Plant Breeding

doi:10.1111/pbr.12353

Genetic evaluation and selection index in tetraploid *Brachiaria ruziziensis*

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With 1 figure and 4 tables

Received September 18, 2015 / Accepted December 21, 2015
Communicated by H.-P. Piepho

Abstract

Half-sib progenies of congo signal grass (*Brachiaria ruziziensis* Germain & Evrard, syn.: *B. eminii*, *Urochloa ruziziensis*) were analysed using a mixed model methodology. The objective was to estimate genetic and phenotypic parameters, predict individual genetic values, estimate correlations between characters associated with biomass production and forage nutritive value and use this information to determine the economic weights to compare three multiplicative selection indices. Individual narrow-sense heritabilities corrected for inbreeding varied from 0.14 to 0.91 for characters associated with biomass production and from 0.04 a 0.24 for nutritional value characters. The correlations among characters associated with annual biomass production were of high magnitude. In contrast, biomass production characters were negatively correlated with crude protein, acid detergent fibre and lignin. Total dry matter yield, crude protein and regrowth ability were weighted separately in evaluations during wet and dry season or annually in selection indices. Index that considered characters annually resulted in better distribution of forage production along the year than indices that separated wet and dry season production.

Key words: *Brachiaria* — forage grass — genetic parameters — mixed models — nutritional value — progeny test — tropical grass — yield

Species of *Brachiaria* are cultivated in over 90% of the pasture area in tropical Brazil, which amounts to about 100 million hectares. Breeding of some species from this genus started in the 1980s in Brazil, based on the evaluation and phenotypic selection of natural ecotypes, collected and introduced from Africa (Valle et al. 2008). Several cultivars have been obtained by this method (Jank et al. 2014), which was successful and simplified by the presence of apomixis, that is asexual reproduction by means of seeds, in *Brachiaria brizantha* (syn. *Urochloa brizantha*), *B. decumbens* (syn. *U. decumbens*) and *B. humidicola* (syn. *U. humidicola*) species (Euclides et al. 2010, Jank et al. 2014), also by high broad-sense heritability in agronomic characters (Resende et al. 2007, Basso et al. 2009).

However, *Brachiaria* forage breeders admit that the cultivars commercially available have limitations in terms of productivity, nutritional value and resistance to pests which can be improved by breeding (Miles 2007, Worthington and Miles 2015). To amplify this genetic variability, recombination is necessary but apomixis impairs intercrossing of apomictic individuals. Therefore, the alternative is to use apomictic individuals as pollen donors in crosses with compatible sexual plants.

In this context, autotetraploid sexual plants of *B. ruziziensis* (Swenne et al. 1981) are used as female parent in interspecific crosses with *B. brizantha* and *B. decumbens* as pollen donors aiming to explore the potential of interspecific hybrids, including heterosis. The ideal strategy of *Brachiaria* breeding is to use reciprocal recurrent selection (Resende et al. 2008) even considering that recombination is not feasible in the apomictic population. Therefore, the sexual population of *B. ruziziensis* should be improved as a function of the apomictic population, that is, of the apomictic individuals in crosses (Resende 2002a, Miles 2007).

The genetic variability available in *B. ruziziensis* as well as estimation of genetic parameters such as heritability, repeatability and genetic correlations are essential for establishing efficient breeding strategies especially for forage species (Hayes et al. 2013). Estimation of genetic parameters is still unknown in *B. ruziziensis* for characters of agronomic importance used as selection criteria and must be investigated for this sexual tetraploid breeding population.

Hybridization methods in forage breeding are commonly based on open pollination, resulting in the development of half-sib progeny for evaluation (Posselt 2010, Walter et al. 2012). In the breeding of *Brachiaria*, *B. ruzizensis* is used as female parent for hybridization with elite apomicts; thus, intraspecific breeding is initially very important to select the best individuals to cross (Simeão-Resende et al. 2013).

Furthermore, individuals should be selected for various important characters simultaneously based on their genetic values and giving effective economic weights in selection indices. Currently, the selection indices in forage breeding adopt procedures without assigning true economic weights to genetic values for the characters. The use of a selection index weighting agronomic characters of biomass production separately for periods of greater and lesser rainfall during the year has been adopted for tropical forages (Figueiredo et al. 2012). Smith and Fennessy (2014) have defined weights to contemplate total dry matter production as a function of the region and of the season. The efficiency of use of economic weights as well as of balancing of characters in indices to obtain greater gains in the *B. ruziziensis* breeding was not evaluated.

Based on these premises, in the first cycle of intrapopulational selection in *B. ruziziensis*, half-sib progenies were analysed using mixed models, with the following objectives: (i) to estimate genetic and phenotypic parameters, (ii) to predict genetic values for all characters evaluated, (iii) to estimate the correlation among characters associated with biomass production and those associates to nutritional quality of the forage and (iv) to use and evaluate the relative efficiency of selection indices to obtain gains in several characters simultaneously.

Materials and Methods

Plant material and phenotypic evaluation: An experimental area was formed in 2010 to obtain open pollination progeny of sexual tetraploid

B. ruziziensis at Embrapa Beef Cattle, in Campo Grande, MS, with 20°28′ of South latitude, 55°40′ West longitude, and 530 m of altitude. Field soil was classified as Haplic Ferralsol (Rhodic) (FAO 2006). The climate according to Köppen′s classification is Aw, humid tropics, with rainy summers and dry winters.

The experiment was set with 140 plants, 20 plants of each of the seven sexual tetraploid *B. ruziziensis* accessions: R30, R38, R41, R44, R46, R47 and R50. The individual plants were randomized in 26 lines and 12 columns, spaced 2 m × 2 m apart. The experimental area was isolated with elephant grass (*Pennisetum purpureum*) planted in lines, to deter pollen contamination of other *Brachiaria* from adjacent areas, as species from this genus are anemophilous. The flowering period and seed production were monitored during the whole first semester of 2011. The seeds were collected weekly in individual plants and processed according to Simeão et al. (2012). Of the 140 plants, 59 were selected to compose the progeny of the breeding population, based on the individual information of viable seed production and flowering synchrony. Thus, a selection of the parents was performed prior to the progeny testing.

For the evaluation of the 59 *B. ruziziensis* progeny, 20 seedlings were produced per parent totalling 1180 individuals. The experiment was planted in November 2012, in a randomized block design with 20 replications and one plant per plot, spaced 1.5 m × 1.5 m. Nine clippings at 15 cm height were performed in the following dates: 1–29 January 2013; 2–4, 5 March 2013; 3–2 April 2013; 4–2 May 2013; 5–10, 11 September 2013; 6–9 October 2013; 7–13 November 2013; 8–16 December 2013; 9–22 January 2014.

Clippings 2, 3, 7, 8 and 9 were performed during periods of average monthly precipitation of 168.32 mm, therefore wet season, while clippings 4, 5 and 6, with 61.13 mm per month, were considered dry season. Clipping 1 was considered establishment and not included in the analysis.

The characters evaluated for all clippings were as follows: green matter yield (GM), total dry matter yield (TDMY), in grams per plant, and regrowth. In clippings 2 and 5, morphological separation into leaves, stems and dead material was carried out for a sample of about 200 g per plant. With this information, it was possible to estimate leaf dry matter yield (LDMY) and stems dry matter yield (SDMY), as well as their proportion in individual plants. Regrowth scores varied from 0 to 6 and were obtained with a combination of density and rate of regrowth visually estimated as described by Figueiredo et al. (2012).

Ground samples from clipping 2 were analysed using near infrared spectroscopy (NIRS) (Marten et al. 1985). Van Soest (1994) sequential method was used to estimate fibre components which are associated with forage nutritional value. Total fibre components (neutral detergent fibre, NDF), lignocelluloses (acid detergent fibre, ADF), cellulose (Cel) and lignin via sulphuric acid (Lig S) and lignin via permanganate (Lig P), and also crude protein (CP) and ADF insoluble ash (ADFinsol) were estimated. Cellulose was estimated as ADF minus lignin and the ash of the residue of the lignin as suggested by Van Soest et al. (1991), even considering it can lead to erroneous values for some forages. Fortunately, it is not the case in tropical grasses, which are almost devoid of non-starch polysaccharides, the main cause of bias in the estimation of cellulose and hemicellulose. The method used to estimate crude protein was Kjeldahl (AOAC, 1990). ADF insoluble ash was measured according to Van Soest and Robertson (1985). For this work, the variables ADF, Cel, Lig S and Lig P were used as a proportion of NDF so as to characterize quality of fibre more than just to estimate absolute values of these components. *In vitro* organic matter digestibility (IVOMD) was also estimated which represents the expected forage digestibility of organic DM in ruminant digestive tract, and it is also useful as a quality index of the biomass (Gouy et al. 2013).

Statistical methods: All univariate analyses were performed using linear mixed models. The following statistical model was used for the analyses of nutritional quality characters (NDF and proportions of ADF, Lig S, Lig P, Cel, PB and ADFinsol):

$$y = Xr = Za + e,$$

where y *is* the data vector, r is the vector for replicate effects (fixed) added to the general mean, a is the vector of individual additive genetic effects (random), and e is the random residual vector. Capital letters represent the incidence matrices for the mentioned effects. Narrow-sense individual heritability (h_a^2) was estimated considering a correction to the coefficient of relationship by Wright (Resende 2002a), due to the proportion of 1 of 7 of crosses among related individuals yielding offspring of the seven initial parents which originated the 59 progeny. The value of an individual under selection is equal to the sum of the average effects of the genes it carries, the summation being made over the pair of alleles at each locus and over all loci, generally termed the breeding value of the individual (Falconer and Mackay 1996). Generally, the predicted breeding value is not equal to the true breeding value of individuals. Accuracy (symbolized here by $\hat{r}_{\hat{a}a}$) is defined as the correlation between true and predicted breeding values. It is equivalent to the square root of the heritability. It can be used directly for genetic gain comparisons among alternative selection methods, which solely the heritability and repeatability do not, because they also depend on phenotypic standard deviation (Bernardo 2010). Higher accuracy values are indicative of better predicted breeding values (Falconer and Mackay 1996) and more efficient selection method (Simeão-Resende et al. 2013). The selective accuracy was estimated according to Resende (2002a), based on the prediction error variance (PEV), via elements of the reverse matrix of the equations coefficients of the mixed model. PEV is related to accuracy through the equation:

$$\hat{r}_{\hat{a}a} = (1 - \mathrm{PEV}/\sigma_a^2)^{1/2}$$

where σ_a^2 is the genetic variation among progeny under evaluation.

The genetic correlation between the characters was estimated as (Falconer and Mackay 1996):

$$r_{a(x,y)} = cov_{a(x,y)}/\sigma_{ax}\sigma_{ay},$$

where $\mathrm{cov}_{a(x,y)}$ is the additive genetic covariance between x and y; σ_{ax} and σ_{ay} are the additive genetic standard deviations for x and y, respectively.

Agronomic characters (GM, TDMY, LDMY and SDMY, in g per plant, and regrowth) were analysed initially for each clipping to evaluate the variance heterogeneity among clippings, using the same model as for the analyses of nutritional quality characters. The determination of heterogeneity of the variances per clipping was obtained based on Hartley′s test. For the characters where the heterogeneity of the residual variance was significant, the data (y) were corrected according to Resende (2007), where

$$y_c = y(h_{ik}/\bar{h}_i)$$

in which h_{ik} and $\bar{h}_i$ are the square root of the heritability of the character in clipping k and the square root of the average heritability of all the clippings.

In the simultaneous analyses of all clippings in each season, the following model was used:

$$y = Xm + Za + Wp + Qi + Ts + e,$$

where y is the data vector, m is the vector of the combination clipping – replicate effects (fixed) added to the general mean, a is the individual additive genetic effects vector (random), p is the permanent plot effect vector (random), i is the interaction individual x clipping effect (random), s is the permanent individual effect vector (random), and e is the residual random vector. Capital letters represent the incidence matrices for the mentioned effects.

The repeatability model used takes into account the repeated measures and the serial correlation among such measures. This is carried out through the fitting of the permanent plot effects and permanent individual effects across measurements. Such model makes use of a covariance structure called compound symmetry, which produces a parsimonious model, being both efficient and less parameterized, and has been largely applied in animal breeding and genetics (Verbeke and Molenberghs 2000, Diggle et al. 2002, Mrode 2005). The relative coefficient of variation was proposed by Vencovsky (1987) based on genetic coefficient of

variation or 'evolvability' as described by Houle (1992) and Hill (2010) and predict the long-time success of directional selection in characters.

The following genetic parameters were estimated: (i) individual narrow-sense heritability (h_a^2) corrected for inbreeding as before, (ii) repeatability or intraclass correlation between repeated measures in the same individual ($\rho = (\sigma_g^2 + \sigma_{ep}^2)/\sigma_y^2$), where $\sigma_g^2, \sigma_{ep}^2$ and σ_y^2 are the genetic variances, the permanent environmental variance and phenotypical variance, respectively) (Falconer and Mackay 1996), (iii) mean genetic correlation through clippings ($\bar{r}_{acut}$), (iv) individual additive genetic coefficient of variation ($CV_{gi} = 100\sigma_a/m$, where m is the general mean of the experiment), (v) genetic coefficient of variation between progeny ($CV_{gp} = 100\sigma_{ap}/m$) and (vi) relative coefficient of variation ($CV_r = CV_{gp}/CV_e$, where CV_e is the environmental coefficient of variation per clipping). Genetic correlations among characters were estimated as before.

In the mixed model methodology context, hypothesis testing is performed through the likelihood ratio test, which is given by the difference between the deviances associated with the fit of two alternative models. This is also called analysis of deviance. The deviance itself is given by the quantity $-2log_{10}L$, where $\log_{10}L$ is the maximum of the logarithm of the likelihood function. The smaller the deviance, the better the goodness of fit of the model (Galwey 2006). Therefore, the difference between deviances of both adjusted models can be used in the test of Wilks (likelihood ratio test – LRT) (Dobson 1990, Resende 2007). In this work, the statistics was used for the tests of hypotheses of the genetic effects. The adjustment of different statistical models to the data was tested using LRT in which the deviance of the saturated model is subtracted from the deviance of the model without this effect and tested on basis of χ^2 test at 1% and 5% of probability, and one degree of freedom. Testing a null hypothesis that a variance is zero put the parameter at the boundary of the parameter space. In such a case, Stram and Lee (1994) proposed to halve the P-value in a model with independent subjects, which do not hold in our case. As there is no simple alternative, the P-values were halved.

All the statistical analyses were performed with the aid of the computational programme in genetics and statistics Selegen – REML/BLUP (Resende 2002b, Colombari-Filho et al. 2013).

The individuals were ranked based of their genetic values for each character. For the simultaneous selection for the characters, three indices were used and compared, which considered the total dry matter production per plant (TDMY), regrowth capacity and crude protein (CP) in the periods of greater and lesser rainfall and the annual production. The indices were as follows:

1) Multiplicative index weighing the target characters as 0.5 in the rainy season and 0.5 in the dry season
   I_1 = (wet season TDMY) (Regrowth) (CP) (0.5) + (dry season TDMY) (Regrowth) (0.5)
2) Multiplicative index weighing the target characters as 0.7 in the rainy season and 0.3 in the dry season, considering that this is the expected distribution of biomass production for *Brachiaria* spp. according to Euclides et al. (2007).
   I_2 = (wet season TDMY) (Regrowth) (CP) (0.7) + (dry season TDMY) (Regrowth) (0.3)
3) Annual multiplicative index:
   I_3 = (annual TDMY) (Regrowth) (CP)

The first two indices consider the effects of selection on the characters evaluated separately in the wet or dry season in comparison with the index using the annual evaluation (third index). Characters in the first index were weighted equally for the production in both seasons; the second index was established according to what is generally observed in terms of annual distribution of forage production in Brazil. Criteria were combined together into a score in the third index named annual multiplicative index.

The individuals were selected based on their rank in each index. Selection intensities used were 1% and 5%, aiming to compose the elite population for crossing with selected apomictic individuals of *B. brizantha* and *B. decumbens* and to compose the breeding population of *B. ruziziensis*, respectively. The relative efficiency of selection for each index was estimated dividing the mean of the selected individuals breeding values for each character in that index by the general mean of the character in the population. To establish graphic representation of individuals selected at 1% selection, differential breeding values of the characters were standardized (Nunes et al. 2005, Figueiredo et al. 2012).

Results

B. ruziziensis progenies presented higher percentage of crude protein, higher digestibility, lower NDF and lignin S concentration (Table 1) than *B. brizantha* cv. 'Marandu' and *B. decumbens* cv. 'Basilisk' (Euclides et al. 2007), both evaluated in the same region, season and based on leaf dry matter. This information corroborates the superior nutritional quality of this grass and its potential contribution to improve apomictic species by hybridization.

High amplitude of genetic variation was observed for the evaluated characters. For all characters, except NDF and IVOMD, genetic variance was significantly higher than zero ($P < 0.005$) based on LRT (Table 1). The narrow-sense heritabilities corrected for inbreeding varied from 0.14 to 0.91 for the agronomic characters and from 0.04 to 0.24 for the nutritional quality characters. The coefficients of genetic variation between individuals (CVgi) and progeny (CVgp) revealed marked difference among characters: SDMY during wet season and regrowth during wet and dry seasons. Nutritional quality characters showed the smallest magnitude (below 16%) for these parameters. Agronomic characters in general presented greater coefficients of genetic variation (>40%), meaning higher genetic variation available for selection in biomass yield than characters of nutritional quality in this forage grass. The goal for high magnitude of accuracy (>85%) was attained for agronomic characters, despite of the fact that the relative coefficients of variation were lower than 1.0. This result could be due to the high number of replications used in the experiment, as accuracy and number of replications are interconnected as explained by Resende and Duarte (2007). This statement could not be extended to the nutritional quality characters, due to the smaller genetic variability expressed in the progeny evaluated.

Repeatability coefficients estimated for TDM yield during wet and dry seasons presented moderate magnitude. Considering the amplitude of repeatability obtained the number of clippings necessary to obtain high selective accuracy varied from 3 to 7, a result that corroborates previous studies with perennial forages, which indicated about 5 to 7 clippings as sufficient for accurate selection (Casler 1999, Shimoya et al. 2002, Figueiredo et al. 2012).

The genotype x clipping interaction was significant, and the inverse of its variance is related to the magnitude of the correlation ($\bar{r}_{acut}$) among the several evaluations, a parameter of real interest and with an applicable information to breeding. The moderate magnitudes found for $\bar{r}_{acut}$ in the sequential evaluations in *B. ruziziensis* indicate a coincidence of approximately 60% of the best individuals in all clippings and furthermore that the character is not genetically the same from one clipping to the next (Falconer and Mackay 1996).

There are two assumptions for the estimate of repeatability according to Falconer and Mackay (1996) and Resende (2002b). The first is that the variances of the different evaluations be equal and that their components have the same proportion. This assumption was met in the analysis as the heterogeneities of variances among evaluations were considered and corrected. The

Table 1: Estimate of genetic parameters as a result of linear mixed models analyses of phenotypic data used for selection of individuals in *B. ruziziensis* progenies

Characters	Season	h_a^2 [1]	ρ^2 [2]	$\bar{r}_{acut}$ [3]	Accuracy	$CV_{gi}\%$ [4]	$CV_{gp}\%$ [5]	CVr [6]	Estimated mean	LRT [7]
Agronomic characters GM	Wet	0.48	0.49	0.67	0.89	54.76	25.68	0.50	479.33	165.98*
	Dry	0.59	0.61	0.60	0.93	79.07	39.47	0.58	242.27	183.45*
	Annual	0.47	0.48	0.66	0.91	63.88	31.94	0.53	388.65	181.62*
TDMY	Wet	0.48	0.49	0.65	0.90	51.91	25.68	0.47	111.99	186.58*
	Dry	0.51	0.52	0.50	0.95	71.79	36.35	0.58	78.34	175.02*
	Annual	0.47	0.47	0.64	0.89	59.36	29.68	0.50	98.99	204.05*
LDMY	Wet	0.52	–	–	0.90	42.70	21.35	0.46	98.47	97.49*
	Dry	0.91	–	–	0.95	107.62	53.81	0.73	59.22	198.93*
	Annual	0.60	0.61	0.52	0.92	75.16	37.58	0.60	78.52	171.81*
SDMY	Wet	0.14	–	–	0.69	23.94	11.97	0.21	31.21	11.23*
	Dry	0.54	–	–	0.92	113.48	56.74	0.53	22.51	72.89*
	Annual	0.04	0.68	0.02	0.81	68.71	34.36	0.37	26.51	8.32*
Regrowth	Wet	0.30	0.31	0.54	0.84	22.40	14.62	0.36	3.19	172.90*
	Dry	0.39	0.40	0.66	0.87	27.72	7.56	0.41	3.12	137.81*
	Annual	0.34	0.34	0.64	0.85	23.94	11.97	0.38	3.16	204.90*
Nutritional quality characters NDF (g/kg)	Wet	0.04	–	–	0.42	0.93	0.47	0.10	653.60	0.82[ns]
ADF (g/kg)	Wet	0.16	–	–	0.68	3.52	1.76	0.20	332.91	9.69*
Cellulose (g/kg)	Wet	0.22	–	–	0.74	3.31	1.66	0.24	193.82	16.70*
Lignin (Lig S) (g/kg)	Wet	0.21	–	–	0.73	8.04	4.02	0.24	27.38	12.89*
Lignin (Lig P) (g/kg)	Wet	0.24	–	–	0.75	15.48	7.74	0.26	97.58	20.00*
Crude protein (%)	Wet	0.14	–	–	0.70	3.23	1.62	0.22	16.42	11.65*
ADFinsol (g/kg)	Wet	0.16	–	–	0.67	11.20	5.60	0.20	62.39	9.35*
IVOMD (%)	Wet	0.04	–	–	0.39	2.21	1.10	0.09	56.93	0.69[ns]

*P-value <0.005.
[ns]Not significant.
[1]Narrow-sense heritability.
[2]Repeatability.
[3]genetic correlation among clippings.
[4]individual additive variation coefficient.
[5]Among progeny genetic coefficient.
[6]relative variation coefficient.
[7]Likelihood ratio test.
–, Not estimated.

Table 2: Genetic correlations among green matter yield (GM), total dry matter yield (TDMY), leaf dry matter yield (LDMY), stems dry matter yield (SDMY) and regrowth evaluated during wet and dry seasons in *B. ruziziensis* progeny

Character	TDMY wet	LDMY wet	SDMY wet	Regrowth wet	GMdry	TDMY dry	LDMY dry	SDMY dry	Regrowth dry
GM wet	1.00	0.85*	0.37*	0.93*	0.85*	0.87*	0.83*	0.56*	0.91*
TDMY wet		0.84*	0.34*	0.93*	0.85*	0.87*	0.84*	0.56*	0.92*
LDMY wet			0.71*	0.78*	0.55*	0.58*	0.54*	0.22*	0.73*
SDMY wet				0.26*	0.04[ns]	0.07[ns]	−0.03[ns]	0.12[ns]	0.23*
Regrowth					0.85*	0.87*	0.83*	0.57*	0.92*
GMdry						0.99*	0.99*	0.88*	0.88*
TDMY dry							0.99*	0.88*	0.89*
LDMY dry								0.89*	0.86*
SDMY dry									0.63*

[ns]Not significant at 1% of probability by Student's *t*-test.
*P-value <0.01.
Underlined: same characters evaluated in different seasons.

second assumption of repeatability is that the different measurements genetically reflect the same character so that the genetic correlation between characters, in this case the same character evaluated in sequential measurements, is close to 1.0. Based on genetic correlations between characters evaluated during the rainy and the dry seasons, they were treated as different (Table 2). Genetic correlations of high magnitude were observed between GM, TDMY, LDMY and regrowth evaluated during the wet or dry season (Table 2). There is a lack of correlation between the genotypic expression for SDMY (r_a = 0.12) in both seasons, which indicates that the genetic expression is different in the two periods. According to Falconer and Mackay (1996), the genetic correlation between characters should be looked upon with care as it is subjected to large experimental errors and are rarely very precise and furthermore differ markedly in different populations. Therefore, the correlation estimates for this *B. ruziziensis* population should not be extended to other populations of the same species or of other species of forage grasses. However, as this population of *B. ruziziensis* is the breeding population available, all parameters presently estimated should be used to decide upon selection.

Genetic correlations were also estimated for annual agronomic as well as nutritional value characters (Table 3). All the correlations between annual agronomic characters (GM, TDMY, LDMY, SDMY and regrowth) were of high magnitude. All biomass components were negatively correlated with the crude protein content (CP), with the proportions of ADF, lignin (P and S) and ADFinsol, and positively with IVOMD and cellulose.

Table 3: Genetic correlations among green matter yield (GM), total dry matter yield (TDMY), foliar dry matter yield (LDMY), stem dry matter yield (SDMY), regrowth, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), *in vitro* digestibility (IVOMD), lignin (S and P), cellulose and ADF insoluble ash (ADFinsol), evaluated in *B. ruziziensis* progeny on an annual basis

	TDMY	LDMY	SDMY	Regrowth	CP	NDF	ADF	IVOMD	Lig S	Lig P	Cel	ADFinsol
GM	1.00*	0.97*	0.77*	0.85*	−0.60*	0.53*	−0.19*	0.11*	−0.29*	−0.38*	0.48*	−0.34*
TDMY		0.97*	0.77*	0.96*	−0.60*	0.54*	−0.21*	0.12*	−0.29*	−0.39*	0.47*	−0.35*
LDMY			0.82*	0.93*	−0.61*	0.51*	−0.23*	0.16*	−0.21*	−0.46*	0.57*	−0.42*
SDMY				0.69*	−0.33*	0.30*	−0.28*	0.26*	−0.19*	−0.43*	0.48*	−0.37*
Regrowth					−0.62*	0.50*	−0.27*	0.19*	−0.18*	−0.50*	0.53*	−0.46*
Crude protein						−0.57*	−0.02^{ns}	0.06^{ns}	0.07^{ns}	0.25*	−0.49*	0.25*
NDF							−0.15*	−0.21*	−0.42*	−0.13*	0.12*	−0.12*
ADF								−0.81*	0.00^{ns}	0.84*	0.04^{ns}	0.87*
IVOMD									0.20*	−0.78*	0.14*	−0.82*
Lig S										−0.29*	0.42*	−0.12*
Lig P											−0.45*	0.91*
Cellulose												−0.29*

^{ns}Not significant at 1% of probability by Student's *t*-test.
*P-value <0.01.

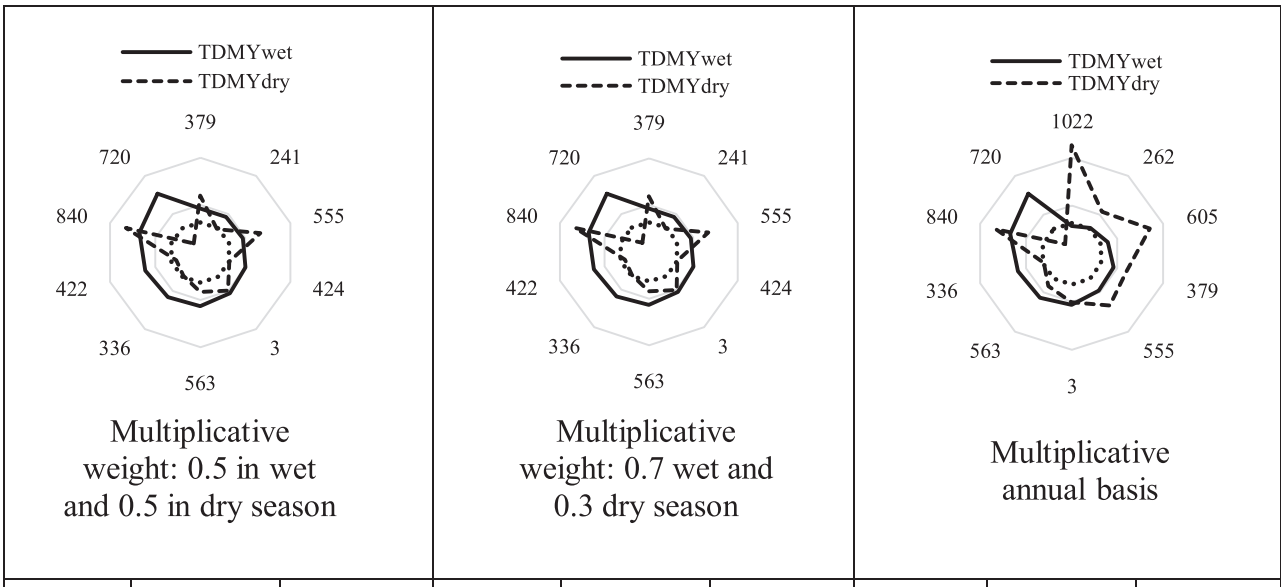
Table 4: Relative response at 1% and 5% selection intensities and mean of selected *B. ruziziensis* individuals by different multiplicative selection indexes in characters directly or indirectly selected and separated in wet and dry seasons or in annual basis

	TDMY wet	LDMY wet	Regrowth wet	TDMY dry	Regrowth dry	TDMY annual	LDMY annual	Regrowth annual	CP (%)
Multiplicative weight 0.5 in wet and 0.5 in dry season									
Selected mean (5%)	228	151	4	175	4	215	155	4	16
Relative response (5%)	2.04	1.54	1.32	2.24	1.37	2.17	1.98	1.36	0.99
Selected mean (1%)	273	160	4	205	4	257	185	4	16
Relative response (1%)	2.44	1.63	1.36	2.62	1.44	2.60	2.36	1.42	0.99
Multiplicative weigh 0.7 wet and 0.3 dry season									
Selected mean (5%)	228	153	4	167	4	212	156	4	16
Relative response (5%)	2.04	1.56	1.32	2.14	1.37	2.15	1.99	1.36	0.99
Selected mean (1%)	273	160	4	205	4	257	185	4	16
Relative response (1%)	2.44	1.63	1.36	2.62	1.44	2.60	2.36	1.42	0.99
Multiplicative annual basis									
Selected mean (5%)	225	149	4	192	4	219	164	4	16
Relative response (5%)	2.01	1.51	1.31	2.45	1.38	2.21	2.08	1.37	0.99
Selected mean (1%)	257	150	4	253	4	264	185	4	16
Relative response (1%)	2.30	1.53	1.33	3.23	1.43	2.67	2.35	1.41	1.00
General mean	112	98	3	78	3	99	79	3	16

Relative response was estimated as a proportion of the breeding value mean of selected individuals, at 1% or 5% intensities, in relation to general mean of the character. TDMY: total dry matter yield and LDMY: leaf dry matter yield, in g per plant; CP: crude protein; Regrowth: scored from 0 to 6.

Selection indices were evaluated and their results can be compared on the basis of the relative gain in each of the agronomic characters and crude protein, including the gain with indirect selection on agronomic characters that do not compose the index (Table 4). The results show that there is no advantage in separating the characters evaluated in the two seasons, as if they were different characters, in terms of average gain for all characters, even if allocating different weights for each season. In addition, with 1% selection differential, exactly the same individuals were selected in the indices 1 and 2 (Fig. 1 and caption). The first two indices selected individuals which had higher TDMY in the wet season, because of their higher mean and larger weights, in detriment of TDMY in the dry season, unlike the multiplicative annual index that selected individuals with high TDMY in the dry season and with TDMY on the average during the wet season (Fig. 1 – individual 1022). First and second indices resulted in higher LDMY in the wet season than the third index, at 1% and 5% selection differential, but were not superior when the target character is annual LDMY (Table 4).

Comparing all three selection indices, there are differences among individuals selected by each one so that only seven individuals were common to all indices evaluated at 1% selection differential (Fig. 1). Selection index 3 resulted in higher selection gains, indicated by higher selected individual mean, and was able to distribute forage yield equitably between wet and dry season, on average. This effect was achieved by selecting individuals with high TDMY during the dry season (individuals 1022 and 605) as well as individuals with better distribution of yield in both seasons (individuals 3, 563, 840 and 379). Better distribution of wet and dry seasons yield in tropical forages is a long-time demand of livestock producers, mostly in central Brazil where animals are bred and finished under grazing. Average daily gain and carrying capacity are both improved when a grass cultivar presents higher yield during the whole growing season in comparison with a grass cultivar that concentrates its production in the wet season (Valle et al. 2013). Selection index 3 has shown plenty of opportunities in *B. ruziziensis*, in terms of genetic variability available for selection and production gain at the same time during wet and dry seasons. Most important is to be able to detect this genetic variability early and use it in future cycles of breeding, without narrowing the genetic base available for the species improvement.



Indiv	TDMY wet	TDMY dry	Indiv	TDMY wet	TDMY dry	Indiv	TDMY wet	TDMY dry
379	244	253	379	244	253	1022	194	409
241	244	167	241	244	167	262	207	236
555	248	274	555	248	274	605	225	328
424	256	172	424	256	172	379	244	253
3	264	225	3	264	225	555	248	274
563	275	201	563	275	201	3	264	225
336	280	167	336	280	167	563	275	201
422	288	157	422	288	157	336	280	167
840	305	321	840	305	321	840	305	321
720	332	113	720	332	113	720	332	113
μ_{IS}	270	186	μ_{IS}	270	186	μ_{IS}	256	244
$\mu_{general}$	112	78	$\mu_{general}$	112	78	$\mu_{general}$	112	78

Fig. 1: Graphic representation of total dry matter yield (TDMY, in grams per plant), in the wet and dry seasons, of the selected individual (Indiv – numbers in each vertices of the decahedron) in relation to the general mean (central dotted line) using five multiplicative selection indexes. (μ_{IS} is the mean of selected individuals and $\mu_{general}$ is the general mean).

Discussion

The improvement of *B. ruziziensis* in the context of this experimentation does not particularly target increases in its economic value towards producing a new cultivar, but to identify parents of high productivity and nutritional quality to hybridize with *B. brizantha* and *B. decumbens*, both apomicts. The starting point to begin a crossing programme is to rely on the best pure individuals in terms of their within population genetic values. After, with the crossing data at hand, the best crossers are kept to establish the reciprocal recurrent selection properly.

Interspecific crossings in *Brachiaria* breeding seeks to combine main forage characters such as biomass production more stable along the seasons, high protein and digestibility and high resistance to pests, especially pasture spittlebugs. Moreover, apomictic interspecific hybrids obtained after crosses between sexual and apomictic species allow heterosis fixation in a cultivar, which is desirable commercially (Savidan and Valle 1999) and has resulted in extensive adoption of *Brachiaria* cultivars in livestock production in Brazil (Jank et al. 2014).

In pastures of *B. decumbens* and *B. brizantha,* animal performance during the dry season is many times unsatisfactory, mostly because of protein deficiency (Euclides et al. 2007). Crude protein content in *Brachiaria* cultivars, even in the wet season, is lower than presently quantified for *B. ruziziensis* (Euclides and Medeiros 2003). Therefore, the contributions of *B. ruziziensis* towards hybrids with higher protein content in the breeding of *Brachiaria* are fundamental. However, the negative correlation between crude protein levels and characters of forage production must be adequately pondered in the selection of parents so as to get genetic gains for both characters.

Breeding involves constant decision-making, either in the choice of method, in the selection of individuals for the final objective or in the use of these individuals in subsequent cycles,

always considering the effective population size so as to assure long-time gains. In reciprocal recurrent selection in *Brachiaria*, the choice should be made based on combining capacity of the selected individuals of tetraploid sexual *B. ruziziensis* in crosses with elite apomictic individuals of the other species of the agamic complex. The high number of *B. ruziziensis* individuals evaluated in this first selection cycle required as a strategy selection based only on individual genetic values, thus exploring only the additive genetic variance of the candidates. Furthermore, to avoid a narrowing of the genetic basis of the sexual population, selection with two intensity levels was adopted: the 5% top individuals in the population were selected to begin the second cycle of intrapopulational breeding, and the top 1% within these 5% were selected to use in crosses with elite apomicts in disconnected factorial or incomplete diallel mating designs, so as to estimate general and specific combining ability and associated parameters to promote new selection.

There are a number of objective and specific criteria in forage breeding which demands selection methods that provide simultaneous gains in several characters of interest. The best procedure of selection will be one that uses all available information of individual breeding values combined into an index of merit (Falconer and Mackay 1996, Bernardo 2010) for the multiple characters adopted as selection criteria (Resende 2002a). The economic value of a forage depends on more than one character, and their mode of inheritance and the correlation between them as well as the economic importance of each character (dry matter yield, quality, persistency) and its impact on production must be considered (McEvoy et al. 2011, Chapman et al. 2012). For the estimation of the index of merit, the characters with the greatest impact on economic performance in the production system must be considered (McEvoy et al. 2011) and the feasibility of its use depends, of course, on the ease with which these can be measured (Wilkins and Humphreys 2003) in many individuals with different genotypes.

Studies to define economic weights in selection in temperate forage species have advanced notoriously. Index models may include seasonal and monthly estimated dry matter yield, silage dry matter digestibility, yield and persistency (McEvoy et al. 2011) or consider only dry matter yield in different seasons (Chapman et al. 2012, Smith et al. 2014). Selection indices using economic weights are unknown in tropical forages. In *B. brizantha*, despite the differences between cultivars in terms of animal response in average daily gain and carrying capacity, during dry period of the year (Valle et al. 2013), little is known about specific forage character contributing to this difference.

The importance of the use of selection indices was to provide the balancing of characters as selection criteria for obtaining the best results in the ultimate goal of improving *B. ruziziensis*. Thus, individuals who will compose the next selection cycle meet two essential conditions for breeding success: the presence of genetic variability and high average on several important characters. Based on the results of the rankings of individuals by each index, the wide genetic variability will allow direct selection aiming at gains in specific characters and achieve better distribution of forage production during the year. Final result of selection indices use certainly will depend on economic advantage contribution of important agronomic characters and the distribution of this production in periods of higher and lower annual rainfall. According to our hypothesis, there are economic advantages in greater forage production throughout the year while maintaining the same capacity of annual support and gain weight more evenly distributed than its concentration in the period of highest rainfall. The answer to this and other issues is still unknown when it comes to tropical forages whose improvement is still in its infancy and for livestock production system that are quite different from temperate forage ones.

What is clear in this work is that the economic weights are just one of the components of a selection index and should be combined with the knowledge of mode of inheritance of the characters through heritability and repeatability, as well as the genetic correlations between them, in decision-making on the best selection criteria. This work has contributed with essential information to the second part component of an index and to the discussion on the first one. The validation of efficiency of such selection procedures is still necessary.

Acknowledgements

We would like to thank the Brazilian Enterprise for Agricultural Research (Embrapa), the Association for the Promotion of Research on Tropical Forage Breeding (UNIPASTO), the Ministério da Ciência e Tecnologia e Inovação/Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Catholic University Dom Bosco (UCDB). And also, we wish to thank two anonymous reviewers for comments on the manuscript and the ideas presented.

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