BEATRIZ TOMÉ GOUVEIA

SELECTION STRATEGIES AND COMBINING ABILITY FOR AGRONOMIC AND NUTRITIONAL TRAITS IN *Urochloa* spp

LAVRAS – MG
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SELECTION STRATEGIES AND COMBINING ABILITY FOR AGRONOMIC AND NUTRITIONAL TRAITS IN *Urochloa* spp

ESTRATÉGIAS DE SELEÇÃO E CAPACIDADE DE COMBINAÇÃO PARA CARACTERES AGRONÔMICOS E DE VALOR NUTRICIONAL EM *Urochloa* spp

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética e Melhoramento de Plantas, para obtenção do título de Doutor.

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GENERAL ABSTRACT

Urochloa breeding requires adoption of efficient strategies for selecting superior genotypes in order to increasing the genetic gain for breeding target traits closely associated with animal performance. Therefore, the aims of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* hybrids, (2) to assess genetic gains for agronomical and nutritional traits based on selection indexes, NLM, and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable, and (3) to estimate the general combining ability (GCA), specific combining ability (SCA) and contribution of additive and nonadditive effects, from a partial diallel cross design between apomictic and sexual parents, for agronomical and nutritional traits in *Urochloa* spp. For the selection strategies comparisons, we evaluated 96 interspecific hybrids (*U. ruziziensis* x *U. brizantha* x *U. decumbens*) in an experiment laid out in a randomized complete block design with four replications. For the combining ability estimation, a total of 1,380 interspecific hybrids from 29 full-sib progenies were evaluated in an experiment laid out in an incomplete block design. In both trials, a series of agronomic and nutritional value traits were measured, and the data were analyzed using a mixed model approach. Path analysis and GYT were performed with the basic variable NLM, and different strategies using selection indexes were adopted. The leaf dry matter yield (LDM) and field green weight (FGW) traits exhibited greater direct effects on NLM. All selection strategies proved to be effective in obtaining gains in the NLM variable. The strategies GYT analysis and the selection index with weights corresponding to the relative direct effects to each trait on the NLM resulted in greater correlated responses for NLM. Indirect selection for NLM via FGW or the index with the FGW and regrowth capacity (REG) traits proved to be viable strategies for selection of *Urochloa* genotypes in the initial stages of the breeding cycles due to their practicality and lower requirements regarding traits to be measured. The GCA effect was observed only for FGW, leaf percentage, leaf/stem ratio and crude protein in sexual parents whereas SCA was observed to all traits. The selection based on NLM provided more favorable gains for agronomic traits FGW, LDM, REG and total dry matter yield than for nutritional traits. In conclusion, all selection strategies proved to be efficient in obtaining genetic gains for NLM, the selection based on FGW showed to be a feasible strategy in the initial phases of breeding program, and there was predominance of nonadditive effects on the phenotypic expression of all traits measured.

Keywords: *Brachiaria* spp. Index selection. Indirect gain. Parental selection. Diallel.

RESUMO GERAL

O melhoramento de *Urochloa* requer a adoção de estratégias eficientes para a seleção de genótipos superiores a fim de aumentar o ganho com a seleção para caracteres alvos do melhoramento intimamente associados com o desempenho animal. Assim, os objetivos desse estudo foram (1) identificar caracteres com maiores efeitos diretos e indiretos na produtividade de massa foliar de alto valor nutricional (PFN) em híbridos de *Urochloa*, (2) estimar o ganho genético para caracteres agronômicos e de valor nutricional baseado em índices de seleção, PFN, e pela análise gráfica genotype by yield*trait (GYT) usando PFN como a variável básica, e (3) estimar a capacidade geral combinação (CGC), capacidade específica de combinação (CEC) e a contribuição dos efeitos aditivos e não aditivos, a partir de um dialelo parcial entre parentais sexuais e apomíticos, para caracteres agronômicos e nutricionais em *Urochloa* spp. Para as comparações entre as estratégias de seleção, foram avaliados 96 híbridos interespecíficos (*U. ruziziensis* x *U. brizantha* x *U. decumbens*) em um experimento conduzido em delineamento de blocos casualizados com quatro repetições. Para as estimações das capacidades de combinação, um total de 1.380 híbridos interespecíficos de 29 progênies de irmãos-completos foram avaliadas em delineamento de blocos incompletos. Em ambos os ensaios, vários caracteres agronômicos e de valor nutricional foram mensurados, e os dados analisados via abordagem de modelos mistos. Análise de trilha e GYT foram realizadas utilizando PFN como variável básica, e diferentes estratégias usando índices de seleção foram consideradas. As características produtividade matéria seca foliar (MSF) e o peso verde de campo (PVC) exibiram maiores efeitos diretos sobre PFN. Todas as estratégias de seleção mostraram-se eficientes em obter ganhos na variável PFN. As estratégias GYT e o índice de seleção com pesos correspondentes aos efeitos diretos relativos de cada característica sobre PFN resultaram em maiores respostas correlacionadas para PFN. A seleção indireta para PFN via PVC ou o índice utilizando as características PVC e capacidade de rebrota (REB) provaram ser estratégias viáveis para a seleção de genótipos de *Urochloa* em fases iniciais dos ciclos de melhoramento devido a sua praticidade e aos requisitos mais baixos em relação aos caracteres a serem mensurados. Efeito da CGC foi observado apenas para PVC, porcentagem de folhas, relação folha colmo e proteína bruta nos parentais sexuais, enquanto CEC foi observada para todos os caracteres. A seleção baseada em PFN forneceu ganhos mais favoráveis para os caracteres agronômicos PVC, MSF, REB e produtividade matéria seca total do que para os caracteres de valor nutricional. Em conclusão, todas as estratégias de seleção foram eficientes para obter ganhos genéticos em PFN; a seleção baseada em PVC mostrou ser uma estratégia viável em fases iniciais do programa de melhoramento, e houve a predominância de efeitos não aditivos na expressão fenotípica de todos os caracteres mensurados.

Palavras-chave: *Brachiaria* spp. Índice de seleção. Ganho indireto. Seleção de parentais. Dialelo.

SUMMARY

FIRST PART

1 GENERAL INTRODUCTION

Cultivated pastures are the basis to Brazilian livestock because they are the main and the most economical source to feed animals, such as beef and dairy cattle (JANK et al., 2014). About beef cattle, 44.23 M heads were slaughtered in the country in 2018, where only 12.6% of cattle were finished under feedlot. Thus, the pastures have contributed to beef cattle market, and also to consolidate the Brazil as the largest world's exporter of beef. In 2018, Brazil exported 1.64 million of tons of beef, a record volume ever exported among exporting countries (ABIEC, 2019).

The forages crops have also importance in the Brazilian seed market, being the third place of market share (11%) which are only lower than maize (37%) and soybean (37%) (ABRATES, 2017). The area occupied by pastures in Brazil is 162.3 Mha. Most of that area (150.5 Mha) is planted in monoculture and 11.8 Mha are used in production systems with grain crops (9.9 Mha) or another crops (1.9 Mha) (ABIEC, 2019). The genus *Urochloa* spp. represent a large area of tropical forage seed production (83%) and of cultivated pastures (85%) in the country, being the *U. brizantha* cv. Marandu, released by Embrapa in 1984, still occupies a large area of cultivated pastures (JANK et al., 2014).

In the public sector, *Urochloa* spp. breeding is primarily conducted at the Embrapa (Brazilian Agricultural Research Corporation) Beef Cattle, which has coordinated the *Urochloa* spp. interspecific breeding program (*U. brizantha x U. decumbens x U. ruziziensis*) and intraspecific programs of *U. decumbens* and *U. humidicola*, and the Embrapa Dairy Cattle has coordinated the intraspecific breeding program of *U. ruziziensis* (JANK; VALLE; RESENDE, 2011, SOUZA SOBRINHO et al., 2016). In 1984 and 1985, the International Center for Tropical Agriculture (CIAT, its Spanish acronym) accomplished a direct collection in the *Urochloa* diversity centers in Africa, and part of that collection was imported by the Embrapa, which holds the main germplasm banks of *Urochloa* spp. in Brazil (with ~450 accessions of 13 different species) (MILES; CARDONA; SOTELO, 2006; RESENDE; VALLE; JANK, 2008). *Urochloa brizantha*, *U. decumbens*, *U. humidicola* and *U. ruziziensis* are the most important species commercially in the genus, being that *U. ruziziensis* reproduces sexually and the other three reproduce asexually by apomixis and sexually (RESENDE; VALLE; JANK, 2008). Usually, apomictic plants are polyploidy and sexual plants are diploid in nature (PINHEIRO et al. 2000). In the apomictic reproduction, unreduced embryo sac develops from a somatic nucellar cell and the embryo is originated by parthenogenesis, and so, apomictic plants are screened to commercial cultivars and also they can be used as male in the breeding programs (MILES, 2007; JANK; VALLE; RESENDE, 2011).

Initially, the cultivar development was carried out by direct selection and commercialization of natural germplasm accessions. This strategy was successful because it allowed to release several cultivars in the Brazilian market, as such as *U. brizantha* cv. Xaraés; *U. brizantha* cv. BRS Piatã, *U. brizantha* cv. BRS Paiaguás, e, *U. humidicola* cv. BRS Tupi (MILES, 2007; VALLE et al, 2004; RESENDE; VALLE; JANK, 2008; UNIPASTO, 2019). The interfertile *U. decumbens* - *U. brizantha* - *U. ruziziensis* agamic complex form a gene pool, and the barriers to recombination in this gene pool were overcome in the late 1980s when a tetraploid sexual *U. ruziziensis* was obtained artificially enabling to start crosses with apomictic *U. decumbens* or *U. brizantha*, used as pollen donors, and so, started the interspecific breeding program of *Urochloa* (MILES; CARDONA; SOTELO, 2006; WORTHINGTON; MILES, 2015). In 2017, the Embrapa Beef Cattle released the first interspecific hybrid, the BRS Ipyporã (EMBRAPA, 2017).

In the 2000s decade, the intraspecific programs of *U. decumbens* ($2n = 4x = 36$) and *U. humidicola* ($2n = 6x = 36$) started at the Embrapa Beef Cattle, and *U. ruziziensis* ($2n = 2x = 18$) at the Embrapa Dairy Cattle. Crossings between tetraploid sexual genotypes of *U. decumbens* (D24/2, D24/27 and D24/45), obtained also by chromosomal duplication, and *U. decumbens* cv. Basilisk enabled to start the breeding program of *U. decumbens* (SIMIONI; VALLE 2009; MATEUS et al. 2015). For *U. humidicola*, a sexual ecotype was found in the germplasm bank (H31) with the same ploidy level of some of the apomictic plants, and this sexual plant was crossed with the best apomictic genotype of the collection, cv. BRS Tupi (FIGUEIREDO; NUNES; VALLE, 2012; FIGUEIREDO et al., 2019). In *U. ruziziensis*, diploid and sexual access have been used in the breeding program (SOUZA SOBRINHO et al., 2016).

In *Urochloa* several agronomic and nutritional traits are evaluated aiming identify superior hybrids focused on animal performance. However, since better animal performance is related with higher leaf dry matter production, protein content, and digestibility, a natural index that combines these traits, yield of high nutritional value leaf mass (NLM), was proposed in this study with the aim of selecting superior genotypes. It is important highlight that this index does not quantify the digestible protein yield because the digestibility measured refer to leaf organic matter digestibility. In addition, the measurement of most traits evaluated is through morphological separation (leaf blade, stem, and dead material) of forage samples, drying, grinding of samples, and then analyzed by NIRS, which consumes time and resources (FIGUEIREDO; NUNES, VALLE, 2012; MATEUS et al., 2015; MATIAS et al., 2016; FIGUEIREDO et al., 2019). Thus, the correlation estimates are useful to help plant breeder making decision on the selection strategies. However, since the correlations do not provide the exact relative importance of the direct and indirect effects of the traits, the path analysis was proposed to eliminate the limitations of simple correlation analysis (WRIGHT, 1921; CRUZ; REGAZZI; CARNEIRO, 2012). Despite of its importance, there are few studies on path analysis in *Urochloa* (BORGES et al. 2011; TORRES et al. 2016).

The selection of superior genotypes is another difficulty in the *Urochloa* breeding program because it is realized for multiple traits and has generally been dependent on a subjective weight given to each trait when using a selection index (FIGUEIREDO, NUNES, VALLE, 2012; MATEUS et al., 2015). Aiming to overcome this problem, Yan and Frégeau-Reid (2018) proposed a new approach of analysis for multiple traits, the genotype by yield*trait (GYT) biplot analysis. In this analysis, there is no attribution of weights to the traits, and the ranking of the genotypes is based on the combination of the basic variable (e.g., yield) with other primary and secondary breeding target traits. In addition, the GYT analysis provides information to detail the genotype profiles regarding multiple traits.

The selection of genotypes with high breeding value per se is not enough to select them as parents for the next breeding cycle, and more reliable parental selection might be accomplished based on their combining abilities (GRIFFING, 1956). The diallel designs are powerful mating designs to estimate the general combining ability (GCA) and specific combining ability (SCA), helping plant breeders choosing the best parents and crosses, and understanding the genetic control of traits (COMSTOCK; ROBINSON; HARVEY, 1949; GRIFFING, 1956). In each breeding cycle is performed crosses, and in *Urochloa* that step involves crosses between apomictic and sexual parents (MATEUS et al., 2015, MATIAS et al., 2018, FIGUEIREDO et al., 2019). There is little information about GCA and SCA for agronomic and nutritional traits in *Urochloa* and these parameters are unknown for the NLM. Matias et al. (2018) did not find a clear predominance of additive or dominance effects for agronomic and nutritional traits in interspecific hybrids of *Urochloa*. In *U. humidicola*, Figueiredo et al. (2019) observed significant GCA only for sexual parents for biomass yield and forage quality traits.

Therefore, the aims of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* hybrids (2) to assess genetic gains for agronomical and nutritional traits by selection based on selection indexes, NLM, and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable, and (3) to estimate the GCA, SCA and contribution of additive and nonadditive effects, from a partial diallel cross design between apomictic and sexual parents, for agronomical and nutritional traits in *Urochloa* spp..

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SECOND PART

MANUSCRIPT 1 – SELECTION STRATEGIES FOR INCREASING THE YIELD OF HIGH NUTRITIONAL VALUE LEAF MASS IN *Urochloa* HYBRIDS

Manuscript written according to the guidelines of the Journal Euphytica

SELECTION STRATEGIES FOR INCREASING THE YIELD OF HIGH NUTRITIONAL VALUE LEAF MASS IN *Urochloa* HYBRIDS

Abstract: Genotype selection in a forage cultivar development program is complex because the ultimate aim is increased performance and sustainability in animal production. The aims of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* sp. hybrids and (2) to assess indirect gain from selection for these traits with greater effects through selection indexes and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable. We evaluated 96 interspecific hybrids (*U. ruziziensis* x *U. brizantha* x *U. decumbens*) in an experiment laid out in a randomized complete block design with four replications. A series of agronomic traits and nutritional value traits were measured. Path analysis and GYT were performed with the basic variable NLM, and different strategies using selection indexes were adopted. The leaf dry matter (LDM) and field green weight (FGW) traits exhibited greater direct effects on NLM. All selection strategies proved to be effective in obtaining gains in the NLM variable. GYT analysis and the selection index with weights corresponding to the relative direct effects to each trait on the NLM were the strategies that resulted in a greater correlated response for NLM. Indirect selection for NLM via FGW or the index with the FGW and regrowth capacity traits proved to be viable strategies for selection of *Urochloa* genotypes in the initial stages of the breeding cycles due to their practicality and lower requirement regarding traits to be measured.

Keywords: *Urochloa*, genetic correlation, indirect selection, path analysis, index selection, biplot analysis.

Introduction

Cultivated pastures are the main and the most economic source to feed animals in the Brazil, which the *Urochloa* genus stands out because it is the most used tropical forage grass genus in cultivated pasture area in the country (Jank et al. 2014). In the interspecific breeding program of *Urochloa*, the crosses began in the late 1980s using *U. decumbens* or *U. brizantha* apomictic, as pollen donors, and artificially tetraploidized sexual *U. ruziziensis* of the same agamic complex (Miles 2007). The breeding program of *Urochloa*, just as for other genera of forage species, aims to improve various traits simultaneously, such as agronomic and nutritional value traits. However, the selection of cultivars in forage species is more complex than crop traits because the final aim is the gain in animal performance, such as meat or milk production (Valle et al. 2008).

In the initial phases of a forage breeding program, the use of animals for selection of the best genotypes is not feasible, due to the large number of genotypes under testing (e.g., progenies, hybrids). In this case, agronomic traits have been measured and chemical analyses have been performed by near-infrared spectroscopy (NIRS) for the purpose of estimating the nutritional value of the best genotypes (Mateus et al. 2015; Matias et al. 2016). These evaluations do not provide values of leaf consumption, digestibility, and protein content available for the animal because there are other factors that affect consumption. These factors include plant architecture, pasture management, and/or anti-nutritional factors, for example, “girder” structures that increase resistance to digestion by the animal (Wilson et al. 1989), as well as factors intrinsic to the animal, such as age and breed. However, these initial evaluations are useful for discrimination of the genotypes and for better understanding of the associations between these variables and animal consumption, which is fundamental for successful development of cultivars.

Cultivars of forage species that have high leaf yield and good nutritional quality, such as high digestibility and protein content, are related to better animal performance (Euclides and Euclides Filho 1998; Torres et al. 2015; Torres et al. 2016). Since animal performance is an indirect product of leaf dry matter production, protein content, and digestibility, an index composed simultaneously of all these traits was proposed in this study, i.e., the yield of high nutritional value leaf mass, with the aim of selecting superior genotypes. It is important highlight that this index does not quantify the digestible protein yield because the digestibility measured refer to leaf organic matter digestibility.

Currently in the forage breeding program, measurement of most traits evaluated in *Urochloa* is through morphological separation (leaf blade, stem + leaf sheath, and dead material) of forage samples, drying, grinding of samples, and then analysis by NIRS, which consumes time and resources (Mateus et al. 2015; Matias et al. 2016). Thus, correlations among traits are of great importance because they assist in choosing the selection strategies to be used in the breeding program. Although the correlations quantify the magnitude and direction of the association between two traits, they do not provide the exact relative importance of the direct and indirect effects of these traits (Cruz et al. 2012). In addition, simple correlation estimates might not represent the true association between two traits since there may be interference from a third trait, or group of traits, that may skew the correlation estimates (Wright 1921). Several factors influence this parameter as such as species, population, environment and estimation method, and so, in the literature there are contrasting correlation estimates for some traits in *Urochloa*, as for example, 0.97 and 0.32 between total dry matter (TDM) and regrowth capacity (REG), 0.00 and -0.71 between TDM and *in vitro* digestibility of organic matter (IVD), and -0.22 and 0.50 between TDM and neutral detergent fiber (NDF) (Figueiredo et al. 2012; Matias et al. 2016).

Path analysis, proposed by Wright (1921), arose with the aim of eliminating the limitations of simple correlation analysis. Path analysis allows decomposition of the simple correlation coefficients in direct and indirect effects of a group of traits on a basic variable of interest (Cruz et al. 2014). This type of analysis has been used in breeding of various crops, such as maize and soybean (Alves and Cargnelutti 2017; Kmail et al. 2017; Machado et al. 2017). However, there are few studies on path analysis in *Urochloa* (Borges et al. 2011; Torres et al. 2016).

Another difficulty in forage breeding, just as in other crops, is selection for multiple traits, because this selection has generally been dependent on a subjective weight given to each trait when using a selection index (Mateus et al. 2015; Yan and Frégeau-Reid 2018). Aiming to overcome this problem, Yan and Frégeau-Reid (2018) proposed a new approach of analysis for multiple traits, the genotype by yield*trait (GYT) biplot analysis. In this analysis, there is no attribution of weights to the traits, and the ranking of the genotypes is based on the combination of the basic variable (e.g., yield) with other primary and secondary breeding targets.

Therefore, the aims of this study were (1) to identify traits with greater direct and indirect effects on yield of high nutritional value leaf mass (NLM) in *Urochloa* hybrids and

(2) to assess indirect gain from selection for these traits with greater effects through selection indexes and by genotype by yield*trait (GYT) biplot analysis using NLM as a basic variable.

Materials and Methods

Genotypes and phenotypic data

We evaluated a total of 99 genotypes: 96 interspecific hybrids of *Urochloa* (*U. ruziziensis* x *U. brizantha* x *U. decumbens*), selected from 1,000 hybrids population obtained by a partial diallel (Matias et al. 2018), and the controls *U. brizantha* cv. Marandu, *U. brizantha* cv. BRS Paiaguás, and the interspecific hybrid cv. Mulato II. The experiment was conducted in the experimental field of Embrapa Beef Cattle, CNPGC, in Campo Grande, Mato Grosso do Sul, Brazil (20°27'S, 54°37'W, and altitude of 530 m AMSL). The trial was set up in 18 Nov. 2014 and conducted in a randomized complete block design with four replications; plots consisted of five clonal plants at a spacing of 0.5 m within rows and 1.0 m between rows.

Seven cuttings were made (cutting 1: 03 Feb. 2015, cutting 2: 10 Mar. 2015, cutting 3: 23 Apr. 2015, cutting 4: 13 July 2015, cutting 5: 13 Oct. 2015, cutting 6: 25 Nov. 2015, and cutting 7: 18 Jan. 2016), in which agronomic and nutritional value traits were evaluated. The agronomic traits evaluated were field green weight (FGW, kg.ha^{-1}), regrowth capacity (REG, score), total dry matter yield (TDM, kg.ha^{-1}), leaf dry matter yield (LDM, kg.ha^{-1}), leaf percentage (%L), and leaf/stem ratio (LSR). In each plot, each cutting was weighed in the field with a dynamometer, to measure FGW. REG was evaluated seven days after the cutting through a scoring scale, as described by Figueiredo et al. (2012). A sample was removed from each plot in the cuttings, and these samples were dried in laboratory ovens at 65°C for 72 hours to estimate TDM. In cuttings 2, 5, and 7, the samples were separated morphologically (leaf blade, stem, and dead material), thus allowing estimation of %L, LDM, and LSR. The FGW and REG traits were evaluated in all the cuttings, and TDM in six cuttings (except in cutting 1).

To evaluate nutritional value traits, the dry leaves from morphological separation of cuttings 2, 5, and 7 were ground and sent for analyses using NIRS (Marten et al. 1989), with curves calibrated, as described by Matias et al. (2018), for the following traits: crude protein in dry matter (CP, %, AOAC 1990), *in vitro* digestibility of organic matter (IVD, %, Tilley and Terry 1963), percentage of neutral detergent fiber (NDF, %, Van Soest et al. 1991), and percentage of lignin in sulfuric acid in the dry matter (LIG, %, Van Soest et al. 1991). The yield of high nutritional value leaf mass (NLM, kg.ha^{-1}) was also estimated. This variable was estimated by the following equation: NLM = LDM x CP x IVD. The NLM was estimated for three cuttings (2, 5, and 7), the same cuttings in which LDM, CP, and IVD were measured.

Statistical analysis

The multi-cutting analysis of the cuttings was conducted on the data through the mixed model approach, considering kinship information of the sexual progenitors, with estimation of variance components using the residual maximum likelihood (REML) method through the ASReml statistical package of R (Butler 2009). Combined analysis of each trait used the following model:

$$y = Xm + Z_1 b + Z_2 g + e$$

where y is the phenotypic data vector; X is the incidence matrix of the fixed effects and the Z's are the incidence matrices in reference to the random effects; m is the fixed effects vector of cuttings, which are added to the overall mean; b is the random effects vector of blocks, in which $b \sim NMV(0, I\sigma_b^2)$ and σ_b^2 is the variance component associated with the effects of blocks; g is the random effects vector of genotypes within cuttings, in which $g \sim NMV(0, G \otimes \mathrm{A})$; and e is the random error vector within cuttings, in which $e \sim NMV(0, R \otimes \mathrm{I})$; R is the residual covariance matrix; G is the genetic covariance matrix; A is the relationship (kinship) matrix; and I is the identity matrix. The Kronecker product is denoted by $\otimes$. For each trait, the R and G matrices were chosen in a sequential manner (Smith et al. 2007; Andrade et al. 2016), first identifying the best covariance structure for R and then for G, considering the previously selected R. The best structure for R and G was indicated by the lowest value of the Bayesian Information Criterion (BIC), proposed by Schwarz (1978).

The significance of the variance components was checked by the likelihood ratio test (LRT) at 5% probability. The BLUP predictions of the random effects were obtained, and then the genetic correlations between the traits were estimated using the BLUPs of the hybrids using the *Agricolae* R package (Mendiburu 2014). The significance of the genetic correlations was checked via Bootstrap by the “bias-corrected and accelerated” (BCa) method, with 9999 simulations, using the *wBoot* R package (Weiss 2016). Genotype mean-based heritability and selective accuracy were also estimated, according to Resende and Duarte (2007).

Path analysis

The multicollinearity test was performed through evaluation in the number of conditions (NC) proposed by Montgomery and Peck (1981), which examines the relation between the highest and lowest eigenvalue of the genetic correlation matrix. Multicollinearity is considered low with NC < 100, moderate to strong if 100 > NC > 1000, and severe if NC > 1000. Multicollinearity was diagnosed using the Genes software (Cruz 2013), and when multicollinearity was above to moderate, a constant of 0.035 was added in the diagonal of the correlation matrix, in a way similar to the ridge regression method (Carvalho and Cruz 1996). Path analysis was carried out using a two-chain causal model, with the NLM trait as the basic variable; CP, IVD, NDF, LIG, and LDM, the primary variables; and FGW, TDM, %L, LSR, and REG, the secondary variables (Figure 2). The causal diagram was chosen based on a theoretical knowledge of causal relationships between traits.

*Genotype by yield*trait biplot*

For the genotype by yield*trait biplot analysis, the NLM variable was taken as the basic or main variable (yield). From this, the BLUP means of the hybrids in regard to NLM from combined analysis of the cuttings were combined with the BLUP means of each one of the other traits for each hybrid to obtain the values of the yield*trait variables, as proposed by Yan and Frégeau-Reid (2018). For FGW, TDM, LDM, %L, LSR, REG, CP, and IVD, in which selection is to increase the trait, the BLUP of the trait was multiplied by the BLUP of NLM for each hybrid, for example, NLM*CP. For LIG and NDF, in which selection is to reduce the value of the trait, the BLUP of NLM was divided by the BLUP of these traits, i.e., NLM/NDF and NLM/LIG. Thus, higher values of GYT are always desirable. Biplots of the yield*trait variables were generated using the *GGEBiplotGUI-R* package (Frutos et al. 2014).

Selection strategies

For selection of the genotypes, six selection strategies were used aiming to increase the NLM trait: strategy 1 – direct selection for NLM; strategy 2 – indirect selection based on the FGW trait; strategy 3 – indirect selection by means of the selection index, with the FGW and REG traits with predefined weights of 60% and 40%, respectively; strategy 4 – indirect selection by means of the selection index including all the traits, except NLM, with predefined weights of 60% for the agronomic traits and 40% for those of nutritional value (the traits had equal weights within each category); strategy 5 – indirect selection by means of the selection index including all the traits, except NLM, with weights corresponding to the relative direct effects related to each trait on the NLM trait from path analysis; and strategy 6 – selection based on GYT analysis, which does not require the establishment of weights, according to Yan and Frégeau-Reid (2018). To compare the different selection strategies, gain from selection (%) was calculated using the selection intensity of 10%, and the *Spearman* correlation of the genotypes among the selection strategies was calculated using the *Agricolae*-R package (Mendiburu 2014).

Results

Genetic parameters

The covariance structures of the *G* and *R* matrices varied according to the traits evaluated. The best structure of the *R* matrix was the unstructured (UN) for FGW, TDM, and CP; compound symmetry (CS) for REG; heterogeneous compound symmetry (CSH) for %L and LIG; and first order autoregressive with heteroscedasticity (ARH1) for LDM, LSR, IVD, NDF, and NLM. As for the *G* matrix, the structure that provided the lowest BIC was ARH1 for FGW, CSH for LSR and LDM, and CS for the other traits.

The genetic variance was significant by the LRT for all the traits evaluated. The variance of the genotype x cutting interaction (σ^2_{GxC}) was non-null for several of the traits as well, except for CP, IVD, NDF, and LIG (Table 1). Genotype mean-based heritability had a magnitude ranging from 31 (%L) to 78% (REG), and for most of the traits, the estimated values were above 51% (Table 1). The heritability estimates were lower than 50% only for %L (31%), IVD (48%), and LIG (45%), which denotes the greater influence of environmental factors on phenotypic expression of these traits. The estimates of selective accuracy were of high magnitude for most of the traits (Table 1), except for %L (55%), IVD (70%), and LIG (67%), which had estimates of moderate magnitude, according to Resende and Duarte (2007). In general, this displays good reliability in the selection of genotypes under testing.

Significant genetic correlations were detected among most of the traits evaluated (Figure 1). The correlations ranged from 0.93 (NLM – LDM) to -0.42 (IVD – NDF). The most highly correlated traits were NLM x LDM (0.93), FGW x LDM (0.85), TDM x LDM (0.86), FGW x TDM (0.84), FGW x NLM (0.83), LSR x %L (0.79), and TDM x NLM (0.77). The correlations among the nutritional value traits, and the nutritional value traits with the agronomic traits or NLM were of moderate to low magnitude.

Path analysis

The genotypic correlation matrix exhibited moderate to strong multicollinearity, detected by the test proposed by Montgomery and Peck (1981). In this case, path analysis was carried out considering multicollinearity, as suggested by Carvalho and Cruz (1996). In analysis of the primary variables on the main variable, an R^2 of 0.94 and an effect of the residual variable (ERV) of 0.25 were observed, indicating that most of the variation was explained by these variables. The LDM had a high correlation estimate and greater direct effect on the main variable NLM. The other traits had a direct effect lower than the ERV, as well as low correlation with NLM (Figure 2).

Considering the effects of the secondary variables on the primary variables, an R^2 and ERV of 0.05 and 0.97 were observed for CP, 0.14 and 0.92 for IVD, 0.22 and 0.88 for NDF, and 0.3854 and 0.7840 for LIG, respectively. Thus, these secondary variables did not satisfactorily explain all the variation for CP, IVD, NDF, and LIG. However, for LDM, they were able to explain a considerable part of the variation ($R^2 = 0.90$), and the ERV was 0.32. The TDM and FGW traits had not only a high positive correlation, but also a direct positive effect on LDM (Figure 2).

Analysis of the effects of the secondary traits on NLM ($R^2 = 0.77$ and ERV = 0.47) showed that FGW had a high magnitude correlation and high direct effects on NLM (Figure 2). TDM had a correlation estimate of 0.77, but with a direct effect lower than ERV. In a similar manner, REG had a moderate correlation estimate with NLM and a small direct effect (Figure 2).

Selection strategies

Direct selection for NLM (strategy 1), as expected, led to the greatest gain for this trait (34.49%) (Table 2). Selection based only on FGW (strategy 2) or FGW plus REG (strategy 3) led to high and favorable gains for NLM, of 28.99% and 27.5%, respectively (Table 2). However, unfavorable gains were found for LSR, IVD, and LIG with strategy 2 and for CP, IVD, and LIG with strategy 3 (Table 2). The Spearman correlation estimates of these strategies with strategy 1 were 0.82 and 0.83, respectively (Table 3).

The strategies based on selection indexes in which all the traits, except NLM, were included (strategies 4 and 5), led to gains greater than those achieved from strategies 2 and 3, but also exhibited unfavorable gains for LIG (Table 2). The Spearman correlation estimate between strategy 6 and direct selection for NLM was 0.99. By biplot analysis, the ten best genotypes based on the NLM-trait combination were 233\10, 778\10, 70\10, 242\10, 1130\10, 658\10, 1243\10, 421\10, 801\10 and 1221\10 (Figure 3). However, by the "which-won-where" biplot, it is possible to describe the profile of the genotypes in a more detailed manner, in which the genotype 233/10 proved to be superior in the combinations of NLM with nearly all the traits, except for LSR (Figure 4), whereas the genotypes 778/10 and 1243/10 had a different profile, and were superior for the combination of NLM with LSR.

Discussion

Genetic parameters

The existence of genetic variation for the traits allows gain to be obtained from selection (Pandolfi Filho et al. 2016). However, the proportion of this genetic variation related to phenotypic variation or heritability varies according to the type of trait evaluated, the estimation method, diversity in the population (genetic variation), and various other factors (Dias et al. 2018; Schmidt et al. 2019). Matias et al. (2018) worked with a population of 1000 interspecific hybrids of *Urochloa* spp., and the heritability estimates obtained were very similar to those found in this study, except for LSR, which exhibited zero genetic variance. The heritabilities reported by Figueiredo et al. (2012) in *U. humidicola* hybrids were also similar for most of the traits, except for %L (68.32%) and IVD (63.93%).

Therefore, the heritabilities in our study indicate the possibility to get high genetic gains with a selection of the best genotypes for most of traits.

Another highly relevant effect for forage breeding is the genotype x cutting interaction. The significant σ^2_{GxC} has implications for selection and has frequently been reported by other authors working with tropical forage species, such as *U. decumbens* and *U. humidicola* (Figueiredo et al. 2012; Mateus et al. 2015; Matias et al. 2016). Evidence of this interaction denotes that the genotypes under testing have a differential response to environmental fluctuations over the performed cuttings. The genotype x cutting interaction might have an impact on selection since genotypes may differ in terms of agronomic stability throughout the evaluation cuts, and it may also affect association among traits.

Estimates of genetic correlations that are of moderate magnitude and positive between CP x IVD and NDF x LIG have been reported in the literature, as well as correlations that are of moderate magnitude and negative between CP x NDF, CP x LIG, IVD x NDF, and IVD x LIG (Matias et al., 2016; Torres et al., 2016). The correlations estimated in the present study were similar, except for correlations between CP x IVD and CP x LIG, which were surprisingly statistically insignificant. In general, for the agronomic traits, the magnitudes of genetic correlations found in this study were similar to those observed in studies with *U. decumbens* and interspecific genotypes (Mateus et al. 2015; Matias et al. 2018). Furthermore, the estimates of the genetic correlations of the agronomic traits with the nutritional value traits CP, IVD, and NDF were low or zero, thus it is possible to select individuals that combine good agronomic performance and good nutritional quality. Similar estimates were found by Matias et al. (2018), who studied interspecific hybrids of *Urochloa*, contrasting to those found in studies with *U. humidicola*, *U. decumbens*, and *U. ruziziensis*, where the increase in production of forage mass increased NDF and decreased CP and IVD (Figueiredo et al. 2012; Matias et al. 2016; Simeão et al. 2016). Matias et al. (2018) suggested that the presence of alleles of *U. ruziziensis* of high nutritional value combined with the alleles of good agronomic production of *U. brizantha* and *U. decumbens*, allowed for an equilibrium or independence of these groups of traits in the interspecific genotypes of *Urochloa*.

Genetic correlations are important in the choice of traits to be evaluated and in use of indirect selection (Matias et al. 2016). In this respect, FGW, TDM, and LDM stood out in this experiment, with high and positive correlation estimates with each other and with NLM, indicating that some of these traits can be used to perform indirect selection for NLM.

Path analysis

It is important to identify the traits of high correlation with the trait targeted in breeding or the basic variable, but, above all, those that have a greater direct effect in a direction favorable to selection, so that the response correlated by means of indirect selection becomes efficient (Cruz et al. 2012). Thus, the results indicate that indirect selection for NLM by means of LDM and FGW is feasible and interesting.

Borges et al. (2011) performed path analysis in *U. ruziziensis* considering dry matter weight (equivalent to TDM) as the main trait, and nine traits in the primary chain, including stem and leaf dry weight, which were derived from fresh matter weight (FMW equivalent to FGW). The authors obtained high magnitudes of the indirect effects through stem and leaf dry weights for FMW, and when these two traits were removed from path analysis, most of their indirect effects migrated to the estimate of the direct effect of FMW on the main trait. In our study, the high indirect effects via TDM for the FGW variable and via FGW for TDM can be explained by the fact that TDM is derived from FGW. Thus, both FGW and TDM can be used for indirect selection for LDM.

Selection strategies

Selection for FGW showed gain for NLM, confirming the possibility of carrying out indirect selection and corroborating the result of the direct effect indicated by path analysis. Selection based on FGW and REG, for its part, showed gain for NLM and high correlation (0.91) with the strategy that provided for an effective gain for NLM by indirect selection (strategy 5), suggesting that selection based on FGW and REG is also efficient for obtaining gains in NLM.

In spite of the gains favorable to NLM and this trait being a product of LDM, CP, and IVD, selection of 10% of the superior individuals based only on FGW resulted in a reduction in IVD, and selection based on FGW plus REG resulted in a gain unfavorable to CP and IVD. These gains unfavorable to IVD (strategies 2 and 3, Table 2) and CP (strategy 3, Table 2) suggest that LDM, as expected, had a greater influence on calculation of the NLM index. It should be noted that selection based on NLM is not expected to contribute to the increase in undesirable protein fractions, such as those connected with fiber, whose availability may be low in digestion by ruminants (Sniffen et al. 1992).

In a population of *U. humidicola*, selection aiming at greater forage production resulted in genotypes with lower crude protein contents (Figueiredo et al. 2012). However, in this study, it was shown that genotypes with greater gains in high nutritional value leaf mass can be selected through selection for FGW or FGW plus REG. These results are very important, since FGW and REG are traits that are more easily measured, and this will result in savings in time and resources for the forage breeding program, especially in the initial steps of the breeding program, in which thousands of hybrids are evaluated.

A common difficulty in plant breeding programs concerning simultaneous selection for several traits is the establishment of economic weights (Mateus et al. 2015). These weights can be predefined in a subjective way by the breeder, as was done for selection strategies 3 and 4. In the case of strategy 4, weight totaling 60% was chosen for agronomic traits and 40% for nutritional value traits, based on experience accumulated from previous evaluations of experiments on *Urochloa* spp., as well as observing the relative variation of the traits and their effect on the target trait of the breeding program.

One way of removing subjectivity in pre-establishment of economic weights is determination in an analytic manner. A reasonable proposal consists in using the relative direct effects to the trait or basic variable NLM, determined from path analysis, such as economic weights. The strategy or selection index 5 was based on this proposal and achieved an effective magnitude of gain for the NLM variable, being superior than the strategies of indexes with predefined weights (strategies 3 and 4), as well as high classification correlation of the genotypes compared to direct selection for NLM and with strategy 6.

GYT analysis allows selection for multiple traits to be performed without the need for assigning subjective weights to the traits (Yan and Frégeau-Reid 2018). This strategy achieved the highest magnitude of gain for the NLM variable, equal to direct selection to NLM, and the greater *Spearman* correlation estimate of the genotypes with direct selection for NLM. It should be noted that the GYT strategy allows ranking and description of the profiles of the genotypes based on the combination of the main trait or basic variable, in this case NLM, with the other traits by means of biplots. Above all, this makes it easier to interpret and understand the results (Yan and Frégeau-Reid 2018).

Nevertheless, although the index with weights based on the direct effect on NLM and of GYT analysis show the greatest gains for NLM, there is the notable difficulty of application in practice due to the requirement of measuring varied traits, which may considerably raise the costs and time of phenotyping. Thus, the viability of these strategies is dependent on refining them and reducing costs on phenotyping, as well as in the use of equipment such as NIRS. These strategies might be more feasible at more advanced steps of the breeding program, in which there is fewer genotypes to be evaluated.

Conclusion

The traits LDM and FGW exhibited the greatest direct effects on yield of high nutritional value leaf mass. The strategy of genotype by yield*trait analysis and the selection index with weights corresponding to the relative direct effects to each trait on the NLM resulted in superior correlated responses for NLM. The strategies of indirect selection via FGW and of the index including only FGW and regrowth proved to be viable for selection of genotypes of *Urochloa* in the initial steps of the breeding cycles because they associated practicality of use and high gain for NLM.

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Table 1 Estimates of variance of genotypes (σ_g^2), variance of the genotype x cutting interaction (σ_{gxc}^2), genotype mean-based heritability (h^2, %), accuracy ($r_{\hat{g}g}$, %), and overall mean ($\bar{Y}_{overall}$) for agronomic traits and nutritional value traits in *Urochloa* hybrids in multi-cutting analysis†

	FGW		**TDM**		**LDM**		**%L**	
	Mean variance	LRT	Variance	LRT	Variance	LRT	Variance	LRT
$\hat{\sigma}_g^2$	10695503.0	260.8*	185950.6	42.9*	117252.8	8.9*	6.3	4.2*
$\hat{\sigma}_{gxc}^2$	-	-	32520.3	23.3*	55501.8	9.3*	20.8	51.2*
h^2	73		65		63		31	
$r_{\hat{g}g}$	85		81		80		55	
$\bar{Y}_{overall}$	12343.6		2676.9		1763.0		63.6	

	LSR		**REG**		**CP**		**IVD**	
	Mean variance	LRT	Variance	LRT	Variance	LRT	Variance	LRT
$\hat{\sigma}_g^2$	0.4	85.4*	0.2	145.9*	0.48	75.8*	2.36	12.2*
$\hat{\sigma}_{gxc}^2$	-	-	0.1	122.4*	0.01	0.4^{NS}	0.00	0.0^{NS}
h^2	72		78		75		48	
$r_{\hat{g}g}$	85		88		87		70	
$\bar{Y}_{overall}$	3.8		2.9		13.50		63.81	

	NDF		**LIG**		**NLM**	
	Variance	LRT	Variance	LRT	Mean variance	LRT
$\hat{\sigma}_g^2$	0.99	16.4*	0.02	11.8*	1609.6	105.1*
$\hat{\sigma}_{gxc}^2$	0.22	0.8^{NS}	0.00	0.0^{NS}	-	-
h^2	54		45		69	
$r_{\hat{g}g}$	74		67		83	
$\bar{Y}_{overall}$	68.01		2.11		146.3	

Legend: FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter yield (Kg.ha^{-1}); LDM = leaf dry matter yield (Kg.ha^{-1}); %L = leaf percentage (%); LSR = leaf/stem ratio; REG = final regrowth capacity (score); CP = crude protein contents in dry matter (%); IVD = *in vitro* digestibility of organic matter (%); NDF = percentage of neutral detergent fiber (%); LIG = percentage of lignin in dry matter (%); NLM = yield of high nutritional value leaf mass (Kg.ha^{-1}). *Significant by the likelihood ratio test (LRT) at 5% probability. †Seven cuttings: FGW, REG; six cuttings: TDM; three cuttings: LDM, %L, LSR, CP, IVD, NDF, LIG, and NLM

Table 2 Gain from selection (%) for each trait using different selection strategies‡ of *Urochloa* hybrids in several cuttings†, with a selection intensity of 10%

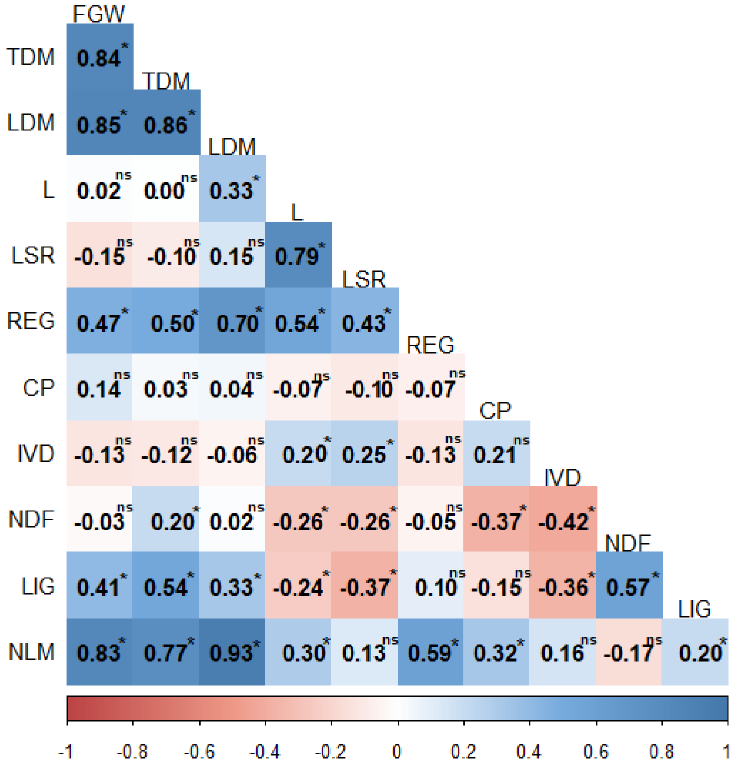
Str ‡	**Traits used**	**Gain from selection (%)**										
		FGW	**REG**	**TDM**	**LDM**	**%L**	**LSR**	**CP**	**IVD**	**NDF**	**LIG**	**NLM**
1	**NLM**	27.06	14.81	19.78	30.67	2.72	4.75	1.44	0.19	-0.07	1.46	34.49
2	**FGW**	31.19	11.92	18.43	25.85	0.78	-1.76	1.06	-0.13	-0.34	2.21	28.99
3	**FGW and REG**	27.09	20.61	18.35	28.81	3.20	6.09	-0.64	-0.17	0.02	2.48	27.50
4	**ALL**	21.99	18.93	14.24	27.76	5.25	13.88	1.00	0.69	-0.50	0.61	31.04
5	**ALL**	28.70	16.36	18.88	30.38	3.26	6.06	1.67	0.20	-0.48	1.43	33.87
6	**ALL**	27.06	14.81	19.78	30.67	2.72	4.75	1.44	0.19	-0.07	1.46	34.49

FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter yield (Kg.ha^{-1}); LDM = leaf dry matter yield (Kg.ha^{-1}); %L = leaf percentage (%); LSR = leaf/stem ratio; REG = final regrowth capacity (score); CP = crude protein contents in dry matter (%); IVD = *in vitro* digestibility of organic matter (%); NDF = percentage of neutral detergent fiber (%); LIG = percentage of lignin in dry matter (%); NLM = yield of high nutritional value leaf mass (Kg.ha^{-1}). ‡Strategies = 1: direct selection for NLM; 2: indirect selection for FGW; 3: index with weight of 60% for FGW and 40% for REG; 4: index including all the traits, except NLM, and weight of 60% for the agronomic traits and 40% for those of nutritional value; 5: index including all the traits and weight based on the direct effect of the traits on the NLM; 6: selection based on GYT analysis. †Seven cuttings: FGW, REG; six cuttings: TDM; three cuttings: LDM, %L, LSR, CP, IVD, NDF, LIG, and NLM

Table 3 Spearman correlation coefficient of the *Urochloa* hybrids between the different selection strategies‡

Str. ‡	**1**	**2**	**3**	**4**	**5**	**6**
1	1	82	83	84	95	99
2		1	80	62	88	80
3			1	85	91	85
4				1	87	88
5					1	96
6						1

‡Strategies = 1: direct selection for NLM; 2: indirect selection for FGW; 3: index with weight of 60% for FGW and 40% for REG; 4: index including all the traits, except NLM, and weight of 60% for the agronomic traits and 40% for those of nutritional value; 5: index including all the traits and weight based on the direct effect of the traits on NLM; 6: selection based on GYT analysis



	FGW	TDM	LDM	L	LSR	REG	CP	IVD	NDF	LIG
TDM	0.84*									
LDM	0.85*	0.86*								
L	0.02^{ns}	0.00^{ns}	0.33*							
LSR	-0.15^{ns}	-0.10^{ns}	0.15^{ns}	0.79*						
REG	0.47*	0.50*	0.70*	0.54*	0.43*					
CP	0.14^{ns}	0.03^{ns}	0.04^{ns}	-0.07^{ns}	-0.10^{ns}	-0.07^{ns}				
IVD	-0.13^{ns}	-0.12^{ns}	-0.06^{ns}	0.20*	0.25*	-0.13^{ns}	0.21^{ns}			
NDF	-0.03^{ns}	0.20*	0.02^{ns}	-0.26*	-0.26*	-0.05^{ns}	-0.37*	-0.42*		
LIG	0.41*	0.54*	0.33*	-0.24*	-0.37*	0.10^{ns}	-0.15^{ns}	-0.36*	0.57*	
NLM	0.83*	0.77*	0.93*	0.30*	0.13^{ns}	0.59*	0.32*	0.16^{ns}	-0.17^{ns}	0.20*

Figure 1 Pearson correlations among BLUPs of the hybrids for field green weight (FGW), total dry matter (TDM), leaf dry matter (LDM), leaf percentage (L), leaf/stem ratio (LSR), regrowth (REG), crude protein (CP), *in vitro* digestibility of organic matter (IVD), neutral detergent fiber (NDF), lignin in sulfuric acid (LIG) and yield of high nutritional value leaf mass (NLM) in the combined analysis. *significant at 5% and ^{ns} not significant at 5% probability by the Bootstrap BCa method based on 9999 simulations

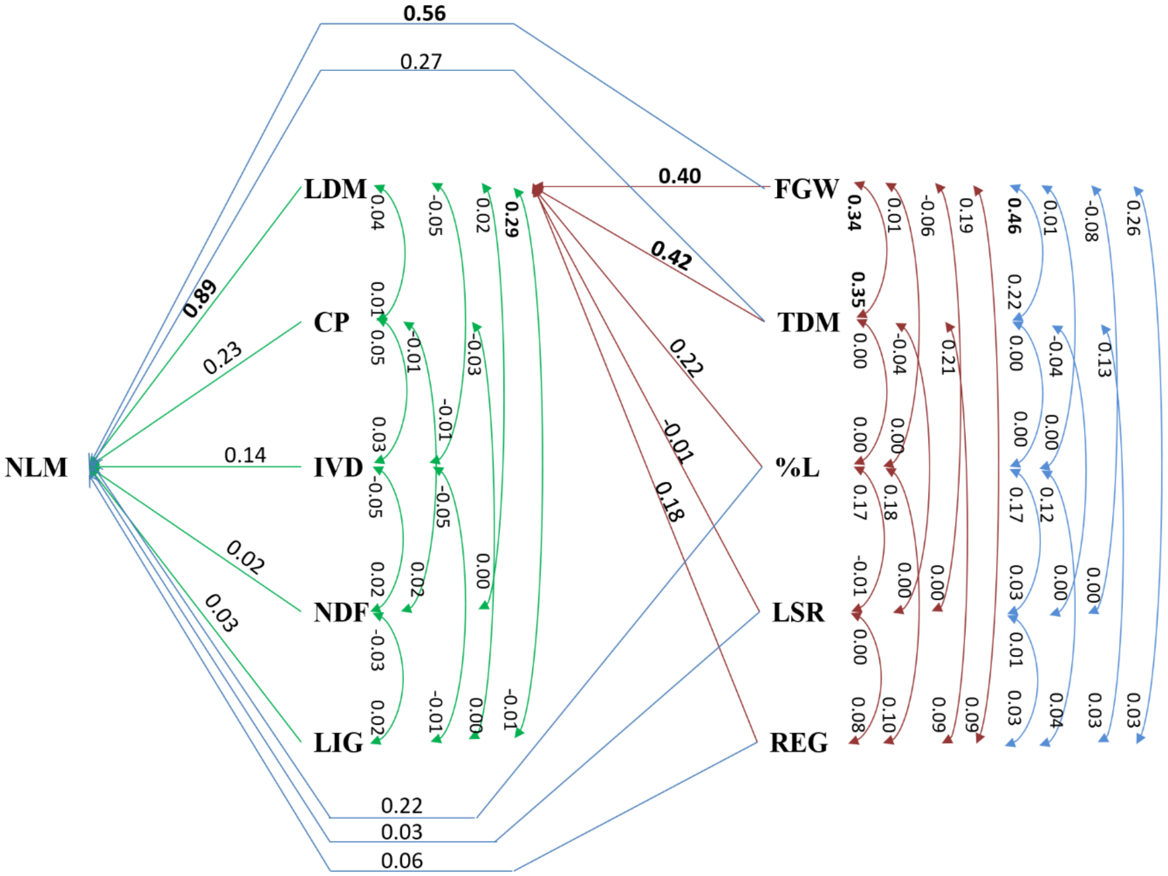


Figure 2. Causal diagram of the direct and indirect effects of the secondary components of field green weight (FGW), total dry matter (TDM), leaf percentage (%L), leaf/stem ratio (LSR), and regrowth (REG), and of the primary component of leaf dry matter (LDM), crude protein (CP), *in vitro* digestibility of organic matter (IVD), neutral detergent fiber (NDF), and lignin in sulfuric acid (LIG) on yield of high nutritional value leaf mass (NLM). Single directional arrows indicate direct effects of the explanatory variables, whereas bidirectional arrows indicate indirect effects. Green lines indicate the direct and indirect effects of the primary variables on NLM, blue lines the direct and indirect effects of the secondary variables on NLM, and red lines the direct and indirect effects of the secondary variables on LDM. Numbers in bold print indicate values superior to the effect of the residual variable.

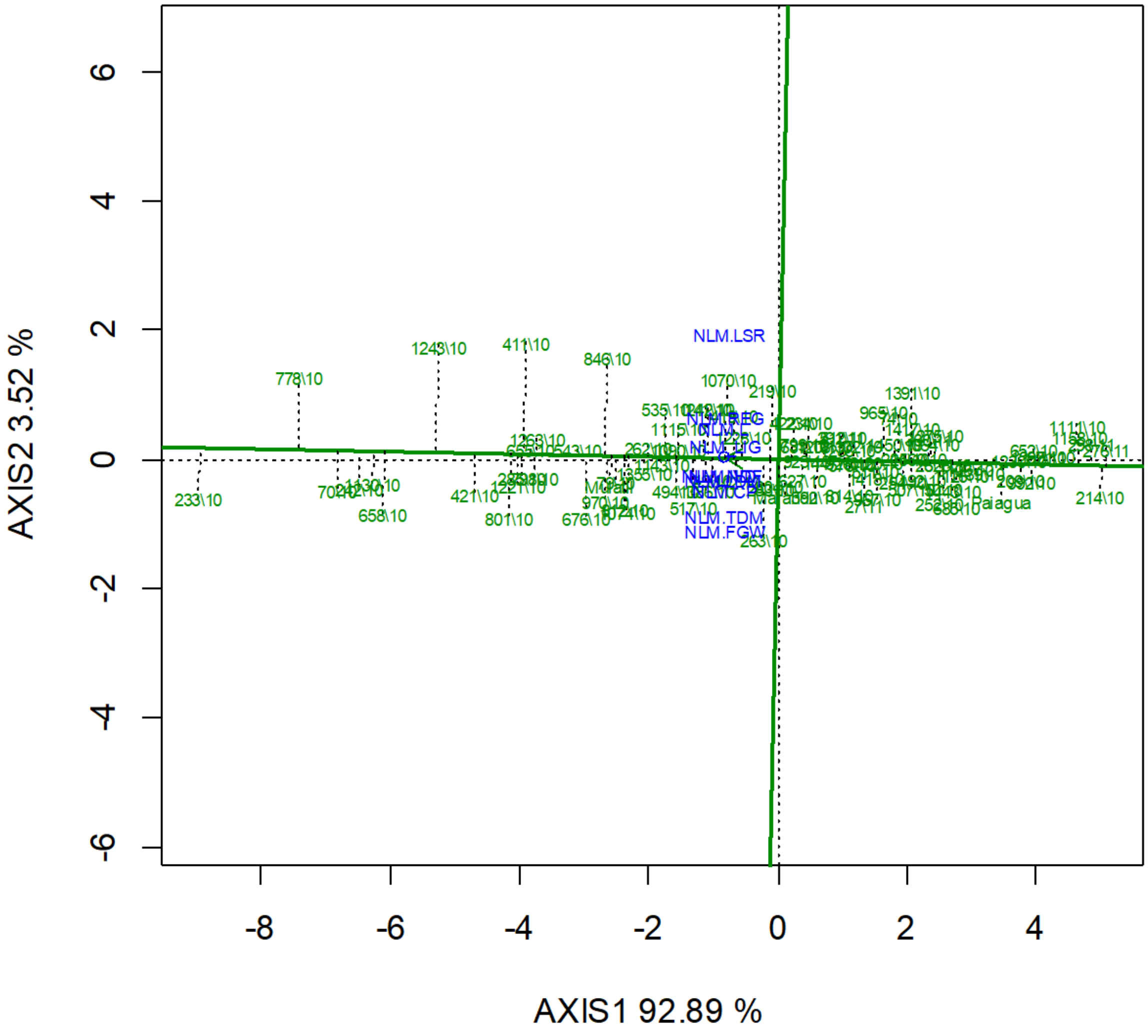


Figure 3. View of mean coordination of the tester of the genotypes by yield*trait biplot constructed using breeding values of each genotype in combined analysis of the cuttings. The biplot was constructed using scaling by standard deviation, centered by 2, and SVP = JK – (row metric preserving). The codes of the traits are FGW: field green weight; TDM: total dry matter; L: leaf percentage; LSR: leaf/stem ratio; REG: regrowth; LDM: leaf dry matter; CP: crude protein; IVD: *in vitro* digestibility of organic matter; NDF: neutral detergent fiber; LIG: lignin in sulfuric acid; NLM: yield of high nutritional value leaf mass.

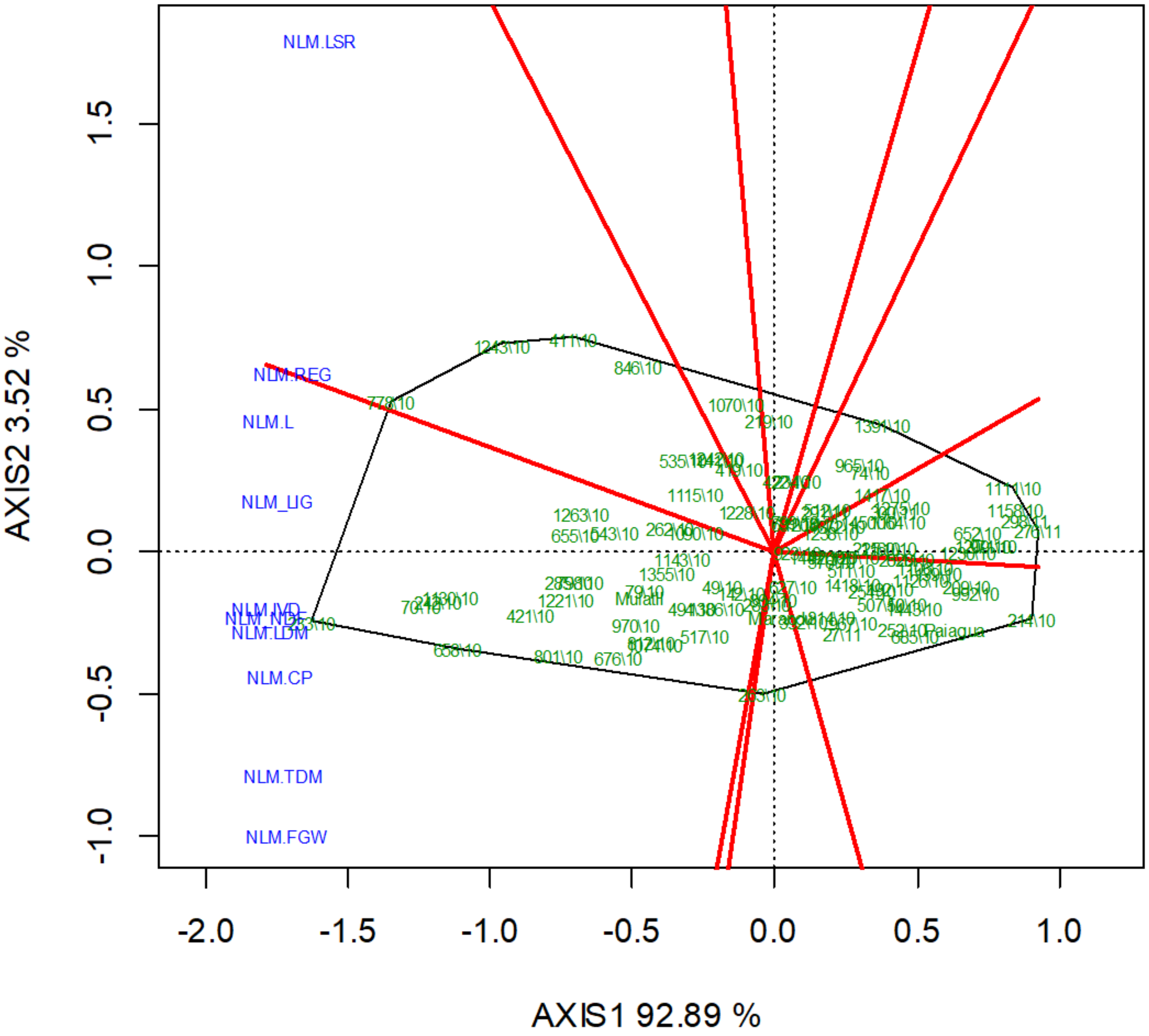


Figure 4. Genotype by yield*trait biplot "which-won-where" constructed using breeding values of each genotype in combined analysis of the cuttings. The biplot was constructed using scaling by standard deviation, centered by 2, and SVP = SQ (Symmetrical). The codes of the traits are FGW: field green weight; TDM: total dry matter; L: leaf percentage; LSR: leaf/stem ratio; REG: regrowth; LDM: leaf dry matter; CP: crude protein; IVD: *in vitro* digestibility of organic matter; NDF: neutral detergent fiber; LIG: lignin in sulfuric acid; NLM: yield of high nutritional value leaf mass.

MANUSCRIPT 2 - COMBINING ABILITY AND HYBRID SELECTION FOR AGRONOMIC AND NUTRITIONAL TRAITS IN *Urochloa* spp

Manuscript written according to the guidelines of the Journal Euphytica

COMBINING ABILITY AND HYBRID SELECTION FOR AGRONOMIC AND NUTRITIONAL TRAITS IN *Urochloa* spp

Abstract: The aim of *Urochloa* breeding program is to development cultivars that provide better animal performance. For this, the breeders have performed crosses between apomictic and sexual parents and following by evaluations of the genetic variability in several cuttings across locations based on agronomic and nutritional values. Thus, the choice of the parents and crosses is crucial for the success of the breeding program and also allow to detail the influence of genetic effects on the phenotypic expression. The aims of this study were to estimate the general (GCA) and specific (SCA) combining ability, and contribution of additive and nonadditive effects, from a partial diallel cross design between 3 apomictic and 10 sexual parents, for agronomical and nutritional traits in *Urochloa* spp., and assess genetic gains for agronomical and nutritional traits by selection based on NLM, and characterize the genotype profiles using GYT biplot analysis. A total of 1380 interspecific hybrids from 29 full-sib progenies were evaluated in an experiment laid out in an incomplete block design. The hybrid selection was performed based on studentized BLUP of NLM and the 10% superior hybrids were used in the GYT analysis, using NLM as basic variable. There was a predominance of nonadditive effects on the phenotypic expression of agronomical and nutritional traits in *Urochloa* spp. The GCA was observed only for some traits in sexual parents whereas SCA was observed to all traits. The selection based on NLM provided favorable gains to most of the agronomic traits and the GYT biplot analysis was efficient for characterization of the genotype profiles.

Keywords: parental selection, diallel, additive variance; nonadditive variance; indirect gain; biplot analysis.

Introduction

Cultivated pastures are the basis to Brazilian livestock because they are the main and the most economical source to feed, for instance, beef and dairy cattle (Jank et al., 2014). The area occupied by pastures in Brazil is 162,3 Mha. Most of this area (150,5 Mha) is planted in monoculture and 11.8 Mha are used in production systems with crops for grains (9.9 Mha) or with another crop (1.9 Mha) (ABIEC, 2019). The genus *Urochloa* is widely used in cultivated pasture area in the country (85%), being the *U. brizantha* cv. Marandu grass, released by Embrapa (Brazilian Agricultural Research Corporation) in 1984, still occupies the largest area (Jank et al., 2014).

The Embrapa holds the main germplasm banks of *Urochloa* spp. in Brazil, with ~450 accessions of 13 different species (Valle et al. 2008). Among the most important species in the genus, *U. brizantha*, *U. decumbens* and *U. humidicola* reproduce asexually by apomixis and sexually, whereas *U. ruziziensis* reproduces only sexually. In the apomictic reproduction the embryo is originated by parthenogenesis, and so, apomictic plants are screened to commercial cultivars and they can be used as male in the breeding program. Apomictic plants are polyploidy and sexual plants are usually diploid, in nature (Pinheiro et al. 2000). The *Urochloa* breeding program at Embrapa Beef Cattle has used diploid-sexual sources ($2n = 2x = 18$) of *U. decumbens* and *U. ruziziensis*, tetraploid-apomictic genotypes ($2n = 4x = 36$) of *U. decumbens* and *U. brizantha*, and sexual and apomictic hexaploidy accesses of *U. humidicola* ($2n = 6x = 36$) (Timbó et al. 2014, Figueiredo et al. 2019).

Initially, the cultivar development was carried out by direct selection and commercialization of natural germplasm accessions. Crossings in the *Urochloa* spp. breeding program began in the late 1980s, when a tetraploid and sexual *U. ruziziensis* was obtained by chromosomal duplication of diploid sexual genotypes, enabling using these genotypes as female in crosses with apomictic tetraploid genotypes of *U. decumbens* and *U. brizantha*, and so, starting the interspecific *Urochloa* breeding program (Valle et al. 2008). In the middle of the 2000s decade, tetraploid sexual genotypes of *U. decumbens* (D24/2, D24/27 and D24/45) were gotten, also by chromosomal duplication, and they were crossed with *U. decumbens* cv. Basilisk to start its intraspecific program (Simioni and Valle 2009; Mateus et al. 2015).

In breeding programs, diallel designs are useful to estimate the general combining ability (GCA) and specific combining ability (SCA), helping plant breeders choosing the best parents and understanding the genetic control of traits (Griffing 1956). There is little information about GCA and SCA for agronomic and nutritional traits in *Urochloa*. Matias et al. (2018) worked with interspecific hybrids of *Urochloa* (*U. ruziziensis, U. decumbens* and *U. brizantha*) and they did not find a clear predominance of additive or dominance effects for agronomic and nutritional traits. In *U. humidicola*, Figueiredo et al. (2019) observed significant GCA only for sexual parents for biomass yield and forage quality traits. Furthermore, these parameters are unknown for the yield of high nutritional value leaf mass (NLM), a natural index that combines leaf dry matter, high digestibility and protein content, which should be better associated with animal gain.

Wide genetic variability is quite often observed from crosses in the forage crops associated mainly with the polyploidy and high heterozygosity (Matias et al. 2016, Simeão et al. 2016, Matias et al. 2018, Figueiredo et al. 2019, Pereira 2019). In this way, the identification of superior genotypes within those crosses is a crucial step to ensure new cultivars and parents in new crosses. In forage breeding, it is more complex than other crops because the final aim is the gain in animal performance (Valle et al. 2008). The hybrid selection has been accomplished by additive indexes with subjective weights for several agronomical and nutritional traits or using biplots with some main traits (Mateus et al. 2015, Matias et al. 2018). However, the selection based on NLM might be more effective for selecting superior genotypes in *Urochloa*. Furthermore, the selection might be performed using graphical approaches as proposed by Yan and Frégeau-Reid (2018) called genotype by yield*trait (GYT). Their approach allows to rank the genotypes and detail their profile regarding multiple traits based on the combination of the basic variable (e.g., yield) with other breeding target traits.

Therefore, the aims of this study were to estimate the GCA, SCA and contribution of additive and nonadditive effects, from a partial diallel cross design between apomictic and sexual parents, for agronomical and nutritional traits in *Urochloa* spp., and assess genetic gains for agronomical and nutritional traits by selection based on NLM, and characterize the genotype profiles using GYT biplot analysis.

Materials and Methods

Genotypes

A partial diallel design was used to cross three apomictic male parents (*U. decumbens* cv. Basilisk, *Urochloa* spp. cv. Mulato II, and *U. brizantha* cv. Marandu) with ten sexual female parents. The female group was compound by seven sexual plants (S20, S29, S59, S92, S115, BS9 and 336T2) from the *Urochloa* interspecific breeding program (*U. brizantha* × *U. decumbens* × *U. ruziziensis*) and other three sexual plants (B13, R33 and R69) from the *U. decumbens* intraspecific breeding program, being both breeding programs coordinated by Embrapa Beef Cattle located in Campo Grande city, Mato Grosso do Sul state, Brazil. These crosses resulted in 29 full-sib progenies and a total of 8,300 hybrids.

The hybrids were submitted to an initial evaluation for spittlebug resistance in greenhouse. For this, around 80,000 adult spittlebugs were released in the greenhouse and hybrids were selected by the mass method based on the lowest leaf damage and non-presence of foam mass. At the end, a total of 1,380 hybrids were selected in the spittlebug resistance evaluation, being variable the number of hybrids per progenies (Table 1). The selected hybrids followed for the field evaluation along three apomictic parents and checks *U. brizantha* cv. BRS Paiaguás and *U.* spp. cv. BRS Ipyporã.

Experimental design

The evaluation of the preselected hybrids was conducted at the Embrapa Beef Cattle experimental field (20°27’ S, 54°37’ W and 530 m asl) in Campo Grande city, Mato Grosso do Sul state, Brazil. The field experiment was laid out in an incomplete block design, formed by 14 blocks of different sizes (ranged from 10 to 30 plots per block). Each plot was constituted by five plants: progeny plots were composed of five hybrids of the same progeny and, for checks and parents the plot contained one seedling of each one. The spacing used between and within rows was 1.5 m, so an area of 11.25 m^2 per plot.

Phenotypic data

The traits were measured in seven cuttings or harvests (cutting 1: 26 Oct. 2016, cutting 2: 05 Dec. 2016, cutting 3: 10 Jan. 2017, cutting 4: 15 Feb. 2017, cutting 5: 21 Mar. 2017, cutting 6: 03 May 2017 and cutting 7: 13 July 2017). The evaluated agronomic traits were field green weight (FGW, $Kg.ha^{-1}$), regrowth capacity (REG, ratings), total dry matter

(TDM, Kg.ha^{-1}), leaf dry matter (LDM, Kg.ha^{-1}), percentage of leaves (%L) and leaves/stem ratio (L:S). For measuring the FGW, the fresh biomass of each plot was weighed in the field after each cutting using the dynamometer. REG was evaluated seven days after cutting using visual scores, as described by Figueiredo et al. (2012). The FGW and REG traits were evaluated in all cuttings. A sample was taken from each plot in the cuttings, except cuttings 1, and it was weighed and then dried at 65°C for 72 hours to estimate TDM. In cuttings 2, 5, and 7, morphological separation of samples was carried out, allowing to estimate %L, LDM and LSR.

For evaluation of nutritional traits, dry leaves from morphological separation were ground and then analyzed using near-infrared reflectance spectroscopy (NIRS, Marten et al., 1989) based on calibration curves for the traits. The nutritional value traits measured were: crude protein content in the dry matter (CP, %, AOAC 1990), *in vitro* organic matter digestibility (IVD, %, Tilley and Terry 1963), neutral detergent fiber (NDF, %, Van Soest et al. 1991) and lignin in sulfuric acid (LIGs, %, Van Soest et al. 1991). The yield of high nutritional value leaf mass (NLM, kg.ha^{-1}) was estimated by equation: NLM = LDM x CP x IVD. The CP, IVD, NDF, LIGs and NLM were estimated for three cuttings (2, 5, and 7).

Statistical Analysis

The phenotypic data were submitted to multi-harvest analysis using the mixed model approach, being the variance components estimation accomplished by the residual maximum likelihood method (REML) and the significance of the variance components verified by likelihood ratio test (LRT). The analyses were performed using ASReml-R v.4 (Butler, 2009) based on two main models: Diallel model to estimate combining abilities, and hybrid selection model to estimate and select the promising hybrids based on their genotypic values. These models are detailed later.

In the diallel model, for each trait was performed the modeling of the covariance matrix of residual (R) effects, whereas in the hybrid selection model it was realized to covariance matrix of R and genetic (G) effects. The covariance structure selection was done sequentially according to Smith et al. (2007). First, it was identified the best structure for R and then for G, considering the R matrix previously selected. The best structure for R and G was indicated by the lower Bayesian information criterion (BIC) proposed by Schwarz (1978).

Diallel model

The diallel analysis to estimate variance components and general combining ability (GCA) of sexual and apomictic parents and specific combining ability (SCA) of the crosses was performed according to following model for each trait:

$$y = X_1c + Z_1b + Z_2a + Z_2s + Z_2i + Z_2p + Z_2q + Z_2t + e \qquad \text{Eq. [1]}$$

where *y* is the vector of phenotypic data; *X* is the incidence matrix of fixed effects and *Z*'s are incidence matrices of the random effects; *c* is the vector of intercept and fixed effects of cuttings; b is the vector of random effects of blocks, $b \sim MVN(0, I\sigma_b^2)$ and σ_b^2 is the variance of blocks; *a* is the vector of random effects of GCA of apomictic parents, $a \sim MVN(0, I\sigma_{\mathrm{APO}}^2)$ and σ_{APO}^2 is the variance of GCA of apomictic parents; *s* is the vector of random effects of GCA of sexual parents, $s \sim MVN(0, I\sigma_{\mathrm{SEX}}^2)$ and σ_{SEX}^2 is the variance of GCA of sexual parents; *i* is the vector of random effects of SCA of the crosses between sexual and apomictic parents, $i \sim MVN(0, I\sigma_{\mathrm{APO\ x\ SEX}}^2)$ and $\sigma_{\mathrm{APO\ x\ SEX}}^2$ is the variance of SCA of the crosses between apomictic and sexual parents; *p* is the vector of random effects of interaction between GCA apomictic parents and cuttings, $p \sim MVN(0, I\sigma_{\mathrm{APO} \times \mathrm{c}}^2)$ and $\sigma_{\mathrm{APO} \times \mathrm{c}}^2$ is the variance component of GCA apomictic parent × cutting interaction; *q* is the vector of random effects of interaction between GCA of sexual parents and cuttings, $q \sim MVN(0, I\sigma_{\mathrm{SEX} \times \mathrm{c}}^2)$ and $\sigma_{\mathrm{SEX} \times \mathrm{c}}^2$ is the variance of GCA sexual parent × cutting interaction; *t* is the vector of random effects of interaction between SCA of the crosses between apomictic and sexual parents and cuttings, $t \sim MVN(0, I\sigma_{\mathrm{APO} \times \mathrm{SEX} \times \mathrm{c}}^2)$ and $\sigma_{\mathrm{APO} \times \mathrm{SEX} \times \mathrm{c}}^2$ is the variance of SCA × cutting interaction; and *e* is the vector of random error within cuttings, $e \sim MVN(0, R \otimes \mathrm{I})$; R is the residual covariance matrix; *I* is identity matrix. The Kronecker product is denoted by $\otimes$.

The additive variance was estimated by the equation $\sigma_{\mathrm{A}}^2 = 4\sigma_{\mathrm{APO}}^2$, the dominance variance by $\sigma_{\mathrm{D}}^2 = \frac{9}{2}(\sigma_{\mathrm{APO} \times \mathrm{SEX}}^2 - 2\sigma_{\mathrm{APO}}^2)$, and the phenotypic variance by $\sigma_{\mathrm{p}}^2 = \sigma_{\mathrm{A}}^2 + \sigma_{\mathrm{D}}^2 + (\frac{\sigma_{\mathrm{e}}^2}{b.c})$, where σ_{e}^2 is the average residual variance for each cutting from Eq. [1], b is the number of blocks and c is the number of cuts, as proposed by Matias et al. (2018). The narrow-sense heritability was calculated by $h^2 = \frac{\sigma_A^2}{\sigma_p^2}$ for Eq. [1]. For significant effects of the SCA or GCA from Eq. [1], estimated the studentized BLUP (t-BLUP) based on the expression $t - BLUP = \frac{BLUP}{SE}$, where BLUP is the Best Linear Unbiased Prediction of each hybrid and SE is the standard error of the respective BLUP (Yan et al. 2002).

Hybrid selection model

The following model was used to hybrid selection:

$$y = X_1c + X_2t + Z_1b + Z_2g + e$$ Eq. [2]

where *y* is the vector of phenotypic data; *X*'s is the incidence matrix of fixed effects and *Z*'s are incidence matrices of the random effects; *c* is the vector of fixed effects of cuttings; *t* is the vector of fixed effects of checks and apomictic parents; *b* is the vector of random effects of blocks, $b \sim MVN(0, I\sigma_b^2)$ and σ_b^2 is the variance of block; *g* is the vector of random effects of hybrids within cuttings, $g \sim MVN(0, G \otimes \mathrm{A})$ and *G* is the genetic covariance matrix, and A is the relationship (kinship) matrix considering information of apomictic and sexual parents; and *e* is the vector of random error within cuts, where $e \sim NMV(0, R \otimes \mathrm{I})$ and *R* is the residual covariance matrix; and *I* is identity matrix. The Kronecker product is denoted by $\otimes$.

The BLUP predictions of the random effects were obtained, and then the genetic correlations between the traits were estimated using the BLUPs of the hybrids using the *Agricolae* R package (Mendiburu 2014). The significance of the genetic correlations was checked via Bootstrap by the "bias-corrected and accelerated" (BCa) method, with 9999 simulations, using the *wBoot* R package (Weiss 2016). The heritability on a hybrid-difference basis (H_Δ^2) was estimated, as proposed by Schmidt et al. (2019), using the expression: $H_\Delta^2 = \frac{2}{n_g\,(n_g - 1)} \sum_i \sum_{j<i} H^2_{\Delta \boldsymbol{ij} BLUP}$, where n_g is the number of hybrids and $H^2_{\Delta \boldsymbol{ij} BLUP}$ is the heritability of the predictor of the difference between hybrids i and j, being $H^2_{\Delta \boldsymbol{ij} BLUP}$ estimated by expression $H^2_{\Delta \boldsymbol{ij} BLUP} = \frac{2\sigma_g^2 - V_{\Delta ij}^{BLUP}}{2\sigma_g^2}$ where σ_g^2 is the genetic variance of hybrids and $V_{\Delta ij}^{BLUP}$ is the prediction error variance of a difference between BLUPs of genotypes i and j. The hybrid selection was carried out by direct selection on NLM, which NLM t-BLUPs

were used to rank the genotypes and an intensity selection of 10% was applied, being then calculated the selection gain (%) for all traits.

The best 10% hybrids, checks and apomictic parents were used in the genotype by yield*trait biplot (GYT) analysis, where the NLM variable was taken as basic variable (yield). From this, the BLUP means of the hybrids over cuttings for NLM were combined with the BLUP means of each other traits to obtain the values of the yield*trait variables, as proposed by Yan and Frégeau-Reid (2018). For FGW, TDM, LDM, %L, LSR, REG, CP, and IVD, where selection is for increasing the trait, the BLUPs of the trait were multiplied by the BLUP of NLM, for example, NLM*CP. For LIG and NDF, where selection is for reducing the value of the trait, the BLUP of NLM was divided by the BLUP of these traits, i.e., NLM/NDF and NLM/LIGs. Biplots of the yield*trait variables were generated using the *GGEBiplotGUI-R* package (Frutos et al. 2014).

Results

Genetic parameters

The covariance structures of the *G* and *R* matrices varied according to the model and traits evaluated (Table 1 and 2, Supplemental material). Regarding to GCA, the variance components of apomictic parents were null ($p < 0.05$) by the likelihood ratio test (LRT) for all traits, whereas the variance components of sexual parents were significant ($p < 0.05$) for FGW, %L, LSR and CP (Table 2). The effect of crosses (SCA) were non-null ($p < 0.05$) for all traits.

The inheritance of all assessed traits was predominantly determined by non-additive genes with proportions of dominance variance varying from 24 to 95% (Table 2). The LSR, CP, IVD, NDF and LIGs presented only dominance effect, whereas the additive variance proportion were low for %F and REG (< 2%) and ranged from 14 to 37% for FGW, TDM, LDM and NLM. Despite of the narrow-sense heritability estimates ranged from 0 to 0.37, the estimates for all traits was not significant (Table 2).

In the hybrid selection model, the hybrid variance was significant by the likelihood ratio test (LRT) at 1% probability for all traits (Table 3). The heritability on a hybrid-difference basis ranged from 0.61 (LIGs) to 0.97 (LSR), which denotes the greater genetic influence on phenotypic expression of these traits and propitious condition to proceed selection of promising hybrids.

Significant genetic correlations ($p < 0.05$) were detected among most of the traits evaluated (Figure 1). The most positively correlated traits were NLM x LDM (0.95), FGW x TDM (0.92), TDM x LDM (0.91), FGW x LDM (0.89), FGW x NLM (0.87), TDM x NLM (0.85), REG x TDM (0.76), REG x LDM (0.77), REG x FGW (0.73) and REG x NLM (0.73). The correlations between the nutritional traits were of moderate magnitude, ranging from -0.67 (CP x NDF) to 0.54 (LIGs x NDF), and correlations between nutritional traits with the agronomic traits or NLM were of moderate to low magnitude.

Selection of parents, crosses and hybrids

There was no sexual parent that provided increase for FGW, %F, LSR and CP simultaneously (Figure 2). However, B13 provided greater FGW gains, significant by t-Student test ($p < 0.05$), and, at the same time, increased CP but the t-BLUP for CP was null. Similarly, in the SCA effect, despite of there was no cross that provided increase for all traits simultaneously, the crosses B13 × Basilisk and R69 × Mulato II stood out because they provided the higher performance to FGW, TDM and LDM and favorable increase for all other traits, except LIGs to R69 × Mulato II and IVD to both crosses (Figure 3). However, t-BLUPs for these crosses were significant ($p < 0.05$) only for LDM, NLM and REG in the R69 × Mulato II cross and for NLM and REG to B13 × Basilisk (Figure 3).

The selection of 10% superior hybrids, based on ranking of NLM breeding values, resulted in the selection gain favorable to FWG, TDM, LDM, REG and NLM, whereas for %L, LSR and nutritional traits the selection gain were unfavorable (Figure 4). The checks and parents were not in the 10% top-performing hybrids: Marandu, Mulato II, Basilisk, Ipyporã and Paiaguás were on 210, 293, 805, 903 and 1385th in the ranking position, respectively. The number of selected hybrids per cross ranged from 0 to 13, where crosses S92 × Marandu (13), S20 × Mulato II (13), S92 × Mulato II (10), B13 × Basilisk (10) and S29 × Basilisk (10) provided the higher number of offspring in the 10% superior hybrids (Figure 5).

The 138 top-performing hybrids, checks and parents were used to create the "which-won-where" GYT biplot (Figure 6). In this biplot the traits were grouped in three sectors, being that REG, LDM, FGW and TDM were placed in one sector, %F, CP, IVD, NDF and LIGs in another one and LSR was its own sector (Figure 6). Thus, it is possible to describe the profile of the hybrids in a more detailed manner, in which the hybrid 1411 (S59 × Marandu) proved to be superior in the combinations of NLM with %F and nutritional traits, whereas the hybrids 48 (R69 × Basilisk), 213 (S29 × Basilisk) and 759 (S20 × Marandu) were superior for the combination of NLM with REG, LDM, FGW and TDM. The top-performing hybrid was the 950 (BS9 × Marandu) to combination of NLM with LSR. The checks and parents were placed in the same sector with no traits.

Discussion

Parents with higher GCA indicates higher probability to produce outstanding hybrids due to presence of favorable additive effect and SCA is related with the performance of crosses and non-additive effects, being both information useful in recurrent selection (Comstock 1949, Ginkel and Ortiz, 2018). The proportion of additive variance relative to phenotypic variance as well as narrow-sense heritability estimates were lower than observed in *Urochloa* spp. by Matias et al. (2018), except to %F and LIGs which both traits were null as observed in our study. All traits presented more non-addictive effects and significant variance to SCA (Table 2). Thus, the selection should be based on the performance of the best cross to improve all traits, where both B13 × Basilisk and R69 × Mulato II provided the higher performance for REG and NLM, and R69 × Mulato II also for LDM.

In *U. humidicola* opposite results were observed, which SCA was not significant between the combinations of sexual or apomictic parents for the evaluated traits (Figueiredo et al. 2019). In interspecific hybrids, unlike our study, Matias et al. (2018) did not find clear trend in the importance of additive and nonadditive effects on agronomical and nutritional traits, where despite of the SCA effect was significant for FGW, TDM, CP, NDF and REG, only for FWG observed high non-additive effect. Matias et al. (2018) suggested the absence of the effect of SCA for some of the traits was due to the population comes from interspecific crosses between polyploid heterozygous species, so that there are many sources of variation within and among progenies, to the point of not being able to clearly discriminate parents or ideal combinations. In our study there were progenies of *U. decumbens* (B13 × Basilisk, R33 × Basilisk and R69 × Basilisk), where Basilisk also is parent of the three sexual parents of *U. decumbens* used in our diallel, and moreover, R69 and R33 are full sibs. In addition, the progenies used in our study were screened for spittlebug before, which may have reduced the wide variation and led to progeny discrimination.

The GCA, related to variance of parents, was significant only for FGW, %F, LSR and CP in sexual parents (Table 2). Similarly, the apomictic parents did not show significant GCA variance in *U. humidicola* (Figueiredo et al. 2019). In *Urochloa* spp., using a diallel design with four sexual parents and five apomictic parents, including Marandu, Basilisk and Mulato II, Matias et al. (2018) observed significant variance for four traits (CP, NDF, REG and density of regrown tillers) in sexual and two traits (regrowth speed and LDM) to apomictic parents. Higher GCA estimates was observed in female parents than male parents in *Panicum maximum*, considering the agronomic traits and the CP (Pereira, 2019). Figueiredo et al. (2019) suggested apomixis can function as a genetic block suffering no alteration over the meiosis, thus, there is the possibility of lack of recombination in the formation of gametes. Nonetheless, in diallel used in *U. humidicola* all sexual and apomictic parents came from a cross between a unique allohexaploid sexual plant in the germplasm bank and the apomictic cultivar BRS Tupi (Figueiredo et al. 2019). The majority of the apomictic parents used in our study came from a natural germplasm collection (Marandu and Basilisk), and Mulato II is an interspecific hybrid, whereas sexual parents have passed by some selection cycles (Matias et al. 2016). The parents used by Matias et al. (2018) and by Pereira (2019) had similar composition, most of apomictic from germplasm bank and sexual parents have passed by selection cycles. Thus, probably, the reproduction mode only favored to breed sexual parents in the breeding programs, and it increased the variability among parents.

The heritability on a hybrid-difference basis (H^2_{Δ}) is focused on genotype "differences", being estimated considering the prediction error variance of a difference between BLUPs of two genotypes (Schmidt et al. 2019). The H^2_{Δ} were higher than 0.61, indicating that genetic variances were the higher proportion of phenotypic variance for all traits. This parameter is variable depending on the environmental condition, species and population variability, so in the literature there are contrasting estimates like for TDM in *Urochloa* spp. (0.88) and *U. decumbens* (0.57), for %L in *Urochloa* spp. (0.31) and *U. humidicola* (0.68), and for LSR in *Urochloa* spp. (0.00), *U. humidicola* (0.50) and *U. decumbens* (0.84) (Figueiredo et al. 2012, Mateus et al. 2015, Matias et al. 2018). However, the heritability can range depending of estimation method, being that the heritability between hybrid means has been used in the *Urochloa* spp. in the literature and there is no information about H_{Δ}^{2} because this method was proposed recently. Comparing with *Urochloa* spp., the H_{Δ}^{2} estimates in our study were higher than heritability between hybrid means estimated by Matias et al. 2018, mainly to %L (0.31) and LSR (0.00) which in our study were 0.77 and 0.97, respectively. This difference can be due to population variability and environment condition as well as estimation method (Schmidt et al. 2019). Schmidt et al. (2019) highlighted that, since the goal in the plant breeding is to select the best-performing genotype(s) to maximize the genetic gain and the ranking of genotypes is uniquely determined by all pairwise differences, to considerate genotypes “differences”, as used in H_{Δ}^{2}, makes more sense than genotypes means or effects themselves which do not inform about the ranking.

An ideal forage plant should be superior in multi-traits, as such as production of dry matter, especially of leaves, regrowth ability and nutritional value; and so, it is important understanding correlations and their implications in the selection (Valle et al., 2008). The traits FWG, TDM, LDM and NLM exhibited high and positive correlation estimates with each other, indicating that the indirect selection can be successful performed, and moreover, LDM has higher influence on NLM, and so, the selection based on higher NLM will select hybrids with higher LDM. High and positive correlation for FWG x TDM in *U. decumbens* (Mateus et al 2015, Matias et al. 2016) and *U.* spp. (Matias et al. 2018) were also observed in the literature. Nevertheless, the FWG is easier trait to be measured and its use for indirect selection for TDM, LDM or NLM in the initial steps of breeding programs will useful to save resources (Borges et al. 2011).

The correlations among FWG, TDM, LDM, REG and NLM with nutritional traits suggested that it is possible to identify hybrids simultaneous with good agronomic and nutritional performance, and moreover, the selection of genotypes with higher NLM, besides higher LDM, can pick hybrids with higher IVD and CP, simultaneous. These results were unlike those observed in *U. decumbens* (Matias et al. 2016) and *U. humidicola* (Figueiredo et al. 2012), where higher magnitudes of negative correlation for TDM x CP and FWG x CP impaired favorable selection gain and hybrid selection with high performance for these traits simultaneals. However, null correlation to TDM x CP and FWG x CP were observed in *Urochloa* spp., which authors suggested to be resulted of the combination between high nutritional value from *U. ruziziensis* and good agronomic yield from *U. brizantha* and *U. decumbens* (Matias et al. 2018).

The selection gains confirmed that LDM has higher influence in NLM because the selection based on NLM provided a favorable gain for agronomic traits LDM and for traits strong correlated with LDM (FGW, TDM and REG), whereas for %F, RFC, PB, DIV, NDF and LIGs presented unfavorable gains. Despite of these unfavorable gains, in the GYT biplot analysis was possible to identify genotypes with profile superior in the combinations of NLM with %F and nutritional traits than to combinations of NLM with agronomic traits FWG, TDM, LDM and REG. Therefore, the selection based on NLM allowed to select superior hybrids with profile more agronomic or nutritional. Furthermore, *U. decumbens* hybrids (48, 1144, 489, 47 and 326) presented profile superior to combination NLM with FWG, TDM, LDM and REG, whereas for combination NLM with %F and nutritional traits only interspecific hybrids were placed in the sector for these NLM-traits. These results support the hypothesis that alleles from *U. ruziziensis* contributed to improve the nutritional value in interspecific hybrids, as suggested by Matias et al. (2018).

The selected hybrids can be sexual or apomictic since the crosses between sexual clones, used as female, and apomictic parent pollen result progenies segregating 1:1 for reproductive mode (Worthington and Miles, 2015). Therefore, in addition of the selected hybrids proceed for next stages of agronomic, nutritional, spittlebug resistance and seed production evaluation, they will be evaluated for reproductive mode, by molecular markers, clonal test and/or embryo sac analysis (Jank et al. 2014, Worthington and Miles, 2015). The apomictic hybrids are potential new cultivars, whereas the sexual are new sexual parents.

Conclusions

There was a predominance of nonadditive effects on the phenotypic expression of agronomical and nutritional traits in *Urochloa* spp. The GCA was observed only for FGW, %F, LSR and CP in sexual parents whereas SCA was observed to all traits. The selection based on NLM provided favorable gains to agronomic traits FGW, TDM, LDM and REG and unfavorable gains to nutritional traits. The GYT biplot analysis was efficient for characterization of the genotype profiles, being possible to identify hybrids with profile more agronomic or nutritional.

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Table 1 Number of hybrids per full-sib progeny of *Urochloa* spp. selected by mass method for spittlebug resistance.

Sexual Parents	**Apomictic Parents**		
	***U. decumbens* cv. Basilisk**	***Urochloa* spp. cv. Mulato II**	***U. brizantha* cv. Marandu**
S 20	50	65	60
S 29	50	0	35
S 59	65	15	80
S 92	50	35	80
S 115	65	20	65
BS 9	55	65	65
336 T2	80	65	65
B13	65	5	50
R33	30	5	15
R69	50	15	15

Table 2 Variance components of GCA of apomictic (σ^2_{APO}) and sexual (σ^2_{SEX}) parents and SCA or crosses effects ($\sigma^2_{\mathrm{APO\times SEX}}$) and their interaction with cuttings ($\sigma^2_{\mathrm{APO\times c}}$, $\sigma^2_{\mathrm{SEX\times c}}$, and $\sigma^2_{\mathrm{APO\times SEX\times c}}$), additive variance ($\sigma^2_{\mathrm{A}}$), dominant variance ($\sigma^2_{\mathrm{D}}$) and narrow-sense heritability (h^2) of *Urochloa* spp. in the combined analysis[†]

Component	**Traits** [‡]										
	FGW	**TDM**	**LDM**	**%L**	**LSR**	**REG**	**CP**	**IVD**	**NDF**	**LIGs**	**NLM**
σ^2_{APO}	6546.93^{ns}	1280.34^{ns}	1549.44^{ns}	0.03^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	16.24^{ns}
σ^2_{SEX}	130848.41*	5413.30^{ns}	575.21^{ns}	6.02*	0.35*	0.01^{ns}	0.09*	0.48^{ns}	0.13^{ns}	0.10^{ns}	0.00^{ns}
$\sigma^2_{\mathrm{APO\times SEX}}$	41416.68*	4105.08*	5383.87*	4.27*	0.07*	0.03*	0.06*	0.58*	0.51*	0.49*	53.10^{*}
$\sigma^2_{\mathrm{APO\times c}}$	33979.67*	513.87^{ns}	162.34^{ns}	0.19^{ns}	0.02^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.06^{ns}	0.00^{ns}	6.09^{ns}
$\sigma^2_{\mathrm{SEX\times c}}$	47449.10*	2662.95*	1651.45*	1.43*	0.08*	0.01*	0.02^{ns}	0.11^{ns}	0.07^{ns}	0.00^{ns}	15.33*
$\sigma^2_{\mathrm{APO\times SEX\times c}}$	52078.28*	2701.96*	1214.24*	1.48*	0.00^{ns}	0.01*	0.06*	0.72*	0.09*	0.01*	18.21*
σ^2_{A}	26187.72	5121.36	6197.78	0.13	0.00	0.00	0.00	0.00	0.00	0.00	64.96
σ^2_{D}	127452.7	6949.82	10282.43	18.93	0.33	0.13	0.28	2.61	2.20	0.02	92.79
σ^2_{A} (%)	14.07	36.60	32.45	0.64	0.00	1.84	0.00	0.00	0.00	0.00	33.74
σ^2_{D} (%)	68.50	49.66	53.83	93.28	23.76	95.19	88.48	82.35	90.44	76.14	48.20
h^2	0.14	0.37	0.32	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.34

[‡] FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter (Kg.ha^{-1}); LDM = leaf dry matter (Kg.ha^{-1}); %L = percentage of leaves (%); LSR = leaf/stem ratio; REG = regrowth capacity (grades); CP = crude protein (%); IVD = *in vitro* organic matter digestibility (%); NDF = neutral detergent fiber (%); LIGs = lignin in H_2SO_4; NLM = yield of high nutritional value leaf mass (Kg.ha^{-1}). [†]Seven cuts to FGW, REG; five cuts to TDM; three cuts to LDM, %L e LSR, CP, IVD, NDF, LIGs and NLM. * and ns: significant and non-significant by the likelihood ratio test (LRT) at 5% probability, respectively

Table 3 Component of hybrid variance (σ_g^2), heritability on an entry-difference basis (H_Δ^2), general mean of hybrids ($\overline{y_H}$) and general mean of apomictic parents and checks ($\overline{y_P}$) of *Urochloa* spp. hybrids in the combined analysis†

	Trait ‡					
	FGW	**TDM**	**LDM**	**%L**	**LSR**	**REG**
σ_g^2	7099480.3**	224759.24**	108047.1**	44.21**	85.50**	0.76**
H_Δ^2	0.95	0.92	0.85	0.77	0.97	0.92
$\overline{y_H}$	4439.22 ± 594.77	908.74 ± 142.14	698.73 ± 147.40	65.85 ± 3.48	3.91 ± 1.38	2.74 ± 0.28
$\overline{y_P}$	4262.97 ± 815.21	840.21 ± 201.41	709.08 ± 226.29	65.85 ± 3.98	4.43 ± 2.67	2.66 ± 0.44
	CP	**IVD**	**NDF**	**LIGs**	**NLM**	
σ_g^2	1.21**	5.28**	4.78**	0.04**	1392.7**	
H_Δ^2	0.81	0.66	0.73	0.61	0.85	
$\overline{y_H}$	14.82 ± 0.54	70.91 ± 1.52	63.37 ± 1.26	1.86 ± 0.15	72.89 ± 15.65	
$\overline{y_P}$	14.76 ± 0.75	70.97 ± 1.73	63.44 ± 1.57	1.88 ± 0.17	74.00 ± 18.15	

‡ FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter (Kg.ha^{-1}); LDM = leaf dry matter (Kg.ha^{-1}); %L = percentage of leaves (%); L:S= leaf/stem ratio; REG = regrowth capacity (grades); CP = crude protein (%); IVD = *in vitro* organic matter digestibility (%); NDF = neutral detergent fiber (%); LIGs = lignin in H_2SO_4. †Seven cuts to FGW, REG; five cuts to TDM; three cuts to LDM, %L e LSR, CP, IVD, NDF, LIGs and NLM. **Significant by the likelihood ratio test (LRT) at 1% probability

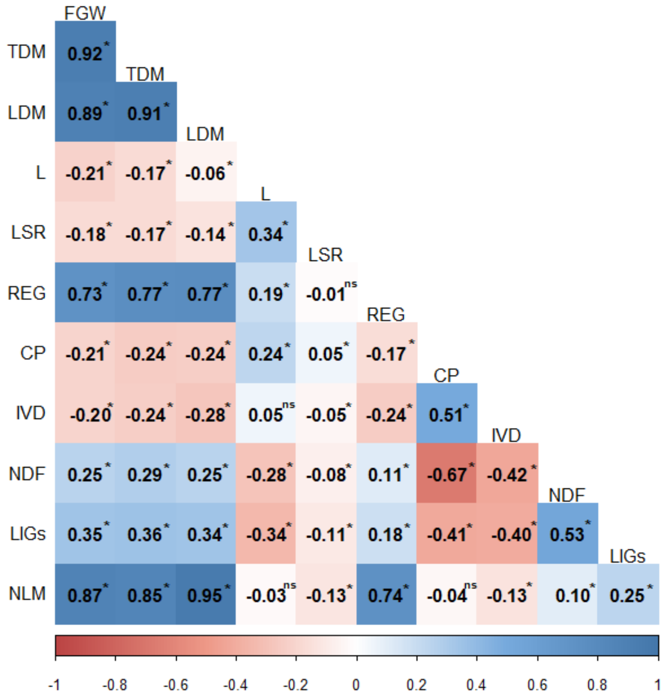

Figure 1 *Pearson* correlations among BLUPs of the hybrids for field green weight (FGW), total dry matter (TDM), leaf dry matter (LDM), leaf percentage (L), leaf/stem ratio (LSR), regrowth (REG), crude protein (CP), *in vitro* digestibility of organic matter (IVD), neutral detergent fiber (NDF), lignin in sulfuric acid (LIGs) and yield of high nutritional value leaf mass (NLM) in the combined analysis. *significant at 5% and ^{ns} not significant at 5% probability by the Bootstrap BCa method based on 9999 simulations

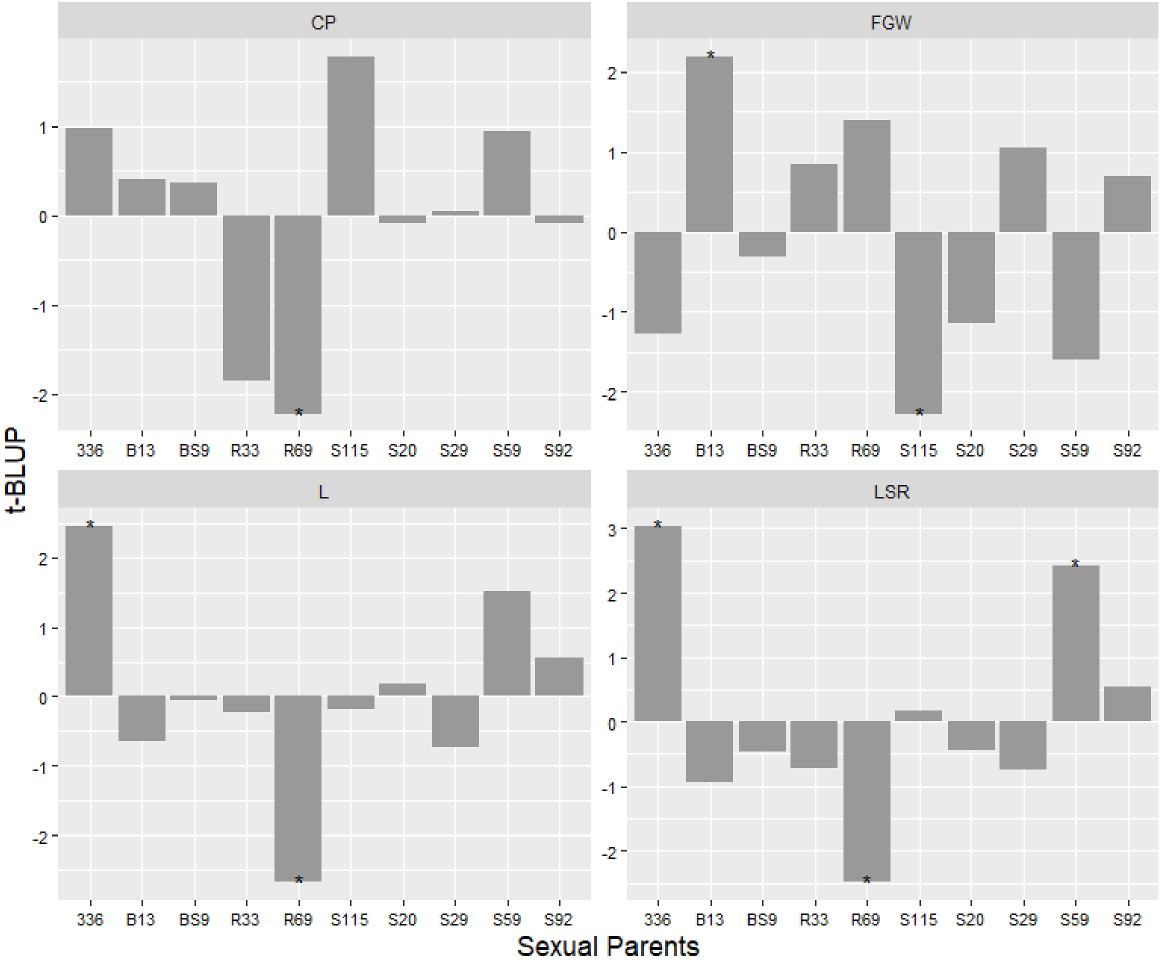

Figure 2 Studentized BLUP (t-BLUP) of the general combining ability of the sexual parents of *Urochloa* spp. for field green weight (FGW), percentage of leaves (L); leaf/stem ratio (LSR) and crude protein (CP) traits. *significant at 5% probability by t-Student test.

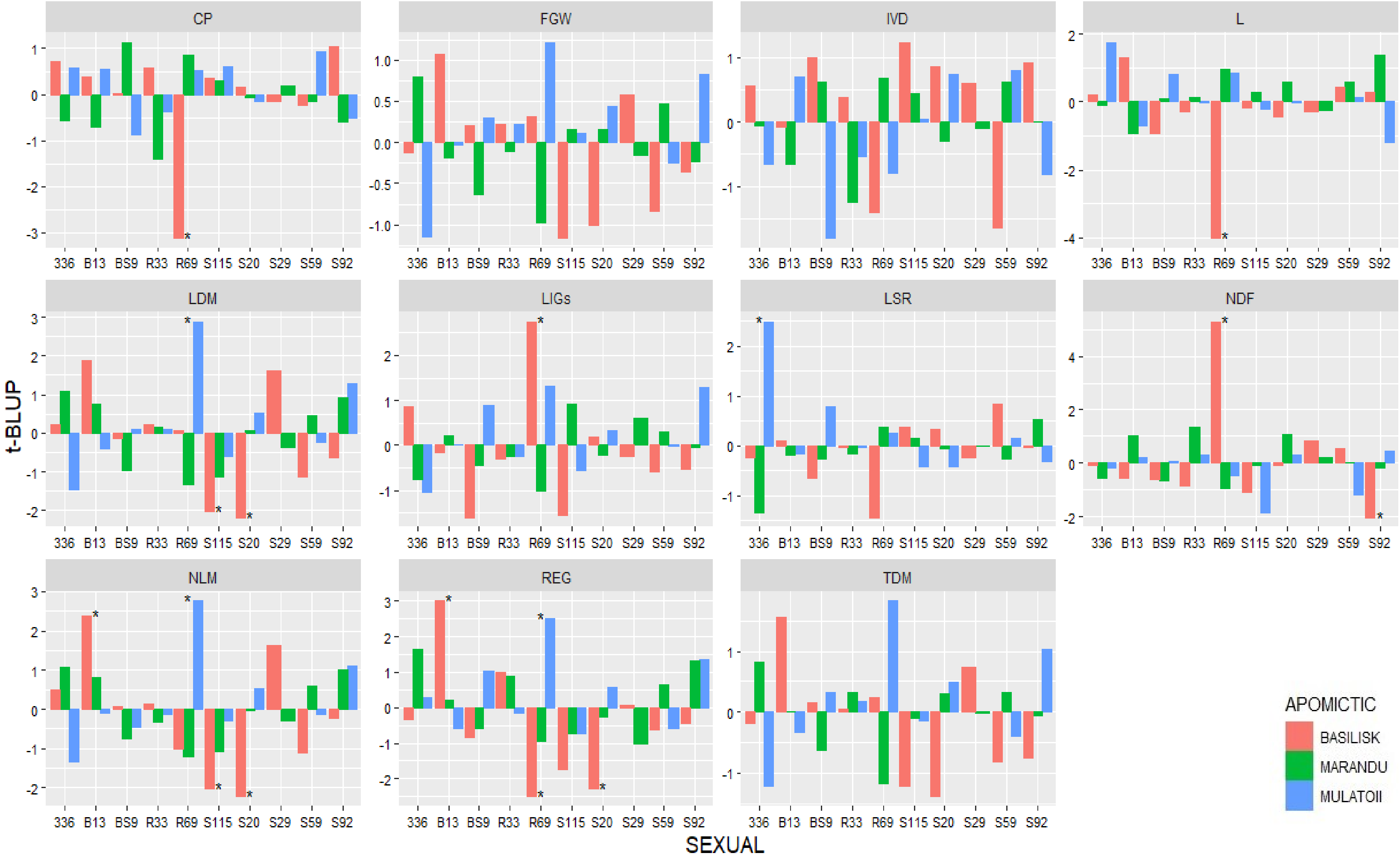

Figure 3 Studentized BLUP (t-BLUP) of the specific combining ability or cross effects estimates of 29 progenies of *Urochloa* spp. for field green weight (FGW), total dry matter (TDM), leaf dry matter (LDM), leaf percentage (L), leaf/stem ratio (LSR), regrowth (REG), crude protein (CP), *in vitro* digestibility of organic matter (IVD), neutral detergent fiber (NDF), lignin in sulfuric acid (LIGs) and yield of high nutritional value leaf mass (NLM) in the combined analysis. *significant at 5% probability by t-student test.

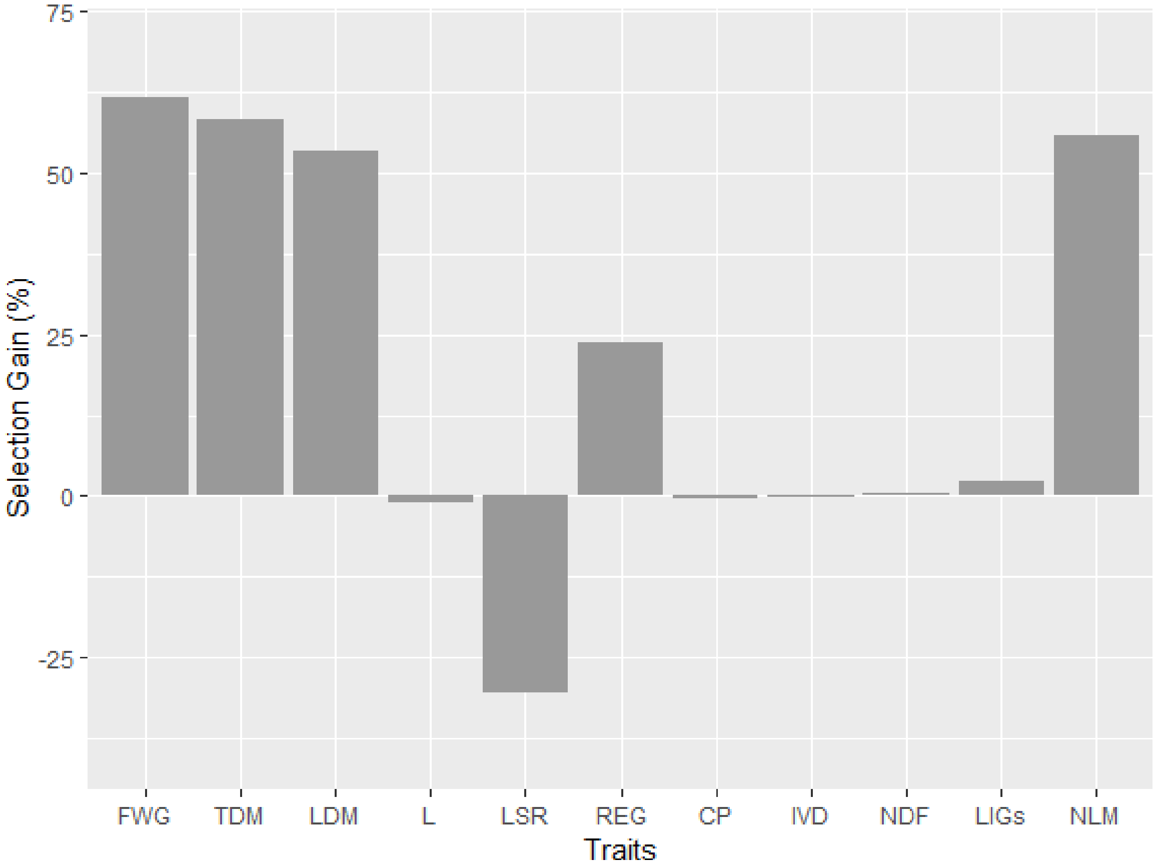

Figure 4 Selection gain (%), based on NLM t-BLUPs and intensity of 10%, for field green weight (FGW), total dry matter (TDM), leaf dry matter (LDM), leaf percentage (L), leaf/stem ratio (LSR), regrowth (REG), crude protein (CP), *in vitro* digestibility of organic matter (IVD), neutral detergent fiber (NDF), lignin in sulfuric acid (LIGs) and yield of high nutritional value leaf mass (NLM).

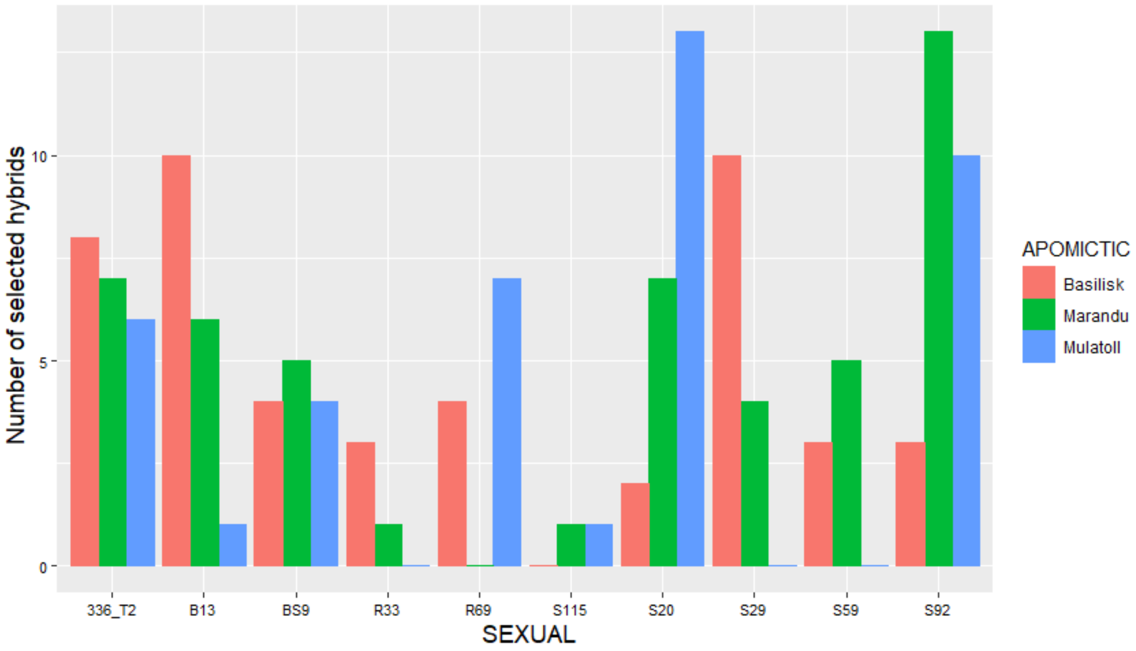

Figure 5 Number of selected hybrids per cross between sexual and apomictic parents using selection based on yield of high nutritional value leaf mass (NLM) t-BLUPs and intensity of 10%.

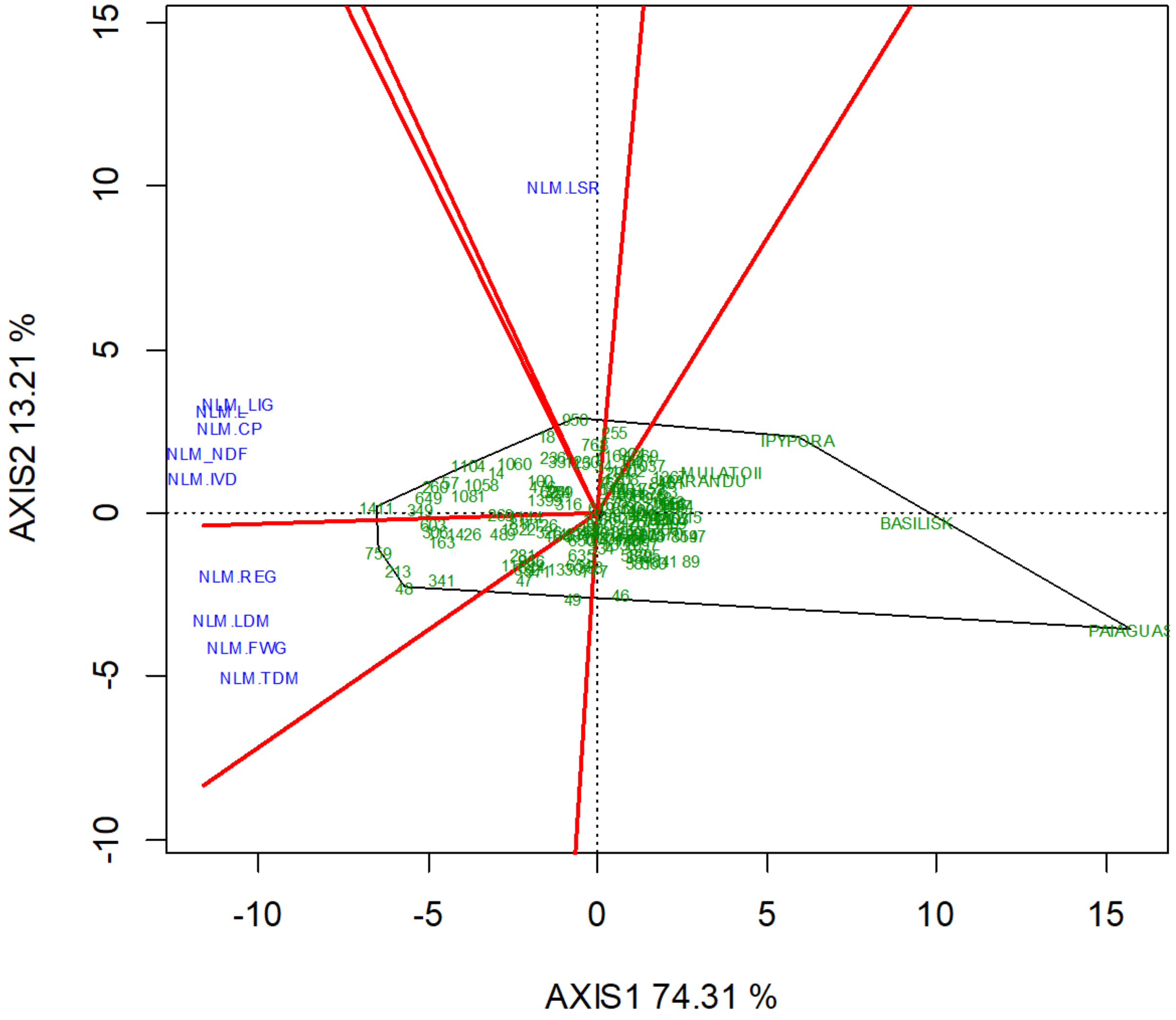

Figure 6 Genotype by yield*trait biplot "which-won-where" constructed using breeding values of each genotype in combined analysis of the cuttings. The biplot was constructed using scaling by standard deviation, centered by 2, and SVP = HJ (Dual Metric Preserving). The codes of the traits are FGW: field green weight; TDM: total dry matter; L: leaf percentage; LSR: leaf/stem ratio; REG: regrowth; LDM: leaf dry matter; CP: crude protein; IVD: *in vitro* digestibility of organic matter; NDF: neutral detergent fiber; LIGs: lignin in sulfuric acid; NLM: yield of high nutritional value leaf mass

Supplemental material

Table 1 Bayesian information criterion (BIC) for the different structures of residual and genetic covariance matrix in the diallel model

		Traits‡					
R†	**G†**	**FGW**	**TDM**	**LDM**	**%L**	**LSR**	**REG**
CS	CS	148391.1	84440.3	48534.8	21554.4	21488.1	4993.4
CSH	CS	145304.8	83375.8	48443	21550.2	15693.1	4911.7
US	CS	144263.6	83106.7	48456.4	21534.6	15684.2	4738.3
ARH(1)	CS	144702	83492.2	48537.4	21581.1	15700.2	5038.0
ARH(2)	CS	144362.5	83251.2	48448.1	21558.5	15700.2	4791.9
DIAG	CS	149080.5	85655.3	49191.7	21670.3	15733.9	6989.6
		CP	**IVD**	**NDF**	**LIGs**	**NLM**	
CS	CS	5479.2	16447.6	12859.9	-1849.4	31681.8	
CSH	CS	5491.4	16461.8	12876.3	-1850.2	31187.5	
US	CS	5498.3	16475.4	12887.3	-1843.3	31197.2	
ARH(1)	CS	5610.7	16473.6	12898.2	-1848.7	31295.5	
ARH(2)	CS	5490.2	16468.3	12882.5	-1844.1	31195.7	
DIAG	CS	5827.8	16476.5	13020.2	-1821.6	31755.6	

†CS = compound symmetry; CSH= compound symmetry heterogeneous; US= unstructured; ARH (1) autoregressive of order 1 heterogeneous; ARH (2) autoregressive of order 2 heterogeneous; DIAG= diagonal; FA1, first-order factor analytic matrix; FA2, second-order factor analytic matrix. ‡FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter (Kg.ha^{-1}); LDM = leaf dry matter (Kg.ha^{-1}); %L = percentage of leaves (%); LSR= leaf/stem ratio; REG = regrowth capacity (grades); CP = crude protein (%); IVD = *in vitro* organic matter digestibility (%); NDF = neutral detergent fiber (%); LIGs = lignin in H_2SO_4; LIGs = lignin in H_2SO_4; NLM = yield of high nutritional value leaf mass (Kg.ha^{-1}). *N.C.=did not converge.

Table 2 Bayesian information criterion (BIC) for different structures of residual and genetic covariance matrix in the hybrid selection model

		Traits‡							
R†	**G†**	**FGW**	**TDM**	**LDM**	**%L**	**LSR**	**REG**	**LIGs**	**NLM**
CS	CS	156272.8	88888.3	50997.9	22635.7	22361.9	5302.7	-1949.3	33278.6
DIAG	CS	153181.7	87693.6	50910.3	22631.6	16263.5	5201.7	-1949.9	32852.7
DIAG	CSH	152593.6	87548.9	50915.7	22625.7	16178.5	5179.0	-1936.7	32790.9
DIAG	US	N.C.*	87530.8	50930.2	22639.5	16159.5	5109.1	-1914.8	32806.2
DIAG	ARH(1)	152151.5	87537.1	50915.0	22624.7	16184.5	5036.5	-1937.2	32792.0
DIAG	ARH(2)	N.C.	N.C.	50922.7	22632.9	16185.7	N.C.	-1904.6	32799.0
DIAG	FA1	152537.8	87509.6	50930.2	22639.5	16159.5	5219.4	-1920.7	32806.2
DIAG	FA2	151996	87524.1	50946.8	22656.1	16301.4	5096.1	-1904.4	32822.9
		CP	**IVD**	**NDF**					
CS	CS	5708.3	17227.3	13468.7					
DIAG	CS	5719.5	17242.3	13485.3					
CS	CSH	5723.4	17243.3	13485.0					
CS	US	5732.7	17256.4	13497.9					
CS	ARH(1)	5727.4	17242.2	13481.5					
CS	ARH(2)	5727.5	17250.5	N.C					
CS	FA1	5732.7	17254.9	13494.5					
CS	FA2	5741.0	N.C.	13509.3					

†CS = compound symmetry; CSH= compound symmetry heterogeneous; US= unstructured; ARH (1) autoregressive of order 1 heterogeneous; ARH (2) autoregressive of order 2 heterogeneous; DIAG= diagonal with heterogeneous variances for each cutting; FA1, first-order factor analytic matrix; FA2, second-order factor analytic matrix. ‡FGW = field green weight (Kg.ha^{-1}); TDM = total dry matter (Kg.ha^{-1}); LDM = leaf dry matter (Kg.ha^{-1}); %L = percentage of leaves (%); LSR= leaf/stem ratio; REG = regrowth capacity (grades); CP = crude protein (%); IVD = *in vitro* organic matter digestibility (%); NDF = neutral detergent fiber (%); LIGs = lignin in H_2SO_4; NLM = yield of high nutritional value leaf mass (Kg.ha^{-1}). *N.C.= did not converge