evaluation of traits by grades is of great utility in plant breeding because of the high correlation with traits of greater importance. In forage species, the selection gains based on grades have been high in each selection cycle for plant shoots (Casler, 2010; Casler & Vogel, 2014).

Conclusion

The expected genetic gains based on regular genotype selection were 1.9% (random), 21.09% (visual), 25.43% (phenotypic mean), and 27.5% (BLUP). Furthermore, the realized heritabilities for these strategies were 0, 15.58, 11.87, and 15.86%, respectively, which might be associated with the genotype by environment interaction.

The visual selection can be a useful strategy in the initial phases of a *U. ruziziensis* breeding program, because the efficiency was moderate to high in relation to the phenotypic mean and BLUP.

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