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ARTICLE

Flowering traits in tetraploid *Brachiaria ruziziensis* breeding

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Abstract: *Tetraploid Brachiaria ruziziensis genotypes which reproduce sexually are essential for the breeding of other species of the Brachiaria genus which reproduce by apomixis. Aiming at studying the available phenotypic and genetic variability in the breeding population of B. ruziziensis, it was estimated the parameters heritability and genetic and phenotypic correlations between the traits associated with flowering, and the traits responsible for forage yield and nutritional quality. Seventeen traits in 1180 individuals from 59 open pollinated families were studied, and the data were analyzed by mixed model methods. Individuals with sparse flowering presented higher breeding values for total dry matter, yield, and total number of panicles per plant than individuals with early or late flowering. Considering breeding population differences on flowering behavior, on individual narrow sense heritability and on genetic correlations between flowering, agronomic quality and nutritional quality traits have to be considered in intrapopulation breeding and in intrapopulational recurrent selection.*

Key words: *Genetic variation, flowering phenology, grass forage, mixed model, signal grass.*

INTRODUCTION

Species of the African forage grasses *Brachiaria* (Trin.) Griseb. are the most important for pastures in the tropics. In Brazil, the *Brachiaria* germplasm collection is maintained by Embrapa Gado de Corte, in Campo Grande, MS, and comprises 475 accessions from 13 species. *Brachiaria ruziziensis* (syn *Urochloa ruziziensis* R. Germ. & Evrard Crins) is represented by 16 diploid accessions (2n = 18) and seven artificially-induced tetraploid accessions (2n = 4x = 36) developed by Swenne et al. (1981) (Valle and Savidan 1996). Both ploidy levels present a sexual mode of reproduction, differently from most naturally-polyploid ones, which are apomictic (Pagliarini et al. 2008). Sexual tetraploid *B. ruziziensis* are also highly self-incompatible (Ferguson and Crowder 1974).

The greatest importance of sexual polyploid accessions of *B. ruziziensis* is their use as female genitor in controlled crosses with apomictic species of economic importance (*B. brizantha* and *B. decumbens*), and thus making breeding viable. Miles (2007) and Resende et al. (2008) proposed recurrent selection (RRS) in specific combining ability, and Worthington and Miles (2015) proposed reciprocal full-sib recurrent selection (RFRS) as appropriate methods for increasing heterotic effects in a sexual *Brachiaria* breeding population. Regardless of the method to be used, breeding goes through cycles of intrapopulational improvement in

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sexual individuals, independently of being the initial sexual population, such as purely tetraploid *B. ruziziensis*, or the interspecific sexual individuals generated in exploratory crosses. Aiming at achieving more efficient breeding methods, high level knowledge on the inheritance and genetic variability for economically important traits associated with yield is necessary, as well as on traits associated to cross ability, such as distribution of flowering in the species, and if there are genetic factors which cause differences.

In breeding programs of tropical forage grasses, the flowering period and the time of the year in which it occurs are not generally target traits for selection. Selection criteria are more associated with traits of forage yield and nutritional quality (Valle et al. 2008) than with flowering time. This is because under cultivation and with grazing pressure, flowering is practically null. According to Santos et al. (2004), by using correct management of cultivated tropical grasses, flowering is controlled (or prevented) once the length of stems is constantly reduced by herbivory. Such management results in higher nutritional value and quality of the forage offered to animals, and also reduces the losses caused by dead matter accumulation (Marcelino et al. 2006).

However, the phenology of reproduction in forage grasses is an important component to be considered in breeding, which involves carrying out controlled crosses to obtain full-sib or open pollinated progeny. The importance is also extended to the production of seeds, both in the quantity and quality needed for commercialization, which will determine success in adopting the improved cultivar.

In this context, the objectives in this work were to determine the flowering period in open pollinated *B. ruziziensis* progenies, in order to estimate genetic and phenotypic parameters of traits associated with flowering, and to estimate the correlation between these traits and those associated with the yield and nutritional quality of forage.

MATERIAL AND METHODS

Progenies and phenotypic evaluation

The 59 open pollination progenies of sexual and tetraploid *Brachiaria ruziziensis* evaluated in this experiment were obtained as described by Simeão et al. (2012). In the progeny test, 1180 individuals germinated from seeds were experimentally evaluated. The experiment was carried out in a randomized block design, with 20 replications, with one plant per plot, spaced at 1.5 m x 1.5 m, and planted in November 2012. The experiment was carried out at Embrapa Gado de Corte, in Campo Grande, state of Mato Grosso do Sul (lat 20° 28' S, long 55° 39' W, alt 530m asl). Field soil is classified as Haplic Ferralsol (Rhodic) (FAO 2006). According to Köppen, the climate type is Aw, humid tropical, with rainy summer and dry winter.

During the phenological evaluation, monthly rainfall was 148 mm, 77.4 mm, 130.8 mm and 42.2 mm, for the months of January, February, March and April, respectively. The mean minimum and maximum temperatures were 20.21 ºC/30.78 ºC, 21.09 ºC/30.92 ºC, 20.29 ºC/30.15 ºC and 20.11 ºC/29.88 ºC, respectively, for the same months.

Nine cuts were carried out to evaluate forage yield, at a height of 15 cm, in individual plants, in the period from January 2013 to January 2014. After the ninth yield evaluation cut, which was carried out on 1/22/14, phenological evaluation of flowering started for the 1180 individuals. This evaluation took place weekly over a period of 84 days, on the following dates: 02/12//2014, 02/19/2014, 02/26/2014, 03/05/2014, 03/12/2014, 03/19/2014, 03/26/2014, 04/02/2014, 04/09/2014, and 04/16/2014.

The following morphological traits associated with flowering were evaluated: number of panicles per plant (NPP), evaluated per week, and total number of panicles in the period; number of ears per raceme (NER), in a sample of five racemes per plant; mode of flower insertion in spikelet (IES) (0 – uni-serial; 1 – bi-serial; 2 – mixed; 3 – complex); stigma color (CEST) (0 – white; 1 – pink; 2 – purple; 3 – dark purple; 4 – black; 5 – other); anther color (CANT) (0 – white; 1 – pale yellow/gray; 2 – yellow; 3 – brown; 4 – bluish/other). The number of days to the first flowering (DTF) was counted from the date of the last cut, and only after the presence of at least three racemes per plant. Individuals which started flowering from the 21st to the 42nd day were classified as early; those that flowered from the 49th to the 70th day, as intermediate; those which started after the 77th day, were considered late; in addition, individuals which flowered over more than one period were classified as of sparse flowering.

Samples of leaves obtained from cut 2, which was carried out on 03/04/13 and 03/05/13, were analyzed using the Near Infrared Spectroscopy (NIRS) (Marten et al. 1985). The Van Soest (1994) sequential method was used to estimate the fiber components, which are associated with the nutritional quality of the forage grass. The total fiber components were obtained: neutral detergent fiber (NDF), lignocelluloses (acid detergent fiber, ADF), cellulose (Cel), lignin content via sulfuric acid (Lig S), lignin via permanganate (Lig P), crude protein content (CP), and silica (Sil). In this study, it was used the variables ADF, Cel, Lig S and Lig P expressed as a proportion of the neutral detergent fiber (NDF), in order to characterize the fiber quality, rather than only estimating the absolute values of these components. The *in vitro* dry matter digestibility (IVDMD) was also determined, which represents the potential for digesting fiber in ruminants, and therefore is useful as an index of biomass quality (Gouy et al. 2013).

Agronomic traits of green matter (GM – in g plant^{-1}), total dry matter yield (TDMY - g plant^{-1}) and regrowth capacity (Reg), as described by Figueiredo et al. (2012), were analyzed for cut 9, carried out on 01/22/14, that is, 21 days before the beginning of the phenological evaluation of flowering.

Statistical methods

All the univariate analyses were carried out using mixed linear models. The following statistical model was used in the analysis of the nutritional quality traits (NDF and proportions of ADF, Lig S, Lig P, Cel, PB, Sil), of the flowering traits (NPP, NER, IES, CEST and CANT), and of the agronomic traits (GP, TDMY and Reg):

$y = Xr + Za + e$, in which y is the vector of data, r is the vector of the effects of replication (fixed) added to the general mean, a is the vector of the (random) individual additive effects, and e is the vector of random residuals. The upper-case letters represent the matrices of incidence for the effects. The individual narrow sense heritability (h^2_a) was estimated considering a correction by the Wright's kinship coefficient (Resende 2002a), due to the 1/7 proportion of crosses among related individuals considering the seven initial genitors that gave rise to the 59 progenies. The selective accuracy was estimated according to Resende (2002a), based on the parameter prediction error variance (PEV), via elements of the inverse coefficients matrix in the mixed model equations. PEV statistics is related to the accuracy by means of the equation: $\hat{r}_{âa} = (1 - PEV/\sigma^2_a)^{1/2}$, in which σ^2_a is the genetic variation among the progenies under evaluation. Genetic correlation among these traits was estimated according to Falconer and Mackay (1996): $r_a = cov_{a(x,y)} / \sigma_{ax}\sigma_{ay}$, in which $cov_{a(x,y)}$ is the additive genetic covariance between traits x and y; σ_{ax} and σ_{ay} are the additive genetic standard deviations for traits x and y, respectively. Student-t test was used aiming to test whether or not the correlation differs significantly from zero (Steel and Torrie 1980).

Deviance statistics was used for genetic effects hypotheses tests. The fit of different statistical models to the data was tested using the Wilks Likelihood Ratio Test (LRT) (Dobson 1990, Resende 2007). All the statistical analyses were carried out using the Selegen software – Reml/Blup (Resende 2002b).

RESULTS AND DISCUSSION

Individuals of *B. ruziziensis* progenies presented high amplitude in number of days to flowering, with an interval that reached 63 days between the earliest and latest flowering (Figure 1). The highest percentage of individuals flowering at the same time occurred at 84 days, and those individuals also presented the highest number of panicles per plant. Of the 1180 individuals evaluated, 22% did not flower during the period under study, and 1% presented early flowering; that is, they flowered from day 21 to day 35. Late flowering, from 70 to 84 days, was observed in 43% of the individuals. Sparse flowering was observed in 34% of the individuals. This information is important for intraspecific breeding in tetraploid *B. ruziziensis*, especially if it is considered that this species is an essential component in crosses with apomictic species of commercial importance. Since

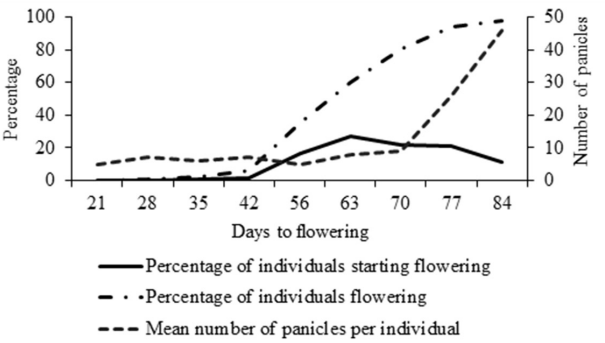

Figure 1. Flowering distribution of *B. ruziziensis* progenies.

pollination progenies generated in intraspecific breeding cycle will be used and, depending on the individuals selected, there may be asynchronous flowering, resulting in inappropriate genetic sampling and compromising the predicted genetic gains in subsequent cycles. Furthermore, knowledge about the flowering period for both the sexual and the apomictic components in interspecific hybridization is an essential condition for planning controlled crosses and achieving success in obtaining hybrids. The effects of genotype x environment interaction in the expression of flowering are well known in grasses (Casler et al. 2013, Arnout et al. 2014), and this interaction may result in the different expression of traits per local. This can be exploited in practice, if necessary, by carrying out crosses between asynchronous elite individuals. However, GxE interaction was not quantified in this research, which was carried out in a single year and only at one site.

Table 1. Mean genetic values in individuals for the traits crude protein content (CP), neutral detergent fiber (NDF), total dry matter yield (TDMY) and total number of panicles per flowering distribution group in open pollination progenies of *B. ruziziensis*

Distribution	CP	NDF	TDMY	Total of panicle
No flower	16.45	0.50	84.84	0
Early	16.53	0.51	77.30	64.18
Late	16.44	0.51	96.96	66.42
Sparse	16.38	0.51	115.42	108.94

Breeding values of the individuals analyzed for the traits crude protein content and acid detergent fiber were similar to those between the previously defined distribution groups for flowering period (Table 1) and were not influenced by them. These results differ from those obtained by Casler et al. (2014), in which sparse flowering cultivars presented 9% more crude protein and 3% less NDF in orchardgrass, but they corroborate with previous studies in which there was no evidence of the effect of flowering on the forage quality in the same species (Berg et al. 1981).

It was observed that for the traits total dry matter yield (TDMY) and number of panicles per plant (NPP) breeding values means differed among groups (Table 1). Individuals with sparse flowering presented mean breeding values for TDMY 19% higher than those in late-flowering individuals, 49% higher than those in early-flowering individuals, and 36%

Table 2. Estimates of genetic parameters resulting from analyses by mixed linear models of phenotypic data associated with the phenology of flowering (number of ears per raceme - NER, mode of flower insertion in spikelet – IES, stigma color – CEST, anther color – CANT, number of days to flowering - DTF and number of panicles per plant – NPP), nutritional quality and agronomic traits for forage yield in *B. ruziziensis* progenies

Traits	h^2_a [a]	h^2_{mp} [b]	Accuracy	CV_{gi}(%)[c]	CV_{gp}(%)	CV_r[e]	Estimated mean	Phenotypic range	LRT[f]
Flowering traits									
NER	0.43	0.77	0.88	46.79	23.39	0.40	1.90	0–9.00	87.88*
IES	0.02	0.07	0.27	0.68	0.34	0.03	0.96	0–2.00	0.10
CEST	0.14	0.48	0.69	3.06	1.53	0.07	0.35	0–4.00	10.69*
CANT	0.90	0.93	0.97	48.23	24.12	0.97	1.42	0–4.00	351.26*
DTF	0.20	0.58	0.76	7.38	3.69	0.26	68.06	21–84	16.13*
NPP - total	0.74	0.86	0.93	84.82	42.41	0.56	84.98	1.00–430.00	134.87*
Nutritional quality traits[g]									
NDF	0.07	0.33	0.57	1.40	0.70	0.16	65.42	48.74–91.02	3.40
ADF	0.14	0.49	0.70	3.78	1.89	0.22	0.51	0.35–0.80	10.97*
Cellulose	0.14	0.49	0.70	3.00	1.50	0.22	0.30	0.20–0.39	11.03*
Lignin (Lig S)	0.13	0.46	0.68	7.75	3.87	0.21	0.04	0.01–0.08	9.08*
Lignin (Lig P)	0.20	0.58	0.76	16.10	8.05	0.27	0.15	0.06–0.46	20.84*
Crude protein	0.14	0.49	0.70	3.28	1.64	0.22	16.40	10.45–21.84	11.36*
Silica	0.12	0.45	0.67	11.19	5.60	0.20	6.25	1.69–22.95	8.84*
IVDMD	0.04	0.23	0.48	2.78	1.39	0.12	56.87	6.58–76.15	1.46
Agronomic traits[h]									
GM	0.75	0.86	0.93	84.82	42.41	0.56	384.28	9.68–1913.68	151.17*
TDMY	0.71	0.86	0.93	59.66	29.83	0.55	103.59	6.28–443.58	140.67*
Regrowth	0.24	0.63	0.79	14.16	7.08	0.29	2.43	0.76–4.55	27.48*

* *P*-value < 0.01; [a] Narrow sense heritability; [b] Family mean heritability; [c] Individual additive variation coefficient; [d] Among progenies genetic coefficient; [e] relative variation coefficient; [f] Likelihood ratio test; [g] NDF – Neutral detergent fiber; ADF – Acid detergent fiber and IVDMD – *in vitro* digestibility of dry matter; [h] GM – Green matter and TDMY – total dry matter yield.

higher than those in individuals that did not flower during the total period considered. For NPP trait, sparse flowering individuals presented 64% more panicles than the late flowerers, and 70% more than the early flowerers. However, it should be emphasized that the reference for forage yield used in this experiment was taken before flowering began. In this case, the valid association is that individuals with greater forage mass in the cut previous to the beginning of the flowering period were those which had more intense flowering for a longer period. Results observed for TDMY and NPP in *B. ruziziensis* differ from those obtained by Casler et al. (2013) in orchardgrass (*Dactylis glomerata* L.), whose cultivars with sparse flowering presented 57% fewer panicles than the cultivars with concentrated flowering. They also presented forage yield 24% to 32% lower, depending on the cutting management.

The main factors of flowering in forage grasses, both temperate or tropical, are the length of the day (photoperiod) and the temperature (Humphreys et al. 2006), and their interaction with genes associated with this trait, activating or deactivating them. In this context, the investigation of genetic variability and its quantification is essential for breeding purposes. Among the studied traits associated with flowering, only IES did not present genetic variability in the studied population (Table 2). The narrow sense heritability corrected for endogamy for the traits NER, CEST, CANT, DTF and NPP presented low (0.14) to high (0.90) magnitudes. Narrow sense heritability for the number of days to flowering (DTF) was of low magnitude (h^2_a = 0.20) in *B. ruziziensis,* and of similar magnitude (h^2_a = 0.17) for the same trait in tall fescue (*Festuca arundinacea* Schreb.), obtained by Amini et al. (2013). For the number of panicles per plant (NPP), narrow sense heritability found in *B. ruziziensis* presented higher magnitude (h^2_a =*0.74*) than that obtained by Amini et al. (2013) for tall fescue (h^2_a = 0.46). The high heritability magnitude of trait CANT (h^2_a = *0.90*) is an indication of few genes determining it. Furthermore, narrow sense heritability varied from 0.24 to 0.75 for the agronomic traits, and from 0.12 to 0.20 for the nutritional quality traits. Low heritability traits demand more efficient and accurate breeding methods, as well as the appropriate use of all genetic information available in the experiments (Simeão-Resende et al. 2013). In this context, the regrowth capacity, all traits associated with forage quality, and the number of days to flowering should use methods such as combined selection and best linear unbiased prediction to obtain greater gains per cycle.

Genetic variation coefficients among individuals (CV_{gi}) and among progenies (CV_{gp}) revealed marked difference among traits. Nutritional quality traits presented the lowest magnitudes for these parameters, below 16%. The traits NER and NPP presented high magnitudes for both genetic variation coefficients. Due to the importance of these last two traits for yield and commercialization of forage seeds, the detection of this variability represents contribution to breeding

Table 3. Phenotypic (above diagonal) and genetic (below diagonal) correlation among traits associated with flowering phenology, nutritional value and associated with yield evaluated in progenies of tetraploid *B. ruziziensis*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
(1) NER	**1.00**	-0.05	0.15*	0.11*	-0.03	0.09	0.17*	0.15*	0.06	-0.08	0.07	0.03	-0.03	-0.01	0.13*	0.01	0.17*
(2) IES	0.84*	**1.00**	0.28*	0.09	-0.11*	0.05	0.09	0.09	0.03	0.00	0.03	-0.06	0.04	-0.02	-0.06	-0.02	-0.05
(3) CEST	0.24*	-0.08	**1.00**	-0.25*	-0.07	-0.03	-0.27*	-0.25*	-0.14*	0.14*	-0.22*	0.05	0.04	0.03	0.05	-0.01	0.07
(4) CANT	0.72*	0.00	-0.19*	**1.00**	-0.19*	0.38*	0.44*	0.43	0.20*	-0.14*	0.19*	-0.06	0.01	-0.08	-0.09	0.08	-0.10*
(5) DTF	-0.09	0.27*	-0.15*	-0.48*	**1.00**	-0.59*	-0.23*	-0.20*	-0.20*	0.04	-0.08	0.07	-0.04	0.04	0.07	0.04	0.05
(6) NPP	-0.07	-0.04	-0.15*	0.66*	-0.60*	**1.00**	0.49*	0.44*	0.34*	-0.12*	0.13*	0.00	-0.02	-0.06	-0.01	0.07	-0.01
(7) GM	0.18*	0.02	-0.31*	0.82*	-0.44*	0.73*	**1.00**	0.97*	0.30*	-0.17*	0.19*	0.01	-0.02	-0.07	-0.03	0.15*	-0.01
(8) TDMY	0.19*	0.02	-0.30*	0.84*	-0.43*	0.70*	0.99*	**1.00**	0.27*	-0.14*	0.16*	-0.01	0.01	-0.06	-0.05	0.14	-0.04
(9) Reg	0.48*	0.14*	-0.21*	0.63*	-0.35*	0.54*	0.74*	0.72*	**1.00**	-0.04	0.05	-0.06	0.04	-0.06	-0.05	-0.02	-0.08
(10) CP	0.45*	0.16*	0.31*	-0.46*	0.31*	-0.19*	-0.53*	-0.55*	-0.45*	**1.00**	-0.50*	-0.61*	0.64*	-0.04	-0.44*	-0.62*	-0.52*
(11) NDF	0.45*	-0.06	-0.32*	0.53*	-0.27*	0.39*	0.51*	0.52*	0.32*	-0.55*	**1.00**	0.14*	-0.47*	-0.41*	0.25*	-0.05	0.28*
(12) ADF	0.47*	0.07	-0.13*	-0.45*	0.36*	-0.41*	-0.22*	-0.22*	-0.06	0.02	-0.14*	**1.00**	-0.89*	0.03	0.90*	0.58*	0.92*
(13) IVDMD	0.45*	-0.11*	0.33*	0.23*	-0.27*	0.25*	0.08	0.09	-0.06	0.02	-0.25*	-0.81*	**1.00**	0.14*	-0.89*	-0.38*	-0.92*
(14) Lig S	0.50*	0.04	0.27*	-0.27*	0.06	-0.43*	-0.33*	-0.31*	-0.16*	0.09	-0.38*	0.03	0.13*	**1.00**	-0.26*	0.53*	-0.16*
(15) Lig P	0.44*	0.13*	-0.17*	-0.53*	0.47*	-0.31*	-0.28*	-0.29*	-0.14*	0.28*	-0.12*	0.85*	-0.79*	-0.25*	**1.00**	0.22*	0.93*
(16) Cellulose	0.47*	-0.12*	0.09	0.26*	-0.29*	0.04	0.27*	0.28*	0.21*	-0.52*	0.12*	-0.04	0.21*	0.43*	-0.49*	**1.00**	0.32*
(17) Silica	0.46*	0.10*	-0.15*	-0.50*	0.41*	-0.31*	-0.27*	-0.28*	-0.17*	0.29*	-0.08	0.86*	-0.83*	-0.11*	0.90*	-0.37*	**1.00**

* *P*-value < 0.01; NER – number of ears per raceme; IES – mode of flower insertion in spikelet; CEST – color of stigma; CANT – color of anther; DTF – number of days to flowering; NPP – number of panicles per plant; GM – Green matter and TDMY – total dry matter yield. Reg – Regrowth; CP – crude protein; NDF – Neutral detergent fiber; ADF – Acid detergent fiber and IVDMD – *in vitro* digestibility of dry matter; Lig S and Lig P – Lignin.

programs for *B. ruziziensis,* and also to its use in directing controlled crosses with apomictic accessions of species of greater commercial importance. The agronomic traits GM and TDMY presented higher genetic variation (>29%) than nutritional quality traits for this forage grass, meaning higher genetic variation available in biomass yield selection. High magnitude accuracy (>85%) for an efficient selection was obtained for the traits GM, TDMY, NER, and NPP, regardless of the fact that relative genetic variation coefficients were lower than 1.0. High accuracy may occur due to the large number of replications used in the experiment, given that accuracy and number of replications are interconnected (Resende and Duarte 2007). This evidence cannot be extended to the nutritional quality traits or to CEST and DTF, due to the lower genetic variability expressed for these traits in the progenies evaluated.

Significant genetic correlations of high magnitude were observed between the traits NPP and TDMY (Table 3) and indicate that direct selection for trait TDMY would be effective in improving panicle yield. It still needs to be investigated whether there is positive and significant genetic correlation between the number of panicles and the production of viable seeds in *B. ruziziensis*. There is strong negative genetic correlation between panicle density and flowering date, which reinforces the evidence of differences between the means of the genetic values for NPP between the flowering distribution groups, as presented above. The phenotypic correlations between the traits of nutritional quality and those associated with flowering were not significant. The genetic correlations between these same traits in many two-on-two combinations presented low to moderate magnitudes, positive or negative, and were significant. Given the importance of genetic correlations between traits for breeding, knowledge of this aspect supports and directs the selection for each trait, and should be considered in practice, especially if the aim is to increase the number of panicles per plant, which will promote reduction in the percentage of crude protein and an increase in NDF. In either case, the result will be undesirable.

An overlap in flowering between individuals selected for the obtainment of open-pollination progenies is essential for the intrapopulational recombination cycle, and also to carry out controlled crosses with these apomictic species. In the first case, it is because all individuals in the population must be able to recombine and maintain the effective population size through various breeding generations (Johnson et al. 2004). In the second, obviously because there can be no cross without overlap. Knowledge on the inheritance mode of the traits associated with flowering, their correlation with other economically important traits, and the variability available in the breeding population of *B. ruziziensis* will allow its proper use in breeding species from the *Brachiaria* genus by interspecific hybridization.

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