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Diversity among elephant grass genotypes using Bayesian multi-trait model

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ABSTRACT. Elephant grass is a perennial tropical grass with great potential for energy generation from biomass. The objective of this study was to estimate the genetic diversity among elephant grass accessions based on morpho-agronomic and biomass quality traits and to identify promising genotypes for obtaining hybrids with high energetic biomass production capacity. The experiment was installed at experimental area of the State Agricultural College Antônio Sarlo, in Campos dos Goytacazes. Fifty-two elephant grass genotypes were evaluated in a randomized block design with two replicates. Components of variance and the genotypic means were obtained using a Bayesian multi-trait

model. We considered 350,000 iterations in the Gibbs sampler algorithm for each parameter adopted, with a warm-up period (burn-in) of 50,000 Iterations. For obtaining an uncorrelated sample, we considered five iterations (thinning) as a spacing between sampled points, which resulted in a final sample size 60,000. Subsequently, the Mahalanobis distance between each pair of genotypes was estimated. Estimates of genotypic variance indicated a favorable condition for gains in all traits. Elephant grass accessions presented greater variability for biomass quality traits, for which three groups were formed, while for the agronomic traits, two groups were formed. Crosses between Mercker Pinda México x Mercker 86-México, Mercker Pinda México x Turrialba, and Mercker 86-México x Taiwan A-25 can be carried out for obtaining elephant grass hybrids for energy purposes.

Key words: *Pennisetum purpureum*; Biomass production; Bioenergy; Biomass quality

INTRODUCTION

Elephant grass (*Pennisetum purpureum* Schum.) is a perennial tropical grass of great importance, whose morphological characteristics present wide variability. It is a highly efficient species in the atmospheric CO_2 fixation, producing more than 60 mg/ha per year (Morais et al., 2009), besides having fast vegetative growth (Gonçalves et al., 2012). Due to being a species with high potential for dry matter production and high photosynthetic efficiency, elephant grass has quantitative traits that favor the use of its vegetal biomass for an alternative energy source.

Vegetable biomass as an energy input has been gaining importance in the development of alternative energies in the energy matrix (Nass et al., 2007). Several crops have been evaluated for the energy generation from biomass, such as sugarcane, sorghum, maize, wheat, triticale, and *Panicum virgatum* L. (Boehmel et al., 2008; David and Ragauskas, 2010; Ra et al., 2012). In this sense, elephant grass stands out in the generation of thermal energy by the biomass combustion, because it has adequate quality characteristics and high biomass production (Morais et al., 2009; Ra et al., 2012; Fontoura et al., 2015).

Embrapa Gado de Leite conserves an elephant grass germplasm bank with 160 accessions, resulting from the introduction of materials from several Brazilian collections. The characterization and evaluation of the germplasm regarding biomass quality may favor its use as a bioenergy source. In this sense, genetic diversity analysis among elephant grass genotypes are important, since the selection of parents for breeding programs and the management of variability in germplasm banks depends on the availability of accurate information on the degree of genetic divergence between the accessions (Pereira et al., 2008).

Currently, some research has been conducted to estimate the diversity among elephant grass genotypes based on morphological and agronomic (Shimoya et al., 2001; Shimoya et al., 2002; Rocha et al., 2017), cytogenetics (Techio et al., 2002) and molecular traits (Daher et al., 2002; Passos et al., 2005; Pereira et al., 2008; Azevedo et al., 2012; Wanjala et al., 2013). However, studies on genetic diversity in elephant grass for bioenergy purposes based on biomass quality are scarce in the literature.

The objective of this study was to estimate the genetic diversity between elephant grass accessions based on morpho-agronomic and biomass quality traits and to identify promising genotypes for obtaining hybrids with high energetic biomass production capacity.

MATERIAL AND METHODS

Deployment of the trial

Trial was installed at experimental area of the State Agricultural College Antônio Sarlo, in Campos dos Goytacazes, RJ, under the geographical coordinates 21°44'47"S and 41°18'24"W, at 12 m in altitude. The region climate, according to Köppen classification, is Aw, hot and humid tropical with dry season in winter and rainy in summer and average annual rainfall of 1152 mm. The soil in the area was classified as Dystrophic Yellow Argisol.

Trial was composed of 52 elephant grass genotypes from the Active Germplasm Bank of Elephant Grass (BAGCE) of the Embrapa Gado de Leite, located in Coronel Pacheco-MG (Table 1). The planting was carried out on March 28, 2008, by means of stem pieces, distributed in furrows of 10 cm depth. At planting, 100 kg/ha of P_2O_5 were added. Irrigation was only offered during emergence of the plants, and at 50 days after planting (DAP) a fertilization with 25 kg/ha of $(NH_4)_2SO_4$ and KCl was done.

Experimental design was a randomized block design with two replicates. Experimental plot was composed of two lines of 3 m spaced 0.5 m and 2 m between plots, considering as useful only 1.5 m of one of the lines, discarding the ends of each line. At 90 DAP, The genotypes were submitted to the uniformization cut (cutting close to the soil) followed by a cover fertilization with 25 kg/ha of $(NH_4)_2SO_4$ and KCl. The first cut for biomass quality analysis was conducted in May 2009, at the end of the rainy season.

Evaluated traits

The following morphoagronomic traits were evaluated: fresh weight per meter (FWM, kg/m), dry matter percentage (%DM), dry weight per meter (DWM, kg/m), number of plants per meter (NPM), plant height (PH, m), and stalk diameter (SD, cm). For the analysis of biomass quality traits, dry material (leaf and stalk) was milled in a Willey mill with a 1-mm sieve and packed in an identified glass bottle. Biomass quality traits evaluated were: acid detergent fiber percentage (%ADF); cellulose percentage (%CEL); lignin percentage (%LIG); ash percentage (%ASH); total nitrogen content (NC); and calorific value (PC), determined by the complete sample oxidation and measured by adiabatic calorimeter IKA model C-200. All biomass quality analyzes were performed according to the Van Soest methodology described in Silva and Queiroz (2002).

Statistical analysis

Components of variance and covariance were obtained using the following Bayesian multi-trait model:

$$Y_{ijk} = \mu_i + b_{ij} + g_{ik} + \varepsilon_{ijk}$$

where in Y_{ijk} is the phenotypic value of the i-th trait evaluated in the j-th block and in the k-th

Table 1. Commercial name, code in the Active Germplasm Bank of Elephant Grass (BAGCE) and origin of the 52 elephant grass genotypes of the Embrapa Gado de Leite (CNPGL) evaluated in Campos dos Goytacazes, RJ.

Code in the BAGCE	Commercial name	Origin
BAGCE -1	Elefante de Colômbia	IPEACO - Colômbia
BAGCE -2	Mercker	IPEACO -Água Limpa - MG
BAGCE -3	Três Rios	UFRRJ - Seropédica -RJ
BAGCE -4	Napier Volta Grande	UFRRJ - Seropédica -RJ
BAGCE -5	Mercker Santa Rita	UFRRJ - Seropédica -RJ
BAGCE -6	Pusa Napier nº 2	Índia
BAGCE -7	Gigante de Pinda	Pindamonhangaba - SP
BAGCE -8	Napier nº 2	Goiás
BAGCE -9	Mercker S.E.A.	UFRRJ - Seropédica - RJ
BAGCE -10	Taiwan A-148 UF	UFRRJ - Seropédica - RJ
BAGCE -11	Porto Rico 534-B	UFV - Viçosa - MG
BAGCE -12	Taiwan A-25	UFRRJ - Seropédica - RJ
BAGCE -13	Albano	Colômbia
BAGCE -14	Híbrido Gig. Da Colômbia	Colômbia
BAGCE -15	Pusa Gigante Napier	Índia
BAGCE -16	Elefante Híbrido 534-A	UFV - Viçosa-MG
BAGCE -17	Costa Rica	Turrialba, Costa Rica
BAGCE -18	Cubano Pinda	UFRRJ - Seropédica -RJ
BAGCE -19	Mercker Pinda	UFRRJ - Seropédica -RJ
BAGCE -20	Mercker Pinda México	UFRRJ - Seropédica-RJ
BAGCE -21	Mercker 86 - México	Colômbia
BAGCE -22	Taiwan A-144	UFRRJ - Seropédica -RJ
BAGCE -23	Napier S.E.A.	UFRRJ - Seropédica -RJ
BAGCE -24	Taiwan A-143	UFRRJ - Seropédica -RJ
BAGCE -25	Pusa Napier nº 1	UFRRJ - Seropédica -RJ
BAGCE -26	Elefante de Pinda	Colômbia
BAGCE -27	Mineiro	IPEACO -Sete Lagoas - MG
BAGCE -28	Mole de Volta Grande	Volta Grande - SP
BAGCE -29	Porto Rico	IPEACO -Sete Lagoas - MG
BAGCE -30	Napier	Pedro Leopoldo-MG
BAGCE -31	Merckeron Comum	Pindamonhangada-SP
BAGCE -32	Teresópolis	UFRRJ - Seropédica-RJ
BAGCE -33	Taiwan A-46 J	UFRRJ - Seropédica-RJ
BAGCE -34	Duro de Volta Grande	UFRRJ - Seropédica-RJ
BAGCE -35	Merckeron Comum Pinda	UFRRJ - Seropédica-RJ
BAGCE -36	Turrialba	UFRRJ - Seropédica-RJ
BAGCE -37	Taiwan A-146	UFRRJ - Seropédica-RJ
BAGCE -38	Cameroon - Piracicaba	ESALQ - Piracicaba
BAGCE -39	Taiwan A-121	UFRRJ - Seropédica-RJ
BAGCE -40	Vrukwona	ESALQ - Piracicaba
BAGCE -49	P-241-Piracicaba	ESALQ - Piracicaba
BAGCE -51	IAC - Campinas	UFRRJ - Seropédica-RJ
BAGCE -52	Elefante Cachoeiro de Itapemirim	UFRRJ - Seropédica-RJ
BAGCE -54	Capim Cana D'África	IPEACS - Linhares-ES
BAGCE -56	Gramafante	Colômbia
BAGCE -57	Roxo	Lavras - ESAL-MG
BAGCE -60	Guaçu/IZ.2	Nova Odessa-SP
BAGCE -64	King Grass	Cuba
BAGCE -65	Roxo Botucatu	UNESP - Botucatu
BAGCE -67	Vruckwona Africana	CENARGEM - Brasília
BAGCE -68	Cameroon	CENARGEM - Brasília
BAGCE -91	Pasto Panamá	Panamá

genotype; μ_i is the systematic effect of the overall genotypic mean for the i-th trait; b_{ij} is the systematic effect of the j-th block on the i-th trait; g_{ik} is the random effect of the i-th trait on the k-th genotype; ε_{ijk} is the random error associated with observation Y_{ijk}. Under the Bayesian approach, the following joint data distribution (likelihood function) was adopted:

$$y_{ijk} \mid \beta, g, G_0, R_0 \sim N\left(x'_{ij}\beta + z'_{ik}g, \hat{\sigma}_e^2\right)$$

Where in: β is the vector of the *a priori* probability of the systematic effects (overall mean and blocks effects); g = (g$_{ik}$) ~ N(0, A×G$_0$) is the vector of the *a priori* probability of genotypic values where A is the relationship matrix between the genotypes and $G_0 = \begin{bmatrix} \hat{\sigma}_{a1}^2 & \cdots & \hat{\sigma}_{a1i} \\ \vdots & \ddots & \vdots \\ \hat{\sigma}_{ai1} & \cdots & \hat{\sigma}_{ai}^2 \end{bmatrix}$ is the genotypic covariance matrix; $\hat{\sigma}_e^2 = (\varepsilon_{ijk}) \sim N(0, I \times R_0)$ is the *a priori* probability vector of residual values, where I is an identity matrix and $R_0 = \begin{bmatrix} \hat{\sigma}_{e1}^2 & \cdots & \hat{\sigma}_{e1i} \\ \vdots & \ddots & \vdots \\ \hat{\sigma}_{ei1} & \cdots & \hat{\sigma}_{ei}^2 \end{bmatrix}$ is the residual covariance matrix taken as homogeneous; x'$_{ij}$ and z'$_{ik}$ are the incidence matrices associated with the systematic and random effects, respectively.

For the systematic effects vector, we assumed the following *a priori* normal distribution: β ~ N(b$_0$, V$_b$) where is a diagonal matrix for the β variance, assuming V$_b$ → . For the genotypic (G$_0$) and residual (R$_0$) covariance matrices, Wishard distribution inverted *a priori* will be assumed: G$_0$ ~ W$_3^{-1}$ ($\sum_g$, n) and R$_0$ ~ W$_3^{-1}$ ($\sum_e$, n) where in $\sum_g$ and $\sum_e$ are scaled matrices and n is the degree of freedom of the parameter. *A posteriori* distribution for all parameters, which are dependent on the genotypic effects of the corresponding matrix but $p(\beta, g, G_0, R_0 \mid y) \propto p(y \mid \beta, g, G_0, R_0) p(\beta \mid b_0, V_b) p(g \mid A \otimes G_0) p(G_0 \mid \sum_g, n) p(R_0 \mid \sum_e, n)$.

As Gibbs sampler is an iterative algorithm, it will be necessary to verify its convergence, which in this work will be verified by applying the Heidelberger and Welch (1983), Geweke (1992) and Raftery and Lewis (1992) criteria.

From the *a posteriori* β (means vector) and R$_0$ (residual covariances matrix) estimates, Mahalanobis distance between each pair of genotypes was estimated, defined by:

$$D_{ii'}^2 = \delta' R_0^{-1} \delta$$

Where in: $D_{ii'}^2$ is the Mahalanobis distance between the genotypes *i* and *i'*; ψ: residual variance and covariance matrix; $\delta' = [d_1 d_2 ... d_v]$, being d$_j$ = $Y_{ij} - Y_{i'j'}$

Where in: Y_{ij} is the measurement of the i-th genotype in relation to j-th variable.

The relative importance of evaluated traits for genetic dissimilarity was made through the D² components relating to each trait in the total dissimilarity observed, using the criteria proposed by Singh (1981) based on statistic S.j', considering, in this case, that:

$$D_{ii'}^2 = \delta' R_0^{-1} \delta = \sum_{j=1}^n \sum_{j'=1}^n \omega_{jj'} d_j d_{j'}$$

where in $\omega_{jj'}$ is the element of the j-th line and j-th of the inverse column of the residual

variance and covariance matrix (R_0). All statistical analyses were performed with the GENES (Cruz, 2013) and R softwares (R Development, 2015).

RESULTS AND DISCUSSION

We considered 350,000 iterations in the Gibbs sampler algorithm for each parameter adopted, with a warm-up period (burn-in) of 50,000 iterations. To obtain an uncorrelated sample, we considered a spacing of five iterations (thinning) between the sampled points, which resulted in a final sample size 60,000. The Heidelberger and Welch (1983), Geweke (1992) and Raftery and Lewis (1992) criteria indicated the convergence of all chains for all parameters. Then, *posteriori* mean estimates were obtained for the components of variance and genotypic values used to estimate the genetic diversity among elephant grass genotypes (Table 2). Estimates of genotypic variance indicated a favorable condition for gains in all traits, which allows the identification of superior genetic material and the achievement of considerable gains with its introduction into a breeding program (Shimoya et al., 2002). Thus, identifying genotypes with satisfactory agronomic performance and biomass quality trait that qualify them as suitable for the bioenergy production is essential for a breeding program, since allied to the information on the genetic diversity of the genotypes, it contributes to the targeting of crosses aiming at obtaining individuals with high energetic potential.

Genetic diversity as the morpho-agronomic traits

Based on the Tocher clustering, it is possible to verify that genotype 21 (Mercker 86-México) is the only one that diverges from the other genotypes regarding the morpho-agronomic traits, which allocated it into group II (Table 3). All other genotypes were allocated into group I, allowing the inference that these materials are genetically similar in relation to the morpho-agronomic traits evaluated. In the same way, by Ward's method two groups were formed, where the genotype Mercker 86-México was also allocated alone into a group and the other genotypes constituted a single group (Figure 1).

The genotype Mercker 86-México presented the highest means for %DM (52%) and DWM (5.44). According to Tew and Cobill (2009), sugarcane breeding programs for energy purposes aim to obtain cultivars containing 12% fiber and 75% water. The results obtained for the Mercker 86-México genotype show its superiority for the biomass production, since the mean %DM obtained for the genotype was 52%, i.e., 48% water. Among the genotypes from group I with better agronomic performance are genotype 36 (Turrialba), which presented higher %DM (44%), and genotype 60 (Guaçu/IZ.2), with higher means of FWM (14.47) and DWM (5.06). Based on these results, crosses between Mercker 86-México x Turrialba and Mercker 86-México x Guaçu/IZ.2 are promising for obtaining superior individuals for biomass production.

Genetic diversity as biomass quality traits

Biomass quality properties (calorific value and fiber, cellulose, lignin, and nitrogen contents) can influence the whole thermal biomass conversion process (Prochnow et al., 2009; Knoll et al., 2012; Na et al., 2016). It is important to mention that the biomass used as thermal energy source in the combustion process must have high concentrations of lignin and

cellulose, high dry matter (i.e., low moisture contents) and calorific value, and low levels of ash and nitrogen (Jaradat, 2010; Na et al., 2016).

Table 2. Means of the traits fresh weight plant[-1] (FWM, kg/m), dry matter percentage (%DM), dry weight plant[-1] (DWM, kg/m), number of plants m[-1] (NPM), plant height (PH, m), stalk diameter (SD, cm), acid detergent fiber percentage (%ADF), cellulose percentage (%CEL), lignin percentage (%LIG), ash percentage (%ASH), total nitrogen content (NC) and calorific value (PC) evaluated in 52 elephant grass genotypes from Active Germplasm Bank of Elephant Grass (BAGCE) of the Embrapa Gado de Leite.

| Genotype	Variable											
	FWM	%DM	DWM	NPM	PH	SD	% CEL	%LIG	%ASH	%ADF	NC	CAL
1	9.45	0.39	3.61	46.63	3.41	11.18	35.29	9.68	0.03	48.24	0.52	3901.04
2	8.73	0.36	3.13	33.97	3.65	12.04	34.42	8.59	0.03	46.37	0.51	3897.44
3	10.65	0.35	3.74	40.80	3.33	12.35	33.55	8.50	0.04	46.20	0.67	3894.47
4	9.57	0.38	3.58	44.82	3.29	11.85	34.47	9.12	0.03	46.36	0.67	3904.34
5	9.72	0.36	3.48	56.91	2.95	11.20	34.76	8.15	0.04	46.46	0.65	3905.28
6	10.45	0.37	3.83	46.83	3.46	11.77	35.56	8.27	0.04	47.46	0.56	3901.61
7	10.27	0.36	3.70	57.99	3.20	10.25	34.66	9.08	0.04	47.81	0.65	3904.19
8	8.09	0.40	3.19	32.74	3.39	12.20	34.38	8.89	0.04	47.16	0.53	3890.81
9	8.09	0.40	3.14	32.64	3.47	11.99	34.58	9.23	0.03	46.99	0.52	3892.66
10	8.19	0.34	2.79	48.92	3.19	11.06	33.74	8.97	0.02	45.31	0.55	3901.71
11	11.25	0.38	4.24	44.55	3.59	11.27	34.63	9.39	0.03	47.05	0.55	3897.64
12	10.52	0.36	3.65	35.02	3.62	13.54	35.13	9.23	0.02	46.78	0.54	3904.11
13	9.74	0.38	3.69	18.91	3.80	14.19	34.83	8.28	0.05	48.22	0.48	3884.48
14	8.66	0.37	3.12	47.71	3.20	11.90	34.76	9.05	0.03	46.48	0.55	3906.78
15	6.89	0.37	2.59	37.98	3.09	10.65	33.53	9.53	0.03	45.95	0.60	3892.00
16	10.42	0.37	3.88	54.38	3.22	12.47	34.52	9.22	0.04	47.87	0.64	3905.65
17	8.24	0.38	3.09	42.82	2.94	11.53	34.14	10.37	0.03	47.95	0.60	3898.14
18	11.14	0.37	4.10	23.08	3.56	13.64	33.53	8.80	0.03	45.71	0.58	3887.45
19	10.60	0.37	3.83	50.13	3.18	11.96	34.88	8.78	0.04	47.24	0.59	3907.57
20	12.62	0.35	4.37	39.30	3.44	12.91	35.56	8.82	0.14	57.72	0.57	3900.96
21	10.76	0.52	5.44	43.76	3.45	11.76	35.46	8.76	0.04	47.73	0.56	3903.52
22	9.99	0.37	3.73	41.55	3.49	10.60	34.38	9.27	0.03	46.60	0.50	3897.21
23	8.59	0.39	3.31	45.71	3.20	10.81	34.44	9.48	0.03	47.25	0.58	3898.72
24	9.68	0.38	3.61	45.84	3.31	11.11	33.85	9.46	0.04	47.39	0.60	3896.96
25	8.97	0.37	3.30	24.09	3.63	14.32	33.37	9.59	0.03	46.11	0.52	3887.24
26	8.66	0.37	3.22	52.64	3.26	11.28	35.50	9.90	0.03	48.36	0.57	3901.63
27	8.24	0.36	3.01	39.87	3.12	10.72	35.32	8.73	0.04	47.69	0.54	3891.75
28	9.28	0.36	3.33	66.35	3.29	10.16	36.56	9.07	0.03	48.46	0.65	3912.03
29	10.07	0.37	3.81	92.78	2.85	8.83	35.72	9.51	0.03	48.70	0.71	3917.01
30	9.12	0.38	3.39	45.29	3.38	10.72	36.64	9.44	0.03	48.72	0.56	3901.36
31	7.73	0.35	2.68	59.70	2.95	9.18	35.14	8.35	0.03	46.48	0.73	3910.11
32	8.82	0.36	3.21	79.50	2.93	8.26	36.26	9.19	0.03	48.24	0.70	3915.21
33	9.79	0.40	3.86	43.01	3.27	10.39	34.96	9.31	0.03	47.72	0.53	3898.05
34	8.67	0.39	3.34	43.33	3.45	11.29	36.10	7.22	0.07	49.68	0.52	3892.71
35	9.67	0.36	3.50	84.12	2.99	8.77	37.06	7.84	0.03	47.69	0.71	3918.60
36	7.94	0.44	3.46	23.96	3.35	13.31	33.49	11.27	0.03	47.97	0.51	3884.97
37	11.00	0.36	3.90	57.55	3.49	11.53	35.68	8.59	0.04	47.65	0.58	3903.70
38	9.92	0.34	3.37	33.86	3.44	13.28	35.26	7.95	0.03	46.20	0.58	3892.73
39	10.05	0.38	3.83	72.40	3.16	9.71	34.60	9.36	0.04	47.75	0.68	3908.42
40	8.15	0.36	2.96	27.22	3.46	12.57	34.38	8.60	0.03	46.10	0.59	3890.09
49	13.72	0.34	4.48	41.23	3.52	13.73	35.90	8.29	0.04	47.97	0.58	3888.85
51	9.64	0.37	3.44	25.30	3.25	13.36	34.90	7.78	0.04	46.26	0.58	3891.50
52	8.35	0.39	3.30	53.22	3.09	11.15	35.36	9.65	0.04	48.56	0.56	3895.54
54	11.72	0.35	4.05	38.26	3.33	13.06	34.10	8.24	0.03	45.53	0.62	3897.48
56	6.77	0.37	2.60	53.25	3.02	10.29	35.20	8.91	0.03	46.61	0.55	3900.55
57	10.69	0.34	3.59	27.25	3.54	13.76	33.41	9.25	0.03	45.81	0.64	3889.32
60	14.47	0.35	5.06	38.87	3.59	14.14	35.81	8.13	0.05	48.85	0.62	3886.49
64	9.11	0.36	3.31	32.32	3.47	12.22	34.66	8.89	0.03	46.39	0.62	3892.90
65	9.00	0.39	3.55	22.49	3.70	14.03	33.63	8.06	0.04	45.59	0.57	3879.31
67	9.88	0.36	3.57	55.07	3.35	12.19	35.63	8.79	0.03	47.67	0.58	3902.59
68	9.41	0.35	3.30	27.27	3.41	13.42	34.23	7.20	0.04	45.03	0.59	3890.62
91	11.26	0.37	4.31	28.30	3.63	13.84	34.98	9.88	0.03	47.91	0.51	3892.24

Similarly, to the clustering for the morpho-agronomic traits, both the Tocher and Ward clustered the genotypes into two groups (Table 4 and Figure 2). Group II consisted only of

genotype 20 (Mercker Pinda México), while the other genotypes were allocated into group I. The genotype Mercker Pinda México presented the highest means for %CEL and %ADF (35.56 and 57.72%, respectively), which may explain the fact that this genotype is divergent from the others based on the biomass quality traits. Thus, promising crosses to obtain transgressive segregating individuals would be those that meet parents with high energy potential and high production, such as Mercker Pinda México x Mercker 86-México and Mercker Pinda México x Turrialba.

Table 3. Tocher cluster applied to Mahalanobis distance of 52 elephant grass genotypes based on morpho-agronomic traits.

| Group	Genotypes
I	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 and 52
II	21
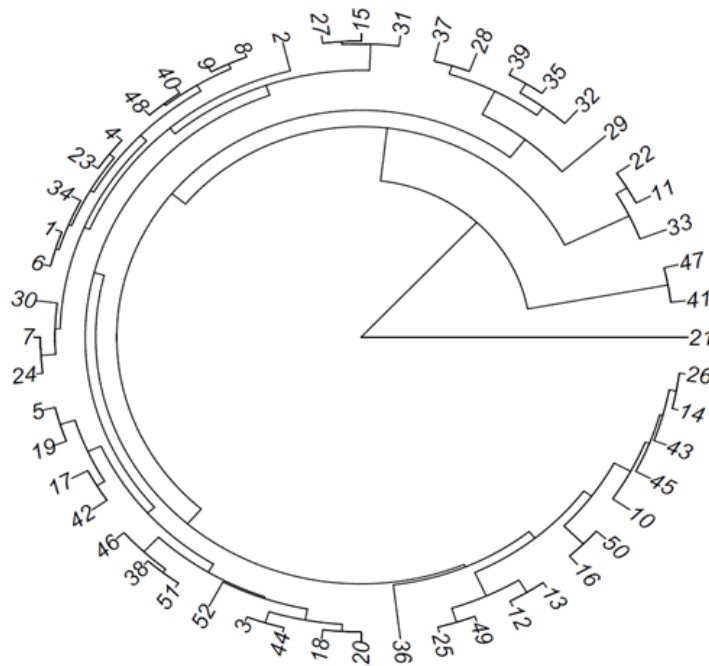
Figure 1. Ward cluster applied at Mahalanobis distance from 52 elephant grass genotypes based on morpho-agronomic traits.

Table 4. Tocher cluster applied to Mahalanobis distance of 52 elephant grass genotypes based on biomass quality traits.

Group	Genotypes
I	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 and 52
II	20
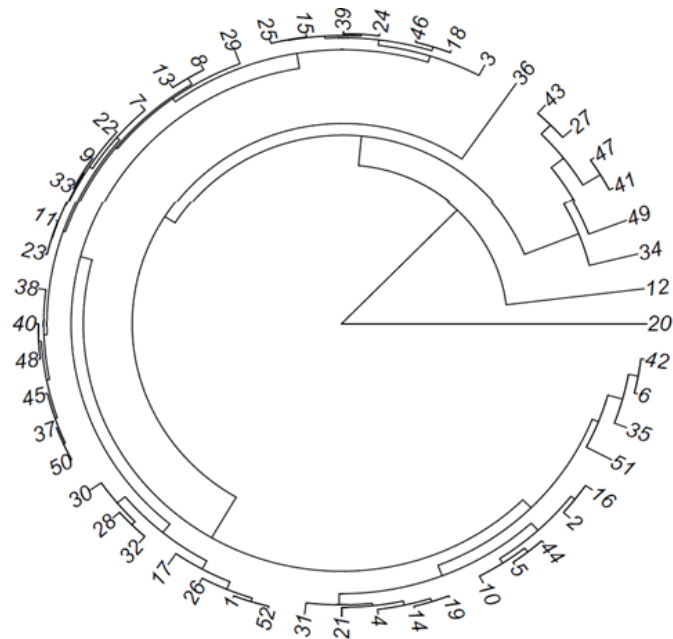
Figure 2. Ward cluster applied at Mahalanobis distance from 52 elephant grass genotypes based on biomass quality traits.

## Genetic diversity as morpho-agronomic biomass quality traits

Tocher clustering based on morpho-agronomic and biomass quality traits allowed the formation of four groups (Table 5). Groups II, III and IV were composed by only one genotype each, constituted by genotypes 12, 20 and 21, respectively. The remaining genotypes were allocated into group I. Although the genotypes differed little in relation to the values of CAL, whose overall mean is 3898.23 cal/g, the genotype 12 (Taiwan A-25), besides presenting satisfactory values of CAL (3904.11 cal/g), it also obtained the lowest %ASH (0.022), Which is a good property for energy use. According to Rossi et al. (2014), biomass production and calorific value are correlated with fiber and ash content, and it is desirable to select materials that have high yield and fiber content, but with low ash content.

Table 5. Tocher cluster applied to Mahalanobis distance of 52 elephant grass genotypes based on morpho-agronomic and biomass quality traits.

Group	Genotypes
I	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 and 52
II	12
III	20
IV	21

Three groups were formed by the Ward cluster, in which group II was formed by genotype 20 (Mercker Pinda México), group III by genotype 21 (Mercker 86-México) and the group I by the other genotypes (Figure 3). Again, by both cluster methods, it is possible to verify that the genotypes Mercker Pinda México and Mercker 86-México are divergent among

themselves and from the others as to morpho-agronomic and biomass quality traits. This fact is confirmed by observing the mean values of the evaluated traits, where Mercker 86-Mexico obtained high means for the agronomic traits %DM and DWM, while Mercker Pinda México presented higher means for the industrial traits %CEL and %ADF.
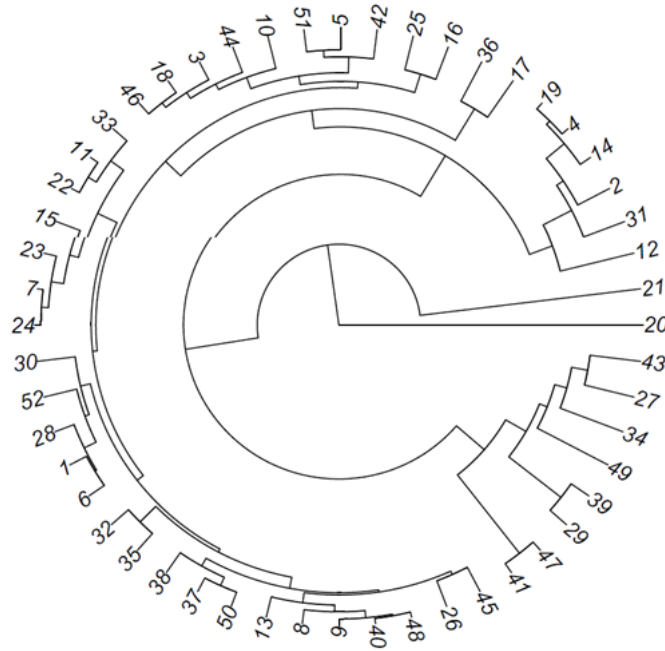
Figure 3. Ward cluster applied at Mahalanobis distance from 52 elephant grass genotypes based on morpho-agronomic and biomass quality traits.

These results differ from those found in the literature, where a classification of the elephant grass germplasm variability is reported in four morphological groups: Napier, Merker, Cameroon, Anão, and interspecific hybrids (Pereira, 1992), or a division considering clustering results based on molecular analysis with the formation of two groups, where group I is constituted by morphologically closer accesses from the cultivars Napier and Merker, while group II presents greater morphological identification with the cultivar Cameroon (Passos et al., 2005; Pereira et al., 2008). From the results obtained in this study, the genotypes Mercker Pinda Mexico and Mexico-86 Mercker do not belong to the same group where Napier's access based on the biomass quality and morpho-agronomic traits have been allocated.

Rocha et al. (2017), evaluating 100 elephant grass accessions from BAGCE regarding the genetic diversity for bioenergy production, verified that they have potential for use as a bioenergetic feedstock, and that the accessions presented variability for the biomass quality traits higher than the morpho-agronomic traits, like the results obtained in this study.

Choosing parents should be made based on allelic complementarity and genetic divergence. Thus, crosses between divergent individuals belonging to different groups constitute the best way to obtain superior clones of elephant grass. Based on the Tocher and Ward clusters, it was possible to obtain information on the genetic variability between the

accessions for agronomic traits and biomass quality, which together with the identification of superior genotypes for traits of interest, contributes to the definition of selection strategies of potential parents in breeding programs. Therefore, crosses between Mercker Pinda México x Mercker 86-México, Mercker Pinda México x Turrialba and Mercker 86-México x Taiwan A-25 can be carried out to obtain elephant grass hybrids for bioenergy production.

Contribution of morpho-agronomic and biomass quality traits to genetic diversity

Analysis of the relative importance of agronomic and biomass quality traits to genetic diversity based on the Singh (1981) criterion indicated that the CAL was the trait that contributed most to the genetic diversity between the accessions, with a relative importance of 36.86% (Figure 4). This shows that the genotypes present variability as to the potential of use as raw material for combustion. The %ASH could have been discarded, since their relative importance is equal to 0. The %ADF (9.77%) had the second highest relative importance followed by NPM, %DM, %LIG and FWM, whose contributions to the genetic diversity were very similar. The other traits presented low contribution for discriminating the genotypes.
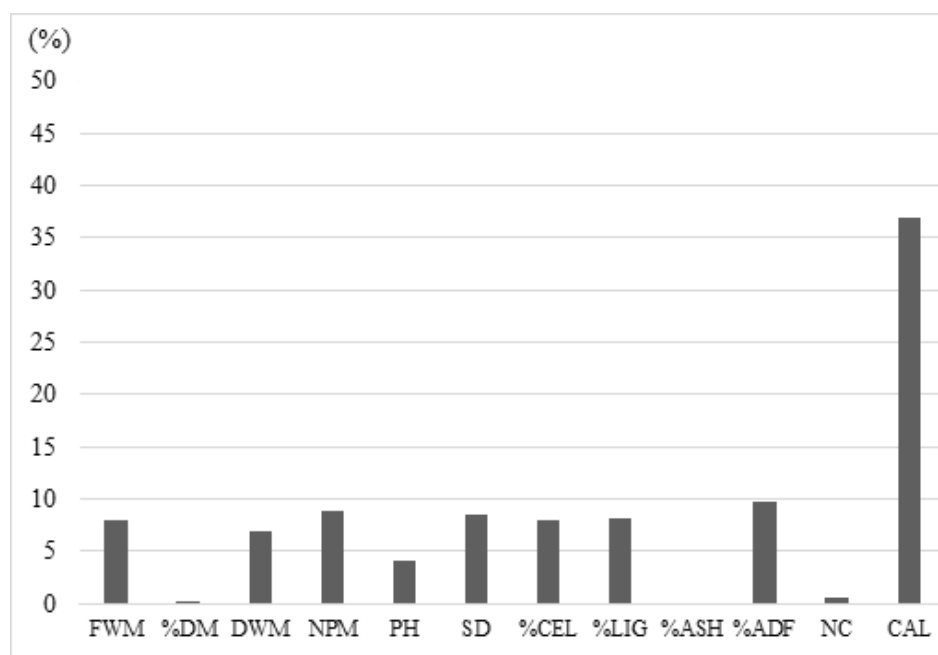
Figure 4. Relative contribution of agronomic and biomass quality traits to the genetic diversity of 52 elephant grass genotypes based on the Singh (1981) criterion.

Thus, it is possible to verify that most of the genetic variability among the genotypes is due to the differences in the traits expression related to the production of biomass. Therefore, the accesses evaluated have potential for use in elephant grass breeding programs for bioenergy purposes.

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