http://dx.doi.org/10.1590/2317-1545v38n1152223

RESEARCH NOTE

Brachiaria decumbens intraspecific hybrids: characterization and selection for seed production[1]

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ABSTRACT – *Brachiaria decumbens* has great importance in the national beef cattle scenario due to its high forage yield potential and high adaptability to acid soils and low fertility. In order to obtain new cultivars, intraspecific hybrids, previously selected by the *Brachiaria* breeding program, were evaluated in 2013 and 2014 for phenological characteristics and seed production potential through their production components, aiming to obtain cultivars with satisfactory production agronomic characteristics and forage yield, resistant to '*cigarrinhas-das-pastagens'* (*Tomaspia* sp.) and good seed production. Estimates of phenotypic correlation and analysis of variance were performed using the SAS 9.3 software. From the results obtained in both consecutive years, it was observed that there was a correlation between the reproductive tiller number (RT) and the pure seeds (PS) weight in the seedbeds of the second year of production around 70% ($p < 0.01$), which can be considered as a parameter to estimate the production of pure seeds before the start of flowering.

Index terms: phenology, apomixis, genotypes, forage improvement.

Híbridos intraespecíficos de *Brachiaria decumbens*: caracterização e seleção para produção de sementes

RESUMO – *Brachiaria decumbens* tem grande importância no cenário nacional da pecuária de corte por apresentar alto potencial produtivo forrageiro e alta adaptabilidade a solos ácidos e de baixa fertilidade. Com o intuito de ser obter novas cultivares, híbridos intraespecíficos, previamente selecionados pelo programa de melhoramento de *Brachiaria,* foram avaliados nos anos de 2013 e 2014, quanto a caracteres fenológicos e potencial de produção de sementes por meio de seus componentes de produção, visando a obtenção de cultivares com características agronômicas satisfatórias de produção e de produtividade de forragem, com resistência às cigarrinhas-das-pastagens e boas produtoras de sementes. As estimativas de correlação fenotípica e análise de variância foram realizadas pelo software SAS 9.3. A partir dos resultados obtidos nos dois anos consecutivos, observou-se que, houve correlação entre número de perfilhos reprodutivos (PR) e peso de sementes puras (SP) nos canteiros do segundo ano de produção em nível de 70% ($p<0,01$), o que pode ser considerado como parâmetro para se estimar a produção de sementes puras antes do início do florescimento.

Termos para indexação: fenologia, apomixia, genótipos, melhoramento de forrageiras.

Introduction

Forage grasses, specially *Brachiaria*, play an important role in Brazilian beef cattle and were responsible for the development of beef cattle in the Central-West, North and Southeast regions. It is estimated that about 85% of pasture land cultivated in tropical Brazil are cultivars of *Brachiaria*, highlighting *B. decumbens* cv. Basilisk, *B. brizantha* cv. Marandu and *B. humidicola* (Valle et al., 2009).

B. decumbens, due to its high forage yield potential and high

[1]Submitted on 7/18/2015. Accepted for publication on 12/7/2015.
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adaptability to acid soils with low fertility, has great importance in the national beef cattle scenario. Even if economically very important, there is only one cultivar available in the market, Basilisk, launched in the 1960s, which has as one of the limitations to its use the susceptibility to *cigarrinhas-das-pastagens* (*Tomaspia* sp.). (Verzignassi, 2010).

At first, the selection of forage crops was based solely on the production potential of mass and the forage quality in grazing conditions. But in recent years there has been a change in the lines and strategies in breeding programs of these plants, aiming superior cultivars in all aspects for the selection of cultivars. The cultivars development process is long, comprising several stages and the traits evaluated should be correlated in order to result in crops that show good performance in all phases and variables of studies and research such as the improvement itself, cytogenetics of the reproductive system, plant nutrition, microbiology, plant health, pasture management and nutritional quality of plants, seeds production and technology, among others (Karia et al., 2006; Valle et al., 2009; Valle, 2010).

Considering that not all the soil, climatic and agronomic management features required for maximum seed yields coincide with those needed to produce fodder, studies on physiological responses and production of tropical forage grass seeds to environmental effects are extremely important (Verzignassi, 2010) and currently scarce.

Just like any other factor, seed production potential is intrinsically linked to environmental factors. As for the other agronomic traits, seed production potential is of great importance in genetic improvement programs, and an indication of this is the recent interest in evaluating it. This potential is taken into account, either for seed production by parent plants used in breeding programs through sex, or to select access or hybrids of the breeding program by apomixis, in order to launch cultivars with high potential for the production of viable seeds (Valle, 2010; Verzignassi, 2010). In this context, the aim of this study was to characterize, evaluate and identify one or more seed production components to predict the production potential of intraspecific hybrids of *B. decumbens,* developed and pre-selected by the *Brachiaria* breeding program at Embrapa Gado de Corte, for the purpose of selection of superior genotypes, which are candidates for new cultivars or potential sex parents to be used in new crossings.

Material and Methods

The genetic materials used to develop this study were selected based on previous experimental results obtained by the Plant Breeding Program of *Brachiaria decumbens* by Embrapa Gado de Corte. Twenty-nine intraspecific hybrids of *B. decumbens* (sexual and apomictic), parents and candidates for new cultivars, were previously selected among 324 hybrids obtained by crossing among three sexual plants of *B. decumbens* artificially in tetraploid (D24/2, D24/27 and D24/45) with cv. Basilisk (apomictic). The selection was based on agronomic characteristics of production and nutritional value of forage. As control, *B. brizantha* cv. Marandu was used, proven resistant to *cigarrinhas-das-pastagens* and it has a good and viable seed production.

The genotypes evaluated were: B006, C001, R025, R033, R041, R044, R071, R078, R087, R091, R101, R107, R110, R120, R124, R126, R144, R181, S018, S031, S036, T005, T012, T026, T054, X030, X072, X117 and Y021. The tests were conducted in the Brazilian city of Campo Grande, MS, at Embrapa Gado de Corte [EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária; Brazilian Corporation of Agricultural Research)], altitude 530 m, geographical location 20º 25’ 03” S and 54° 42’ 20” W and tropical savanna Aw Köppen climate classification, characterized by rains in summer and dry season in winter (Köppen and Geiger, 1928). The tests were conducted under normal environmental conditions without artificial irrigation and the site soil was classified as clayey-textured distroferric red latosol (53% clay, 38% sand and 9% silt).

The seedlings were transferred to the field in early 2012. Each plot consisted of two seedlings, with a floor area of 2 m^2 and spacing between plots measuring one meter. Evaluations were carried out between December 2013 and the first quarter of 2014 with second-year plants. In the first year (2012/2013 harvest), the plants standardization cut was held in October 2012. For evaluations in the second year, in order to produce seeds, standardization was held on October 15, 2013. The experimental design was a complete block one with two replications and two observations per plot.

After the 2012 standardization cut, plant fertilization was carried out, based on soil chemical analysis. Therefore, 40 $kg.ha^{-1}$ of K_2O (potassium chloride), 70 $kg.ha^{-1}$ of nitrogen (ammonium sulfate) and 1 $kg.ha^{-1}$ of boron (boric acid) were used. In the second year, aiming to provide nutrients for the 2013/2014 harvest and after the standardization cut was carried out in 10/2013, the fertilization of the plants of the experimental area was held, based on new soil analysis, and 80 $kg.ha^{-1}$ of P_2O_5 (phosphorus pentoxide) (MAP – monoammonium phosphate), 50 $kg.ha^{-1}$ of nitrogen (ammonium sulfate) and 1 $kg.ha^{-1}$ of boron (boric acid) were used.

Also in the plots, evaluations were carried out in the beginning of the formation of the first reproductive tillers, before the beginning of the formation of the first inflorescences in 0.25 m^2: number of vegetative tillers and number of reproductive tillers.

In each plot, two seeds collectors were installed and within them five inflorescences were inserted in complete anthesis and without threshing. After the end of the seeds threshing of the inflorescences, the collectors were removed from the plots with their respective seeds and inflorescences. The material collected was taken to the Seeds Laboratory at Embrapa Gado de Corte and assessed for the number of pedicels, number of seeds primordia and number of grain-free seeds.

To determine the number of pedicels, count of existing pedicels in inflorescence racemes was carried out. To determine the number of seeds primordia, the difference between the number of grain-free seeds and the number of pedicels was considered.

To determine the seeds yield and quality, the harvest of the total area of the plot (2 m^2) was performed. To this end, in the maturation point of each plot, plant cut was done for the entire plot (except the inflorescences that were inserted in the collectors). The cutting was done manually at 20 cm from the ground by means of a rice cutting machine and was carried out at the start of the threshing, at the touch of 15 to 20% of seeds of inflorescences. The material collected was bagged in paper bags and these were closed and dried in the shade up to 10-12% moisture. After drying, the samples were subjected to manual separation of the inflorescences seeds. The seeds were then subjected to processing by pre-cleaning operations and cleaning by sieves and air column blower. To obtain pure seeds, there was a separation of full seeds from empty ones. To this end, blower model Seedburo South Dakota Seed Blower was used, with a 3.0 opening for *B. decumbens,* and a 6.0 opening for *B. brizantha* cv. Marandu, in both cases, for 30 seconds.

After processing the seeds, the following evaluations were carried out, in accordance with the Regras para Análise de Sementes [RAS (Rules for Seed Testing)] (Brasil, 2009): weight of pure seeds, thousand seed weight, germination test, physical purity and tetrazolium test, in addition to the weight of empty seeds.

The variables were subjected to analysis of variance by the software SAS version 9.3 (SAS Institute Inc, 2012), the means compared by the Scott-Knott test by the software GENES 7.0 (Cruz, 2009) at 5% probability or Duncan's test by the software SAS 9.3. Also, the variables of the assessments of number of grain-free seeds, number of pedicels, number of seeds primordia, number of reproductive tillers, number of vegetative tillers, purity, tetrazolium test, germination test, thousand seed weight, weight of empty seeds, weight of seeds harvested and weight of pure seeds of the seedbeds were analyzed for phenotypic correlation by means of software SAS 9.3.

Results and Discussion

There were significant differences among genotypes assessed for number of grain-free seeds and number of pedicels. For number of seeds primordia there were no differences (Table 1). Hybrid R181 showed the highest values for both number of grain-free seeds in collectors as the number of pedicels, but without differing from control cv. Marandu. In the assessments made in the seedbeds, it was observed that the genotypes evaluated showed no significant differences, but numerically it is noted that there were low levels of germination, due to likely seed dormancy of the genotypes tested (Castro et al., 1996; Silva et al., 2014). For the thousand seed weight (TSW), control cv. Marandu differs from the genotypes evaluated, showing the largest weight, followed by hybrids C001 and R041 (Table 2).

Table 1. Number of grain-free seeds (GFS), number of pedicels (NP) and number of seeds primordia (PRIM) of seed collectors for intraspecific hybrids of *B. decumbens* evaluated during the second year of production.

GENOTYPE	GFS[1]	NP[2]	PRIM[3]
B006	613.00 B*	778.33 B	165.33
C001	726.50 B	859.75 B	133.25
MAR	969.00 A	1069.50 A	100.50
R041	721.00 B	866.75 B	145.75
R087	322.00 C	400.00 C	78.00
R091	755.25 B	938.25 B	183.00
R110	688.25 B	803.75 B	115.50
R120	753.50 B	1194.25 A	440.75
R124	662.50 B	714.75 C	52.25
R126	682.00 B	864.25 B	182.25
R181	1160.75 A	1302.00 A	141.25
S031	759.75 B	1054.25 A	294.50
S036	783.75 B	898.25 B	114.50
T012	686.50 B	887.25 B	200.75
T026	726.50 B	844.00 B	117.50
T054	393.25 C	544.25 C	151.00
X030	826.00 B	887.00 B	61.00
X072	686.25 B	847.50 B	161.25
X117	715.00 B	892.00 B	177.00
Y021	488.50 C	536.00 C	47.50
CV (%)	24.84	19.13	38.19

[1]Comparing means by the Scott-Knott test at 5% probability. Data transformed ARC SEN $\frac{(X+0,5)^{\frac{1}{2}}}{100}$. [2]Comparing means by the Scott-Knott test at 5% probability. [3]Comparing means by the Scott-Knott test at 5% probability. Data transformed $(X+0.5)^{1/2}$. The table data are original. *Means followed by the same letter in the columns do not differ by the tests comparing means.

Table 2. Germination (G %) test and thousand seed weight (TSW) of the seedbeds for intraspecific hybrids of *B. decumbens* evaluated during the second year of production.

GENOTYPE	G %[1]	TSW[2]
C001	2.75	4.15 B*
Marandu	8.25	6.13 A
R041	2.00	2.81 C
CV (%)	31.88	4.48

[1]Comparing the means by the Duncan's test at 5% probability. Data transformed ARC SEN $\frac{(\mathbf{X}+\mathbf{0,5})^{\frac{1}{2}}}{\mathbf{100}}$. [2]Comparing means by the Duncan's test at 5% probability. The table data are original. *Means followed by the same letter in the columns do not differ by the tests comparing means.

Regarding the number of vegetative tillers, genotypes X030, Y021, T026, R126, R110 and R087 showed the highest production in terms of biomass, which is an important character in the development of new cultivars (Souza-Sobrinho et al., 2005; Souza-Sobrinho et al., 2011). However, these genotypes did not show high seed yield (Tables 1, 3 and 4).

Table 3. Number of vegetative tillers (VT) of the seedbeds for intraspecific hybrids of *B. decumbens* evaluated during the second year of production.

GENOTYPE	VT[1]
B006	43.25 B*
C001	26.50 B
Marandu	36.75 B
R041	44.50 B
R087	57.50 A
R091	22.50 B
R110	52.00 A
R120	46.75 B
R124	24.75 B
R126	55.75 A
R181	31.00 B
S031	36.00 B
S036	37.75 B
T012	41.75 B
T026	58.00 A
T054	45.50 B
X030	65.25 A
X072	41.00 B
Y021	62.50 A
CV (%)	34.45

[1]Comparing means by the Scott-Knott test at 5% probability. *Means followed by the same letter in the columns do not differ by the tests comparing means.

Table 4. Weight of pure seeds (PS), weight of empty seeds (ES), weight of seeds harvested (SH) and percentage of empty seeds (ES %) produced in the seedbed for intraspecific hybrids of *B. decumbens* evaluated during the second year of production.

GENOTYPE	PS[1] ($g.m^{-2}$)	ES[1] ($g.m^{-2}$)	SH	ES %
B006	0.30 d*	9.47 b	0.08 C	96.93
C001	49.27 a	38.40 a	11.23 A	43.80
Marandu	23.95 b	14.59 b	8.65 B	37.86
R041	1.54 c	11.59 b	0.41 C	88.27
R091	0.07 d	11.25 b	0.02 C	99.38
R110	0.05 d	4.53 c	0.02 C	98.91
R120	1.12 c	10.17 b	0.31 C	90.08
R124	0.32 d	9.29 b	0.10 C	96.67
R126	0.10 d	10.12 b	0.03 C	99.02
R181	0.00 d	5.70 c	0.00 C	100.00
S031	0.16 d	17.39 b	0.02 C	99.09
S036	0.47 d	8.23 c	0.13 C	94.60
T012	0.55 d	15.06 b	0.12 C	96.48
T026	0.06 d	3.81 c	0.02 C	98.45
T054	0.16 d	3.71 c	0.05 C	95.87
X030	0.82 c	4.48 c	0.18 C	84.53
X072	0.00 d	2.71 c	0.00 C	100.00
Y021	0.57 c	7.64 c	0.13 C	93.06
CV (%)	31.5	26.7	20.76	

[1]Comparing means by the Scott-Knott test at 5% probability, data transformed $(x+0.5)^{1/2}$. [2]Comparing means by the Duncan's test at 5% probability, data transformed $(X+0.5)^{1/2}$. The table data are original. *Means followed by the same letter in the columns do not differ by the tests comparing means.

The hybrids had similar responses for the variables weight of pure seeds and weight of empty seeds for the different genotypes (Table 4), and these variables showed a correlation around 75% ($p < 0.01$) from each other (Table 5). Hybrid C001 presented the highest values for weight of pure seeds, surpassed only by Marandu. For weight of empty seeds, C001 and Marandu showed the highest values, so that the percentages of empty seeds were lower for the genotypes mentioned, 43.80% and 37.86%, respectively. The other genotypes showed up to 100% of empty seeds among the seeds produced.

The high magnitude production of empty seeds in hybrids is very common and can occur due to the abortion of flowers because of genetic crossing (Mendes-Bonato et al., 2001; Mendes-Bonato et al., 2002; Mendes-Bonato et al., 2007; Felismino et al., 2010), or also by infestation of *cigarrinhas-das-pastagens*, which may impair the plant development and consequently seeds production (Auad et al., 2009). The yields observed in this study were lower than 1000 $kg.ha^{-1}$ estimated by Hopkinson et al. (1996). One of the correlation analysis objectives was to verify the possibility of selecting one or more variables making up seeds production that were likely to be used as indicators to predict the production potential and thus increase the genotypes selection speed by improvement programs.

Table 5. Phenotypic correlation results between physical purity (Purity %), number of grain-free seeds (GFS), number of pedicels (NP), number of seeds primordia (PRIM), number of reproductive tillers (RT), number of vegetative tillers (VT), weight of pure seeds (PS), feasibility by the tetrazolium test (TZ), germination (G %), thousand seed weight (TSW), number of racemes (NRAC), weight of empty seeds (ES) and weight of seeds harvested (SH) for hybrids of *Brachiaria decumbens* evaluated in seedbeds during the second year of production.

	Purity %[1]	GFS[1]	NP[1]	PRIM[2]	RT[2]	VT[2]	PS[2]	Purity %[2]	TZ[2]	G %[2]	TSW[2]	NRAC[2]	ES	SH
Purity %		0.0194*	-0.0884	-0.1832	0.1438	0.1252	0.5638	0.7463	-0.019	0.3901	0.9572	-0.1393	0.3381	0.37488
GFS			0.8235	-0.2166	-0.1145	-0.1305	0.0946	0.1913	0.0768	0.3075	0.6824	0.0949	0.1586	0.1549
NP				0.3753	-0.1458	-0.2260	0.0472	0.1252	-0.3201	0.1601	0.6318	0.3459	0.1895	0.1770
PRIM					-0.0648	-0.1776	-0.0720	-0.0960	-0.3845	-0.2204	0.0255	0.4210	0.0623	0.0474
RT						-0.3084	0.7004	0.5414	0.5045	-0.4676	-0.2954	-0.3218	0.6035	0.6317
VT							-0.1920	-0.1550	0.1778	-0.1411	0.2058	-0.0371	-0.2122	-0.2155
PS								0.9112	0.2769	-0.5265	0.0817	-0.2080	0.7545	0.8050
Purity %									0.0394	-0.1362	0.6881	-0.2403	0.5962	0.6510
TZ										-0.2602	-0.2444	-0.469	0.3623	0.3546
G %											0.5789	0.3184	-0.5449	-0.5481
TSW												0.1009	-0.3008	-0.2534
NRAC													-0.2033	-0.2094
ES														0.9967
SH														

[1]Hybrids of *B. decumbens* evaluated in seed collectors during the second year of production. [2]Hybrids of *B. decumbens* evaluated in beds during the second year of production.
*Significant at 1% probability by the Pearson coefficient.

The number of reproductive tillers, evaluated at the beginning of their formation, before the beginning of the formation of the first panicles (inflorescences), showed a correlation of 70% ($p < 0.01$) with the weight of pure seeds (Table 5), indicating that the variable can be used to predict the production before the plants flowering beginning, which is in accordance with what was mentioned by Lopes and Franke (2011). In *Bromus auleticus*, seed production is similar in the first three reproductive tillers that develop in a plant, which are responsible for about 75% of the final yield (Maia et al., 2006).

Conclusions

There is a correlation (0.70) between the number of reproductive tillers (RT) and weight of pure seeds (PS) in the seedbeds of the second year of production ($p < 0.01$), which can be used as a parameter to estimate the pure seeds production potential, even before the start of flowering.

Acknowledgments

Instituto Federal Goiano– Campus Rio Verde, Embrapa Gado de Corte, FAPEG [Fundação de Amparo à Pesquisa do Estado de Goiás, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FUNDECT (Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), UNIPASTO (Associação para o Fomento à Pesquisa de Melhoramento de Forrageiras), FUNDAPAM (Fundação de Apoio à Pesquisa Agropecuária e Ambiental) and the members of the Tropical Forage Seeds Production and Technology team of Embrapa Gado de Corte, specially Mr. Luiz de Jesus, Mr. Hugo Corado and Mr. Vagner Martins.

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