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Genetic parameters in *Stylosanthes* using different statistical methods

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Genetic parameters in *Stylosanthes* accessions were estimated through (ANOVA) and REML/BLUP (Restricted Maximum Likelihood /Best Linear Unbiased Prediction), to compare them for the genetic values in order to select superior accessions. Twenty five genotypes were evaluated in two environments in a randomized blocks experimental design with four replications. The genetic parameters were estimated for 12 descriptors by the two methods. Both methods indicating that the accessions presented genetic variability for the descriptors, but, the ANOVA and the REML/BLUP presented divergent values for the unbalanced data and for descriptors with high environmental influence, as it happens with mass descriptors, what, in turn, indicates that the method REML/BLUP leads to a more accurate predictions and allows for the selection of *Stylosanthes* accessions. It allows the inclusion of characters, even if they present heterogeneity of the residual variance. The indirect selection may be used, because the primary stem length descriptor revealed itself as a good option due to the correlation with the total dry mass. The accessions BGF-016 and BGF-015 are the most promising ones to be taken up by a Stylosanthes plant breeding program for fodder in the Brazilian Semiarid region.

Key words: Semiarid, forages, breeding.

INTRODUCTION

The *Stylosanthes* genus calls our attention due to its number of species with extraordinary fodder potential with excellent nutritional quality and their easy adaptation to different environmental conditions. Even though Brazil is the centre of diversity of these species, Australia, African and Asian countries have released cultivars with higher impact on the production system than the releases made in Brazil (Resende et al., 2006). In Brazil the most used cultivar is the Estilosantes Campo Grande, which belongs to the species *Stylosanthes capitata* (Embrapa, 2007), however this variety was not developed for the Semiarid. A survey done in the herbariums from the universities of Bahia and Embrapa Genetic Resources and Biotechnology indicated the existence of different species in the State of Bahia, revealing the great diversity for the genus (Costa, 2006), and thus demonstrating that there is a great potential for the study of this germplasm, so far quite neglected, in order to develop cultivars for the

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Brazilian Semiarid.

The procedures normally used for the selection of fodder plants is done by the evaluation of accessions and the selection of the superior ones (Chakraborty, 2004). However, this selection is more efficient when it is based on the genetic values estimate and in association with variables of economic importance (Assis et al., 2010). Due to the lack of *Stylosanthes* cultivars developed for the region, it is desirable to establish a plant breeding program for this fodder plant, and thus the genetic parameters estimation is needed to identify the best strategies to be used in the selection of *Stylosanthes* individuals which are tolerant to droughts, has high fodder productivity and high nutritional value.

For a long time, the variance analysis (ANOVA) method has been the most used in plant breeding to estimate variance components, but, in situations where there are unbalanced data, environmental variance, variance heterogeneity among experiments, competition between genotypes due to the difference in aggressively and sensibility of different genetic materials (Resende, 2007) its use is limited.

This study had the objective to estimate the genetic parameters through the statistic models (ANOVA and REML/BLUP), to select the best method of identifying the most prominent accessions in the selection for superior materials for starting a breeding program of this fodder in the Semiarid of Bahia, which is also very important for in the Brazilian semiarid.

MATERIALS AND METHODS

Two field experiments were carried out between July 2012 and January 2013. The first one was done at the experimental station Horto Florestal (12° 16'087''S; 38° 56'346"W; 243 m) which belongs to the State University of Feira de Santana - UEFS in Feira de Santana, BA (A_1) and the other one at the experimental field of the Technology and Social Sciences Department (09° 24' 50"S; 40°30' 10"W; 368 m) of the University of Bahia - UNEB in Juazeiro, BA (A_2).

For the experimental set up, 25 accessions of *Stylosanthes* spp., from collections acquired between the years of 2008-2011 in three semiarid regions in the state of Bahia (Sisaleira, the Middle Lower São Francisco River Basin and the micro region of Feira de Santana) were used along with a commercial variety, Estilosantes Campo Grande, as a control (Table 1).The fruits of each accession obtained in the collecting expeditions were sent to the Evolutive Ecology Laboratory - LEE from the Estate University of Feira de Santana, Feira de Santana, BA for manual processing, consisting of the removal of loment by friction using rubber devices of four mm of thickness. The seeds from each accession were put in labelled envelopes, and kept in air tight bottles with silica gel as a humidity indicator and were kept in Lab conditions (temperature of 25 °C). Between the months of April and May 2012, 54 seeds of each accession were mechanically scarified with wood sandpaper n° 150 and sown in polyethylene tubes with dimensions of 6 x 20 cm filled with the commercial substrate. They were kept under greenhouse conditions, and watered twice daily. The seedlings in both experiments were produced in green house of the State University of Feira de Santana (UEFS) with the purpose of standardizing the procedures for obtaining the seedlings and after three months they were transplanted to the field.

The soil, in both experiments, was prepared by disk harrow and the marking of the plots were marked and, then pitting was performed with the aid of a manual digger. A complete randomized block design with four replications and four plants per useful area of each plot was used, with a total of 16 plants per treatment and the spacing of 3.0 m between rows and 0.8 m between plants was adopted. There was a basal fertilization, in both trials, with the application of 30 kg ha^{-1} P$_{,}$ 30 kg ha^{-1} K and 20 kg N, and after around 35 to 40 days of the transplanting, topdressing fertilization with 30 kg ha^{-1} K and 20 kg ha^{-1}N was used. Both experiments were irrigated using the drip irrigation system whenever necessary.

The evaluations were performed between four and five months after transplanting. For morphological descriptors the measurements were made on all the plants of useful area from each treatment. They were: PD - Plant Diameter (mm) measured at the base of the plant; PH – Plant Height (cm), measured from the ground level to the highest leaf on the stem; PBL - Primary branch length (cm), its measurement was taken from the insertion of the primary branch at the bottom of the stem up to the last leaf; PAL- Length of the central axis of the plants (cm), measured from the ground level to the highest leaf of the main stem; NS - Number of stems (units), counting of the number of stems from ground level to the last stem inserted in the main axis; CLL - central leaflet length (mm) and CLW - width of the central leaflet (mm), measured in the longitudinal part of the central leaflet of the third definitive leaf of the plant, inserted in the first ten centimeters of the central axis; SLL - Side leaflet length (mm) and LLW- Lateral width of leaves (mm), measured in the longitudinal and latitudinal part, respectively, of the right of the third leaf stage of plant, inserted in the first ten centimeters from the central axis lateral leaflet. The TDM descriptors (total dry mass - g), DSM (dry stalk mass - g) and DLM (dry leaf mass - g) were evaluated in one plant per plot.

Initially the individual variance analysis was done for each place to check if the accessions differed significantly among themselves. Then, the homogeneity of variance was tested (F maximum - ratio between the largest and smallest mean squared residual for each descriptor) following the test of Hartley (1950) recommended by Cruz et al. (2004), excluding descriptors that presented F maximum superior to 7.

In this study, each accession was formed by a group of individuals which represented a given population, and therefore, the genotypes effects were regarded as random and the environment effects as fixed using the following model (Cruz et al., 2004): $Yijk = \mu + (B / A)jk + Gi + Aj + GAij + Eijk$ where: $Yijk$ = observation in the k-the block, reported in the i-the genotype and j-the environment; μ is the general mean of the test; (B/A) the block effect k within the environment j; Gi = effect of genotype i; Aj = effect of environment j; GAij = effect of the interaction between genotype i and environment j; and Eijk = random error associated with the ijk observation.

From the joint analysis, the genetic parameters were estimated (Cruz et al., 2004), thus the decomposition of the mean squares estimate of the interaction in simple and complex parts was measured (Cruz and Castoldi, 1991).

Genetic parameters were also estimated using the methodology of mixed models REML/BLUP (Restricted Maximum Likelihood /Best Linear Unbiased Prediction) to observe possible differences from ANOVA (Resende, 2007). In this analysis each individual has an individual genotypic value allowing for a more accurate estimate of the genetic value and more adequate ordering of superior individuals, leading to a better selection (Martinez et al., 2011).

In the analysis of mixed models with unbalanced data such as the present one, the effects of the model were tested via LRT (likelihood ratio test) over the F test which is used in the analysis of variance and in replacement of the framework of ANOVA a similar framework called analysis of *deviance* (ANADEV) was performed. All these effects along with the estimates of the variance components and genetic parameters were obtained by the

Table 1. Origin and description of *Stylosanthes* accessions stored in BGF/UEFS.

Acession	City	Geographic coordinates	Year	Species
BGF 08-001	Araci	11°36'20"S e 39°09'52.1"O	2008	*S. viscosa* (L.) Sw.
BGF 08-002	Araci	11°27'24.5"S e 39°26'43.6"O	2008	*S. scabra* Vogel
BGF 08-003	Ichu	11°42'24.2"S e 39°09'59.4"O	2008	*S. scabra* Vogel
BGF 08-004	Serrinha	11°40'29.9"S e 39°04'38.1"O	2008	*S. scabra* Vogel
BGF 08-005	Serrinha	11°47'46.8"S e 38°53'24.5"O	2008	*S. scabra* Vogel
BGF 08-006	Serrinha	11°26'36.4"S e 39°12'00.8"O	2008	*S. scabra* Vogel
BGF 08-007	Valente	11°22'13.0"S e 39°17'28.1"O	2008	*S. scabra* Vogel
BGF 08-010	Nova Soure	10°29'36.7"S e 39°20'44.0"O	2008	*S. scabra* Vogel
BGF 08-011	Valente	11°27'12.6"S e 39°25'24"O	2008	*S. scabra* Vogel
BGF 08-012	S. Domingos	11°27'27.1"S e 39°32'46.4"O	2008	*S. scabra* Vogel
BGF 08-014	Tucano	11°01'59"S e 38°48'17"O	2008	*S. scabra* Vogel
BGF 08-015	Queimadas	10°54'40"S e 39°12'17"O	2008	*S. scabra* Vogel
BGF 08-016	Queimadas	10°54'40"S e 39°12'17"O	2008	*S. scabra* Vogel
BGF 08-017	Queimadas	11°19'26"S e 39°49'13"O	2008	*S. scabra* Vogel
BGF 08-018	Candeal	11°49'49.8"S e 39°07'08.5"O	2008	*S. scabra* Vogel
BGF 08-019	Cansanção	09°50'78.7" e 39°28'05.1"O	2008	*S. scabra* Vogel
BGF 08-020	Candeal	11°49'49.8"S e 39°07'08.5"O	2008	*S. scabra* Vogel
BGF 08-021	Casa Nova	09°16'50.5"S e 41°29'15.5"O	2008	*S. humilis* Kunth
BGF 08-023	Casa Nova	09°21'36"S e 41°47'17.5"O	2008	*S. humilis* Kunth
BGF 08-024	C. A. Lourdes	09°35'15.1"S e 42°54'02.1"O	2008	*S. capitata* Vogel
BGF 08-026	Casa Nova	09°10'33.3"S e 40°50'17.1"O	2008	*S. viscosa* (L.) Sw.
BGF 08-029	Canudos	09°54'29.9"S e 39°03'17.2"O	2008	*S. viscosa* (L.) Sw.
BGF 08-032	Sento Sé	10°09'11.3"S e 41°39'01.1"O	2008	*S. scabra* Vogel
BGF 08-033	Sento Sé	10°10'22.6"S e 41°58'24.0"O	2008	*S. humilis* Kunth
BGF 08-034	F. Santana	12°09'719"S e 38°57'696"O	2011	*S. scabra* Vogel

BGF - Number in the Bank of Germplasm of Forage.

SELEGEN-REML/BLUP software (model 23 - selection based on various replications, taking into consideration one or more plants per plot, evaluation in more than one location and experiments in completely randomized blocks) .

According to Resende (2007) using the following statistical model $y = Xr + Zg + Wi + e$, where:y= data vector;$r$is the vector of the effects assumed to be fixed (repetition + overall mean);$g$is the vector of genotypic effects,$i$is the effects vector of the genotype x environment interaction, where: $e$is the vector of errors or residues (random), where: X, $Z$and $W$are the incidence matrices for the referred effects. Based on the genetic parameters it was possible to estimate the selection genetic gain calculated by the formulae $G_{si(j)} = h^2_{g_i} * ds_{i(j)}$ where $G_{si(j)}$ is the gain in environment i, with selection based on environment j; dsi (j) is the selection differential in the environment i in which the individuals selected have the best performance in the environment j; and h^2_g is the heritability of the character in the environment i, adopting a selection intensity of 20% for each analysis. To estimate the gain selection percentage it was used the following formulae: $G_s\% = (G_s/\bar{x})*100$.

RESULTS AND DISCUSSION

The joint analysis, using ANOVA, showed that the mean squares of the effects of genotypes and environments presented high ($P \leq 0.01$) for almost all variables except lateral leaflet width (LLW), for environment ($P \leq 0.05$). The effects of G x E interaction were also great ($P \leq 0.01$), except plant diameter (PD) and lateral leaflet width (LLW) (Table 2). When considering the values between the two environments, most of the descriptors presented F maximum below seven (Table 2), except the total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) descriptors and the genetic variances (V_g) were higher in comparison to variances of the interaction GxE (V_{gxe}) and environmental variance (V_e) for almost all descriptors except for side leaflet length (SLL) (Table 3) indicating genetic diversity among the accessions evaluated and also demonstrated that the genetic variability among the accessions is partially due to differences among individuals within each accession and, also, due to characteristics of different species, since the 25 treatments comprised four species (*Stylosanthes scabra, Stylosanthes viscosa, Stylosanthes capitata* and *Stylosanthes humilis*).

The relative coefficient of variation (CV_r) was almost equal to 1.0 side leaflet length (SLL) and higher than 1.0 for the remaining variables. As for broad-sense

Table 2. Joint variance analysis in genotypes of *Stylosanthes.*

SV	DF	Means square								
		PD	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW
G	25	56.20**	994.66**	2124.10**	883.27**	108.36**	127.72**	14.86**	45.01**	6.99**
E	1	13.51**	790.35**	4230.96**	1378.72**	58.55**	272.69**	9.85**	157.36**	3.76*
GxE	25	2.92^{NS}	151.39**	128.58**	156.36**	14.05**	11.81**	0.99^{NS}	15.44**	2.25**
Res.	150	2.28	36.75	51.97	42.32	4.48	4.85	0.69	3.81	0.62
CV	-	12.95	13.60	11.58	17.72	12.98	15.6	12.54	17.55	16.48
M	-	11.66	44.57	62.24	36.72	16.31	14.11	6.61	11.11	4.79
Fmax	-	1.71	1.53	1.09	1.87	1.02	2.94	1.69	1.45	1.13

Excluded descriptors

Fmax	TDM	DSM	DLM
	8.64	16.00	8.17

G - Genotype; E - Environment; GxE - interaction Genotype x Environment; CV - Coefficient of Variation; M - Mean; Fmax - relation between the biggest and smallest square mean of the residue. SV - Source of variation; DF - Degree of freedom; PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central Leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. **, * = significant at 1 and 5% of probability respectively; ^{NS} = not significant.

Table 3. Estimates (Est) of genetic parameters using ANOVA in accessions of *Stylosanthes.*

Est	PD	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW
V_g	6.66	105.41	249.44	90.86	11.79	14.49	1.74	3.70	0.59
V_e	0.25	31.35	61.30	32.11	0.55	7.71	0.31	3.81	0.12
V_{gxa}	0.08	14.33	9.58	14.26	1.20	0.87	0.04	1.45	0.20
V_f	6.99	151.09	320.32	137.23	13.54	23.07	2.09	8.96	0.91
h^2_g	0.95	0.70	0.78	0.66	0.87	0.63	0.83	0.41	0.64
r_{12}	0.91	0.70	0.84	0.63	0.56	0.83	0.75	0.55	0.45
r_g	0.99	0.88	0.96	0.86	0.91	0.94	0.98	0.72	0.74
CV_g	22.13	23.04	25.38	25.96	21.05	26.98	19.93	17.30	16.07
CV_e	4.26	12.56	12.58	15.43	4.56	19.68	8.46	17.57	7.34
CV_r	5.19	1.83	2.02	1.68	4.62	1.37	2.36	0.98	2.19
%C	-	17.31	1.55	7.41	45.91	7.28	-	12.89	15.56
%S	-	82.69	98.45	92.59	54.09	92.72	-	87.11	84.44

V_g - Genotypic Variance; V_e - Residual Variance; V_{gxa} - genotype x environment interaction variance; V_f - individual phenotypic variance; h^2_g - heritability of the total genotypic effects; r_{12} - genetic correlation he genotypes in both environments; r_g - genetic correlation between both environments; CV_g - genotypic variation coefficient ; CV_e - residual variation coefficient; CV_r - relative variation coefficient; *%C* - Part of the complex interaction; *%S* - part of the simple interaction. PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. (-) - Descriptor not significant at 5% of probability by F test.

heritability (h^2_g), the values ranged from 0.41 to 0.95. The results also showed that all characters expressed interaction of the simple type, except for central leaflet width (CLW) and plant diameter (PD) that were not significant and, therefore, the correlation between environments (r_{12}) were relatively high (Table 3).

On the other hand, the values of ANADEV (Analysis of *Deviance*) show that seven descriptors plant diameter (PD), plant height (PH), primary branch length (PBL), length of the central axis of the plants (PAL), number of stems (NS), central leaflet length (CLL) and central leaflet width (CLW) presented effects of genetic variances (V_g) and broad-sense heritability (h^2_g) high ($P \leq 0.01$) and the heritability ranged from 0.59 to 0.83. Regarding the descriptors side leaflet length (SLL), lateral leaflet width (LLW), total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM), the effects of genotypes were not significant via the LRT test. For the interaction effects of GxE and for the coefficient of determination (r^2int) the values of the LRT test for all descriptors were high ($P \leq 0.01$), except for plant diameter (PD) and central leaflet width (CLW) (Tables 4 and 5).

Table 4. Analysis of Deviance (ANADEV) evaluated in *Stylosanthes.*

	Descriptors					
Effects	**PD**	**PH**	**PBL**	**PAL**	**NS**	**CLL**
Genotype	35.46**	15.58**	29.80**	13.89**	19.39**	25.62**
G x E	1.61^{NS}	25.56**	9.68**	21.60**	17.15**	9.57**

	Descriptors					
Effects	**CLW**	**SLL**	**LLW**	**TDM**	**DSM**	**DLM**
Genotype	28.94**	3.28^{NS}	2.58^{NS}	2.24^{NS}	1.419^{NS}	2.08^{NS}
G x E	1.36^{NS}	28.99**	23.10**	47.23**	46.21**	26.93**

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. Table chi-square - 3.84 (*) and 6.63 (**) for the levels of significance of 5 and 1%, respectively; ^{NS} = not significant at the level of 5% probability.

Table 5. Estimates (Est) of genetic parameters by REML/BLUP in accessions of S*tylosanthes*

Est	**PD**	**PH**	**PBL**	**PAL**	**NS**	**CLL**	**CLW**	**SLL**	**LLW**	**TDM**	**DSM**	**DLM**
V_g	10.43	126.26	314.59	107.73	14.48	22.41	2.93	4.48	0.69	26513.08	7232.43	2489.26
V_e	0.26	31.45	21.02	31.14	2.68	2.01	0.09	4.87	0.71	46962.39	17515.12	4332.75
V_{gxa}	1.99	36.99	51.51	42.52	4.30	4.90	0.71	3.83	0.63	33216.22	12664.96	5195.29
V_f	12.68	194.70	387.13	181.40	21.47	29.32	3.72	13.18	2.03	106691.70	37412.51	12017.29
h^2_g	0,83	0.65	0.81	0.59	0.68	0.76	0.79	0.34	0.34	0.25	0.19	0.21
h^2_{mg}	0.96	0.86	0.95	0.83	0.88	0.93	0.96	0.60	0.61	0.47	0.40	0.45
r^2_{int}	0.02	0.16	0.05	0.17	0.13	0.07	0.02	0.37	0.40	0.44	0.47	0.36
r_{gg}	0.98	0.92	0.97	0.91	0.94	0.97	0.98	0.78	0.78	0.69	0.63	0.67
r_g	0.98	0.80	0.94	0.78	0.84	0.92	0.97	0.48	0.49	0.36	0.29	0.37
CV_g	28.96	25.40	29.35	28.46	23.70	33.99	25.95	19.21	17.38	38.30	44.90	33.97
CV_e	12.65	13.75	11.88	17.88	12.91	15.90	12.77	17.78	16.62	42.87	59.41	49.07
CV_r	2.29	1.85	2.47	1.59	1.84	2.14	2.03	1.08	1.05	0.89	0.76	0.69
m_g	11.15	44.24	60.43	36.47	16.06	13.93	6.59	11.02	4.78	425.11	189.42	146.88

V_g - Genotypic Variance; V_e - Residual Variance; V_{gxa} - genotype x environment interaction variance; V_f - individual phenotypic variance; h^2_g - coefficient of heritability in the broad sense; h^2_{mg} -mean genotype heritability; r^2int - coefficient of the determination of interaction G x E; r_{gg} - genetic correlation he genotypes in both environments; r_g - genetic correlation between both environments; CV_g - genotypic variation coefficient ; CV_e - residual variation coefficient; CV_r - relative variation coefficient; m_g - overall mean; m_1 - overall mean of environment A_1; m_2 -overall mean of environment $A_{2;}$ PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass; m_g - mean of both environments.

When comparing values for the variance components obtained by ANOVA with the ones acquired by REML/BLUP, there was divergence in value, once for the ANOVA method the central leaflet length (CLL) and lateral leaflet width (LLW) were high ($P \leq 0.01$), whereas for the REML/BLUP these descriptors showed no significant differences. The mean values of the descriptors that were possible to assess through ANOVA were higher when compared to those obtained by REML/BLUP, thus indicating elevation of means by ANOVA in the joint analysis. As such, this may induce a wrong selection of the best individuals.

It is noteworthy that the descriptors mass total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM), the most important ones from the forage point of view, had to be excluded from the joint analysis by ANOVA due to this method`s limitations on heterogeneity of residual variances (Cruz et al., 2004), thus preventing the evaluation of their genetic parameters, the potential in relation to the genetic gains and consequently the indication of possible accessions for the selection of superior materials for the forage production.

Therefore, the methodology REML/BLUP, despite being a method little used for annual plants, can be used to detect variability among accessions in different species of *Stylosanthes* particularly for descriptors that exhibit heterogeneity of variance and unbalanced data, thus showing, that these factors are not limiting to estimate genetic predictions for descriptors that have a strong environmental influence. This reassures this methodology as essential for breeding programs of forages that need to be grown in different environments, as it is the case for *Stylosanthes* in the Semiarid of Bahia. The methodology of mixed models was also used efficiently to quantify the variation and estimate genetic predictions in some annual species (Borges et al., 2010; Neto et al., 2013; Resende et al., 2006).

It is also worth mentioning that apart from mass descriptors, other relevant traits in *Stylosanthes* need to be considered since in a breeding program the accessions selected by genetic merits of mass production should also be assessed in the association with grasses, produce enough seeds and present tolerance to diseases such as anthracnose – *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Resende et al., 2006), although not yet reported in the state of Bahia. On the other hand, the mass descriptors were not significant among the accessions evaluated using REML/BLUP, but the broad-sense heritability and mean of dry matter found in this study was similar to the values found for *Stylosanthes* (Resende et al., 2006; Santana et al., 2013).

The CV_g% ranged from 17.38 to 44.90%, with the lowest value observed for the lateral leaflet width (LLW) descriptor, while the highest variation was for dry stalk mass (DSM); the environment coefficient of variation (CV_e) were superior for all descriptors, except for the mass ones; the relative variation coefficient (CV_r), were above a unit for all descriptors, except for mass descriptors (Table 5). Regarding the mean heritability of the genotype (h^2_{mg}), the values obtained were high for all the descriptors, although lower for mass descriptors. A very high precision, the selective accuracy (r_{gg}, Resende and Duarte, 2007), was observed for most descriptors of the plant and only two of them (side leaflet length -SLL and lateral leaflet width -LLW) presented only high accuracy, however, the mass descriptors showed moderate accuracy.

The genetic correlations (r_g) between the accessions and the environments showed that the plant diameter (PD), plant height (PH), primary branch length (PBL), length of the central axis of the plants (PAL), number of stems (NS), central leaflet length (CLL) and central leaflet width (CLW) descriptors showed higher coefficients than 0.78 and plant diameter (PD), central leaflet width (CLW) and primary branch length (PBL) were highlighted. On the other hand, the characters side leaflet length (SLL), lateral leaflet width (LLW), total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) displayed genetic correlation between both environments(r_g) values below 0.50 but the lowest levels were observed for total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) (Table 5).

From the values of genetic variances, heritability and relative coefficient of variation obtained in this work for mass descriptors, it can be inferred that the genotypes demonstrated potential for obtaining genetic progress with the selection in the environments of study. By comparing the results found here with other annual forages commonly used in the Brazilian Semiarid region, the potential of the genus *Stylosanthes* is confirmed (Assis et al., 2008, 2010; Cunha and Lima, 2010).

Moreover, the values of heritability in the broad sense were also overestimated by the method ANOVA, because nearly all descriptors were superior to the REML/BLUP values, except for the central leaflet length (CLL), confirming that the method of ANOVA for more complex situations leads to inaccurate estimates of the variance components and consequently inaccurate predictions of breeding values (Resende, 2007).

The behavior of the variance components when decomposed to G x E interaction by the two methods (ANOVA and REML/BLUP) were similar to some descriptors except side leaflet length (SLL) and lateral leaflet width (LLW) that showed simple interaction type by ANOVA and complex interaction by REML/BLUP. Then, the choice of the best accessions should happen through the REML/BLUP, because it was more viable for descriptors that exhibit different behavior in different environments (side leaflet length - SLL), lateral leaflet width - LLW, total dry mass - TDM, dry stalk mass - DSM and dry leaf mass - DLM). This method facilitates the evaluation and selection in this situation, similar to what happens with other forages (Cargnin et al., 2006; Luz et al., 2010; Pereira et al., 2010) and studies using this

Table 6. Estimate of simple correlations in *Stylosanthes* accessions.

Desc.	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW	TDM	DSM	DLM
DP	0.53**	0.59**	0.49**	0.55**	0.25**	0.21**	0.22**	0.16*	0.33**	0.29**	0.30**
PH		0.60**	0.96**	0.84**	0.55**	0.07^{NS}	0.11^{NS}	0.01^{NS}	0.14^{NS}	0.13^{NS}	0.11^{NS}
PBL			0.56**	0.63**	0.55**	0.36**	0.48**	0.29**	0.68**	0.68**	0.57**
PAL				0.83**	0.14^{NS}	0.04^{NS}	0.06^{NS}	-0.01^{NS}	0.10^{NS}	0.09^{NS}	0.09^{NS}
NS					0.38**	0.22**	0.30**	0.17*	0.27**	0.25**	0.24**
CLL						0.84**	0.93**	0.75**	0.46**	0.45**	0.42**
CLW							0.81**	0.89**	0.26**	0.25**	0.25**
SLL								0.85**	0.43**	0.42**	0.40**
LLW									0.22**	0.22**	0.22**
TDM										0.99**	0.95**
DSM											0.90**

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. **, * = significant at 1% and 5% of probability respectively; ^{NS} =not significant.

methodology in selecting genotypes (Arantes et al., 2013; Bastos et al., 2007; Zeni-Neto et al., 2008; Verardi et al., 2009).

The estimate of the simple correlations revealed that the majority of descriptors showed significant correlations ($P \leq 0.01$), but of moderate to low magnitude from one character to the other, except for plant height (PH) and length of the central axis of the plants (PAL) that showed no significance with the majority of the characters. For the primary branch length (PBL) it was observed a positive association from moderate to low intensity for most descriptors, however, there was a significant and moderate correlation between PBL with the mass descriptors (Table 6).

Therefore, a possibility to improve the selection of genotypes with efficiency for higher dry matter would be through indirect selection. Moreover, indirect selection can lead to superior gains over direct selection, when the auxiliary character displays heritability above the main one, a positive correlation and of high magnitude (Falconer and Mackay, 1996). Therefore, the indirect selection can be used, once the primary branch length (PBL) is a good option for selection due to the correlation with total dry mass. This is a feature of great significance to the breeding of *Stylosanthes* in the Brazilian Semiarid region with the purpose of increasing the total dry mass, since there is a great variation in the environmental conditions.

The selection of the best five accessions by ANOVA generated total gains ranging from the minimum and the maximum equal to 20.81%, and total dry mass (TDM) presented the smallest increase, while the largest was observed in central leaflet length (CLL). By the REML/BLUP method the amplitude of variation was approximately 38.02%, where the central leaflet length (CLL) had the highest and the lowest value was for dry leaf mass (DLM). As for primary branch length (PBL), it was observed that the two methods presented similar high values to gain selection and the estimation of genetic gains by the two methods showed that the BGF-016 and BGF-015 accessions were the top among the materials evaluated (Table 7).

Thus, the REML/BLUP method allowed the elimination of accessions that had negative genotypic effects (values below the overall mean of the experiment), and this increased the probability of selecting superior individuals; it provided the estimate for the number of individuals to be selected by accession for each variable and also enabled the identification of the plots of the best individuals and the number to be selected in each one (Martinez et al., 2011; Resende et al., 2006).

Besides all these advantages presented here, it is known that the REML/BLUP method is little used for annual species in the area of plant

Table 7. Overall genetic gain (Gt %) and gain of selection (G_S %) by genotype in *Stylosanthes.*

PD				PH				PBL				PAL			
Anova		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**	
G	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***
002	31.94	002	36.73	016	26.89	016	32.59	024	44.74	016	45.80	016	31.00	016	33.36
010	24.77	010	29.42	026	24.39	026	29.64	016	36.24	024	44.50	026	26.13	026	28.20
026	24.77	026	29.42	029	20.12	024	25.56	015	26.68	015	34.80	015	23.08	024	26.13
012	18.90	012	23.40	015	19.76	029	24.56	010	19.93	010	26.70	029	21.07	015	24.97
016	17.68	016	22.19	014	16.98	015	24.13	014	19.31	014	26.00	024	19.41	029	22.83
Gt=23.61		Gt = 4.41		Gt=21.63		Gt =18.48		Gt=29.38		Gt=30.08		Gt =24.14		Gt= 9.26	

NS				CLL				CLW				SLL			
Anova		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**	
G	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***
016	43.85	16	41.10	T	79.56	T	105.00	T	69.82	T	71.41	T	41.92	T	30.49
015	42.73	15	40.05	024	59.20	024	65.20	024	68.56	024	65.06	024	34.98	024	18.62
026	28.59	26	27.18	015	21.43	015	31.00	034	16.07	034	17.50	015	12.40	015	11.38
T	16.80	14	15.45	026	15.45	026	22.60	004	6.15	002	4.94	026	5.72	026	5.39
014	15.68	24	15.45	016	14.20	016	20.90	002	4.39	006	4.37	016	5.72	016	5.39
Gt=29.53		Gt=21.52		Gt=37.97		Gt=39.97		Gt =33.00		Gt =27.74		Gt=20.15		Gt=8.08	

LLW				TDM				DSM				DLM			
Anova		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**		**Anova**		**Blup**	
G	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***	**G**	***Gs%***
024	57.32	T	23.90	016	25.05	016	31.08	016	27.65	016	28.79	15	29.19	015	28.62
T	51.31	024	22.50	015	24.88	015	30.88	015	26.54	015	27.68	16	25.77	016	25.43
034	12.83	034	7.66	024	19.15	011	12.90	024	26.03	024	11.00	11	14.90	011	15.26
006	10.96	006	5.74	011	9.57	014	10.08	011	7.55	011	8.86	24	12.85	014	7.79
011	8.42	011	4.08	014	7.17	024	9.04	020	7.54	020	8.85	14	6.91	020	5.74
Gt=28.17		Gt=7.11		Gt=17.16		Gt=10.00		Gt=19.06		Gt= 8.09		Gt=17.93		Gt=1.94	

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. G - genotype; (-) absence of value; T - Control.

genetic resources, but the results presented here show that this methodology can be used routinely in the selection of accessions since it is easily handled, it estimates the genetic values with greater accuracy, increases the efficiency of selection, and consequently decreases the cost of breeding programs to increase mass descriptors for the species of *Stylosanthes*.

Conclusions

The methods (ANOVA and REML/BLUP) presented divergent values, indicating that the REML/BLUP method estimated the genetic values with greater accuracy, increases the efficiency of selection and decreases the cost of breeding programs. The indirect selection may be used, because the primary stem length descriptor revealed itself as a good option due to the correlation with the total dry mass. The accessions BGF-016 and BGF-015 are the most promising ones to be taken up by a *Stylosanthes* plant breeding program for fodder in the Brazilian Semiarid region.

Conflict of Interests

The authors have not declared any conflict of interests.

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