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Bioenergetic potential and genetic diversity of elephantgrass *via* morpho-agronomic and biomass quality traits

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ABSTRACT

Elephantgrass has been a notable option as bioenergy plant. However, for its bioenergetic use, the quantification of genetic diversity based on biomass quality traits has not been commonly reported in the literature. The objective of this study was to quantify the genetic diversity among 100 accessions of the Active Elephantgrass Germplasm Bank (BAGCE), by means of morphological (flowering, height, vigor and stalk diameter), agronomic (total dry biomass) and biomass quality traits (dry matter concentration, cellulose, lignin, hemicellulose, *in vitro* digestibility, nitrogen, ash, and calorific value), and the ultimate goal was to use the elephantgrass as a bioenergy feedstock. By using mixed model methodology and genetic diversity analyses, it was found genetic variability between elephantgrass accessions, which is the basic premise to start any breeding program. The BAGCE presented greater genetic variability for the biomass quality traits, when compared with morpho-agronomic traits. The accessions were divided into 6 clusters of genetic similarity, with potential for use in second generation ethanol production and direct biomass combustion, besides forage uses. Furthermore, to potentiate elephantgrass as bioenergetic plant, crosses among divergent individuals from distinct clusters were recommended. Thus, the genetic variability of BAGCE can be exploited to produce superior combinations that can maximize second generation ethanol conversion and biomass direct combustion. In addition, these actions can increase the contribution of elephantgrass for a sustainable energetic matrix diversification.

1. Introduction

Elephantgrass (*Pennisetum purpureum* Schum.) has been a notable option for bioenergy production. The species, which is traditionally used as forage plant, has attracted considerable attention as one of the promising crops for use as bioenergetic feedstock, especially for its photosynthetic efficiency (C4 carbon fixation mechanism), high biomass production, longevity, rapid growth, broad adaptation, in addition to its chemical properties (Anderson et al., 2008; Morais et al., 2009; Zeng-Hui and Hong-Bo, 2010; Ra et al., 2012; Fontoura et al., 2015), and for its biological nitrogen fixation ability, since its contributions ranges from 18 to 70% of the nitrogen used by the plant (Morais et al., 2009, 2012).

Many of the cultivars which are currently in use were selected for animal feed, placing an emphasis on high percentage of leaves, high nitrogen concentration, and low fiber levels. Biomass production was often a secondary factor in regards to obtaining increased nutritional quality (Rengsirikul et al., 2013). On the other hand, for bioenergy production, the objective is to obtain the maximum biomass yield, with adequate quality for direct combustion or for biofuel conversion (Strezov et al., 2008; Prochnow et al., 2009; Naik et al., 2010; Na et al., 2016a).

The biomass used as a source of thermal energy in the combustion process should present high concentrations of lignin and cellulose (Gani and Naruse, 2007), high carbon/nitrogen ratio,

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high calorific value, and low levels of moisture, ash, and nitrogen (McKendry, 2002; Long et al., 2006; Jaradat, 2010). For the production of cellulosic ethanol, high cellulose/lignin ratio, and high hemicellulose content are desirable to provide high ethanol production per ton of biomass. Moreover, in the fermentative processes, it is desirable that the biomass presents high concentration of carbohydrates with low molecular weight in unpolymerized state (Porter et al., 2007).

Embrapa Dairy Cattle Research Center maintains an Active Elephantgrass Germplasm Bank (BAGCE) with 160 accessions. Of these, 101 accessions are of the *Pennisetum purpureum*, 19 accessions are of the species of the tertiary gene *pool* of the genus (*Pennisetum* spp.), and 40 accessions are of a work collection of *P. glaucum*. The pre-breeding expansion efforts of elephantgrass, such as the activities of characterization and evaluation of the germplasm in regards to biomass quality, favor its use as a bioenergy source.

In the pre-breeding stage, genetic diversity analyses are noteworthy, and in relation to elephant grass, it should be mentioned studies of genetic diversity based on morphological and agronomic traits (Van de Wouw et al., 1999; Shimoya et al., 2001, 2002), cytogenetic traits (Techio et al., 2002), and molecular traits (Struwig et al., 2009; Harris et al., 2009; Azevedo et al., 2012; Wanjala et al., 2013; López et al., 2014). However, for bioenergetic use, the quantification of genetic diversity in elephantgrass based on biomass quality traits has not been commonly reported in the literature.

The objective of this study was to quantify the genetic diversity among elephantgrass accessions of the BAGCE, aiming at using it as bioenergetic feedstock, by means of the description of morpho-agronomic and biomass quality traits.

2. Material and methods

2.1. Experiment conduction

The experiment was carried out at the experimental field of Embrapa Dairy Cattle Research Center, located in the municipality of Coronel Pacheco, MG, Brazil (21°33′18″s, 43°15′51″W, at 417 m asl). The planting was carried out in December, 2011, in 0.20 m deep furrows, and 80 kg ha^{-1} P_2O_5 fertilizer was applied at planting. After the establishment stage, 30 days after planting, elephantgrass were cut to 0.30 m stubble height (uniformity harvest). The first of two 250 day growth periods started at this time. Maintenance fertilization was carried out with 300 kg ha^{-1} of the N-P_2O_5-K_2O formulation (20:05:20 blended granular fertilizer), after the uniformity harvest, and after the first evaluation cutting. Fertilization was carried out according to the soil analysis.

Two evaluation cuttings were carried out for this study. Aiming at using them as bioenergetic feedstock, we adopted cuttings every 250 days.

2.2. Genetic material and experimental design

One hundred accessions of the Active Elephantgrass Germplasm Bank (BAGCE) were evaluated. Plots (1.5 m × 4 m) consisted of a single 4 m row. The rows were planted side by side, spaced 1.5 m apart. Plots were allocated in a simplex lattice design, with two replications. Simple lattice design is a partially balanced lattice, a type of resolvable incomplete block design that was developed for the comparison of large number of *treatments* (clones) in agricultural experiments.

2.3. Morpho-agronomic traits

The following morpho-agronomic traits were measured: Flowering (days) – was determined by the number of days from the standardized harvest until the flowering of 50% of the experimental plot; mean height (m) – was obtained from the arithmetic mean of the height of three randomly selected plants, in each plot, measured from the ground level to the curve of the last completely expanded leaf; phenotypic vigor (1–5) – was obtained using a grading scale, which ranged from 1 to 5 (5 = high vigor; 1 = low vigor); stalk diameter (mm) – was obtained from the arithmetic mean of five plants in the useful plot, taken at random, measured at 10 cm from the ground level with a digital caliper rule; total dry biomass (Mg ha^{-1}) – was obtained from a cut at 7.5 cm stubble height in a 3 m section from the middle of rows, using a gasoline powered trimmer, and after that, it was harvested by hand. The 3 m section was immediately weighed in the field to provide estimates of fresh biomass. Total dry biomass was quantified by multiplying the fresh biomass and the dry matter concentration given as percentage.

2.4. Biomass quality traits

Before cutting the experimental plots, random samples of three complete plants from each plot were collected. Then, these samples were dried in a forced air circulation oven at 56 °C for 72 h. After drying, samples were ground (1 mm) in a Wiley type grinder and sent to the biomass analysis laboratory for the chemical analysis described below:

Cellulose (g kg^{-1}), lignin (g kg^{-1}) and hemicellulose (g kg^{-1}) – were determined following the methodology proposed by Goering and Van Soest (1967). *In vitro* digestibility of the dry biomass (g kg^{-1}) – was determined following the methodology used by Tilley and Terry (1963). Nitrogen (g kg^{-1}) – was determined following the methodology proposed by the *Association of Official Analytical Chemical* (AOAC, 1975). Ash (g kg^{-1}) – was determined according to the methodology proposed by Silva and Queiroz (2002). Calorific value (MJ kg^{-1}) – was determined using a IKA C-5000 calorimeter. Dry matter concentration (g kg^{-1}) – was obtained by the sampling of three complete plants from each plot, which were dried in a kiln after weighing (fresh weight) until weight stabilization. Samples were weighed (dry weight) again, and then the dry matter concentration was determined by the ratio between dry weight and fresh weight. This trait was used as a common denominator for the estimation of cellulose, lignin, hemicellulose, *in vitro* dry matter digestibility, nitrogen, ash, and calorific value.

2.5. Statistical analyses

Due to the structural complexity of the data (repeated measures throughout time or longitudinal data), it was adopted the mixed model statistical analyses *via* REML/BLUP (restricted residual maximum likelihood and best linear unbiased prediction), according to Patterson and Thompson (1971) and Henderson (1975).

For the deviance analysis, it was used the statistical model denoted by: $y = Xm + Zg + Wb + Ti + Op + \varepsilon$, in which

y = data vector

m = vector of the effects of the measurement-replication combination (assumed to be fixed) added to the overall mean;

g = vector of the genotypic effects (assumed to be random);

b = vector of the effects of blocks (assumed to be random);

i = vector of the effects of the genotype × measurements;

p = vector of the permanent environment (random);

ε = vector of errors or residuals (random)

X, Z, W, T, and Q represent the incidence matrices for these effects.

For the random effects of the model, the significance for the Likelihood ratio test (LRT) was tested using the chi-square test with one degree of freedom. BLUP (Best Linear Unbiased Prediction) means were estimated for each of the 100 accessions based on the 13 traits evaluated.

Table 1
Joint deviance analysis in two cuttings for the 13 traits evaluated in 100 elephant-grass accessions.

Traits	Effects	
	Clones^{a}	Clones × Cuttings^{b}
Flowering (days)	70.74^{**}	120.09^{**}
Average height (m)	15.79^{**}	22.76^{**}
Phenotypic vigor (1–5)	22.02^{**}	0.89^{NS}
Stalk diameter (mm)	23.58^{**}	11.42^{**}
Total dry biomass (Mg ha^{-1})	30.31^{**}	11.80^{**}
Dry matter concentration (g kg^{-1})	12.06^{**}	34.08^{**}
Cellulose (g kg^{-1})	5.67^{*}	4.60^{*}
Lignin (g kg^{-1})	8.61^{**}	1.86^{NS}
Hemicellulose (g kg^{-1})	1.77^{NS}	3.28^{NS}
In vitro digestibility of the dry biomass (g kg^{-1})	10.59^{**}	1.87^{NS}
Nitrogen (g kg^{-1})	12.02^{**}	0.44^{NS}
Ash (g kg^{-1})	10.03^{**}	1.79^{NS}
Calorific value (MJ kg^{-1})	12.56^{**}	2.61^{NS}

Chi-squared tabulated: 3.84 and 6.63 for levels of significance of 5% and 1%, respectively.

**,* significant to 1 and 5% respectively, by the chi-squared test.

^{NS} not significant to 5%, by the chi-squared test.

[a] Likelihood ratio test, using the chi-squared test with one degree of freedom, reduced model without clones effects.

[b] Likelihood ratio test, using the chi-squared test with one degree of freedom, reduced model without clones × cuttings effects.

The Tocher clustering method (Rao, 1952) was used in the quantification of the genetic diversity among 100 accessions of the BAGCE, based on the genetic dissimilarity matrix, obtained by the mean standardized Euclidean distance of the BLUP means. For deviance analysis, estimates of the BLUP means and dissimilarity matrix were obtained using the *software* Selegen-REML/BLUP (Resende, 2007).

The relative importance of the traits in quantifying genetic divergence was estimated based on the method proposed by Singh (1981), using the GENES *software* (Cruz, 2013).

To show the genetic diversity of BAGCE, graphical analysis dendrograms (graphical analysis) were constructed based on the hierarchical methodology of single linkage (neighbor joining), using the R program (R Development Core Team, 2015).

3. Results and discussion

3.1. Genetic variability

Significant clone effects ($p < 0.05$) were detected by the joint deviance analysis in the two cuts evaluated for the morpho-agronomic traits and for the biomass quality trait, with the exception of hemicellulose (Table 1). These results indicate elevated genetic variability among 100 accessions (clones) of the BAGCE.

Regarding the clones x cuttings interaction, it was observed significant effect ($p < 0.01$) for four (flowering, mean height, stalk diameter, and total dry biomass) of the five morpho-agronomic traits. For the traits of biomass quality, there was significant effect ($p < 0.01$) for two (dry matter concentration and cellulose level) of the eight evaluated traits (Table 1). Non-significant clones × cuttings interaction indicates coincident performance of clones in relation to the different cuttings. Therefore, it can be inferred that selection can be carried out in any of the cuttings, especially when the traits of biomass quality are taken into account. Since the clones effect, based on the means of the cuts, is given by the means of the clones effects in each cutting subtracted by the interaction effect (clones × cuttings), it may be stated that significant effect of clones in the presence of significant interaction confirms the high variability of the 100 accessions of the BAGCE.

3.2. Morpho-agronomic clustering

Considering the variability of the morpho-agronomic traits evaluated in the 100 accessions, the formation of three clusters was observed by using the Tocher method (Fig. 1). Ninety-six accessions were allocated in the first cluster. The ten most similar accessions were: Mercker S.E.A, Elefante Cachoeiro Itapemirim, Taiwan A-25, Híbrido Gigante da Colômbia, IJ 7125, Mercker 86 México, IJ 7126, 11 AD IR, Gramafante, and 05 AD IRI. According to the Tocher clustering method, the mean intracluster distance must be lower than the mean intercluster distance. The cluster criterion is given by the maximum value (θ) of the dissimilarity measure found in the cluster of smaller distances that involve each pair of individuals (Rao, 1952). Thus, accessions which were extremely divergent from the others, such as Mott (Fig. 1), affect the Tocher clustering method. This accession showed greater relative distance than the other accessions. Therefore, the reunification of the accessions classified in cluster I enabled the formation of 19 sub-clusters, which indicates high variability of these accessions.

It is worth noting that the accession Mott, uniquely assigned to cluster II (Fig. 1), presents reduced mean plant height (dwarfing genes), low biomass production, and consequently low potential for bioenergy production.

In a study with molecular markers, Struwig et al. (2009) reported that the RAPD (*Random Amplification of Polymorphic DNA*) markers were incapable of separating elephantgrass accessions in regard to mean plant height. Likewise, Azevedo et al. (2012) noted that microsatellites (SSR) markers were also inefficient in determining a clustering standard with low height accessions. This highlights the importance of using morpho-agronomic traits for the genetic diversity of elephantgrass. Nevertheless, was reported that SSR markers may be important to the development of tools for elephantgrass genetic breeding (Azevedo et al., 2012), and for managing the invasion risk of elephantgrass (López et al., 2014). The clones BAGCE 2, King Grass and BRS Capiaçu (cluster III) have higher total dry biomass production, stalk diameter, mean plant height, phenotypic vigor, and late flowering. BRS Capiaçu is more divergent than the clone Mott. Recently, the clone BRS Capiaçu has been registered and protected as cultivar by the Ministry of Agriculture, Livestock and Food Supply (MAPA). Thus, Embrapa Dairy Cattle has recommended BRS Capiaçu to farmers.

3.3. Biomass quality clustering

When considering the variability of the traits of biomass quality evaluated in 100 accessions, it was possible to distinguish 11 clusters by the Tocher optimization method. Sixty five percent of the accessions were concentrated in the first cluster, among which the ten most similar were: Kizozi, Ibitinema, IJ 7126, IJ 7139, Cameroon, Elefante Cachoeiro Itapemirim, Guaco, Guaçu, Taiwan A-121 and BRS Canará. High genetic variability was also observed (Fig. 2). Therefore, biomass quality analyses routinely used in breeding programs and in the evaluation of nutritive value of forage assist in the selection and distinction of divergent clusters, and can contribute to the characterization of elephantgrass biomass for energetic use (Ampong-Nyarko and Murray, 2011).

3.4. Morpho-agronomic and biomass quality clustering

The genetic diversity analysis among the 100 accessions based on both morpho-agronomic and biomass quality traits revealed the formation of six clusters, and 91 accessions were allocated in the first cluster (Fig. 3). Azevedo et al. (2012), while studying the genetic diversity of these accessions, by means of microsatellite molecular markers, obtained only one cluster of genetic similarity among the evaluated *P. purpureum* accessions. These results

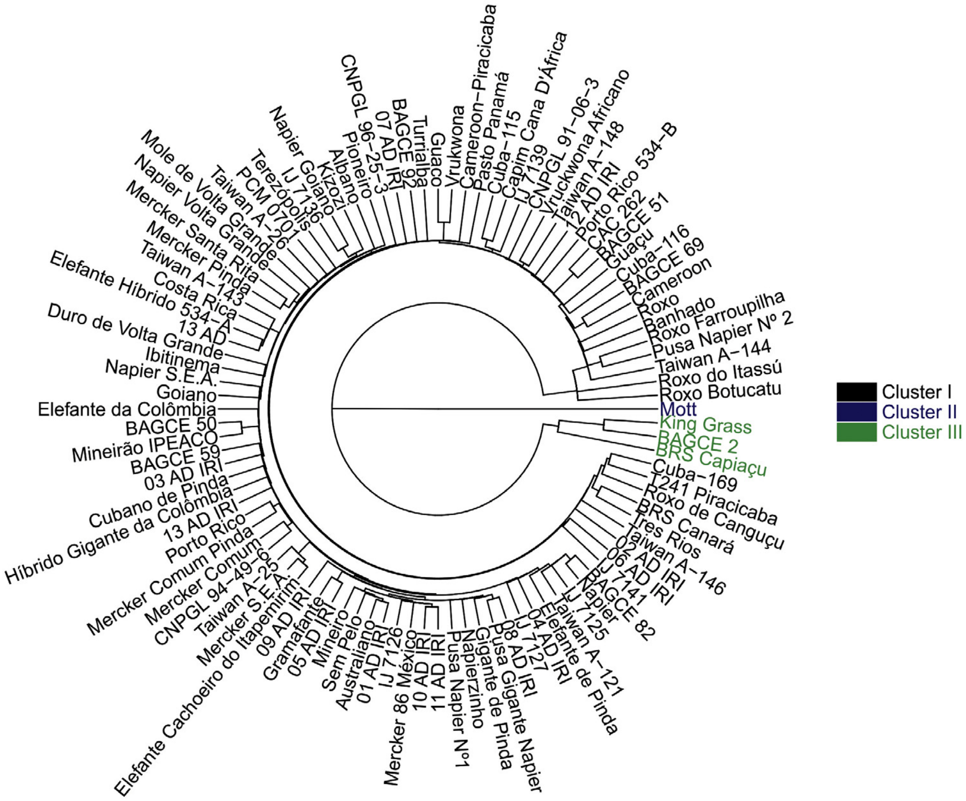

Fig. 1. Dendrogram of 100 accessions of *P. purpureum* of the BAGCE, based on the neighbor joining method, obtained from BLUP means of elephantgrass morpho-agronomic traits. Different colors represent the clusters of similar accessions formed using the Tocher clustering method.

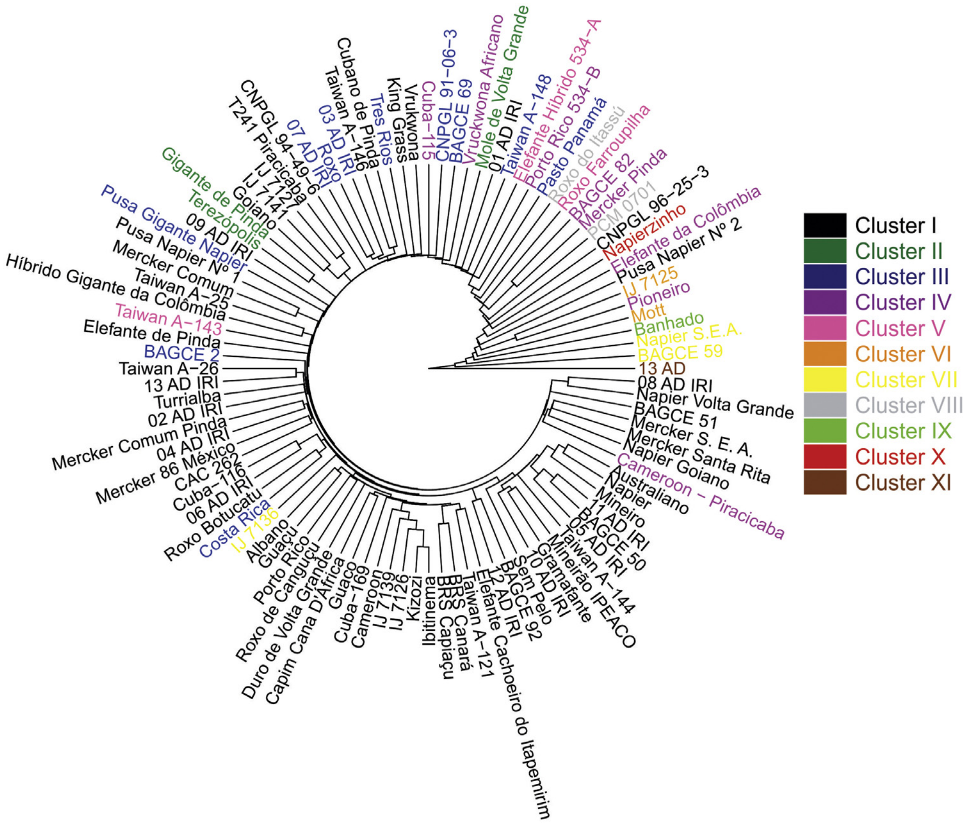

Fig. 2. Dendrogram of 100 accessions of *P. purpureum* of the BAGCE, based on the neighbor joining method, obtained from BLUP means of elephantgrass biomass quality traits. The different colors represent the clusters of similar accessions formed using the Tocher clustering method.

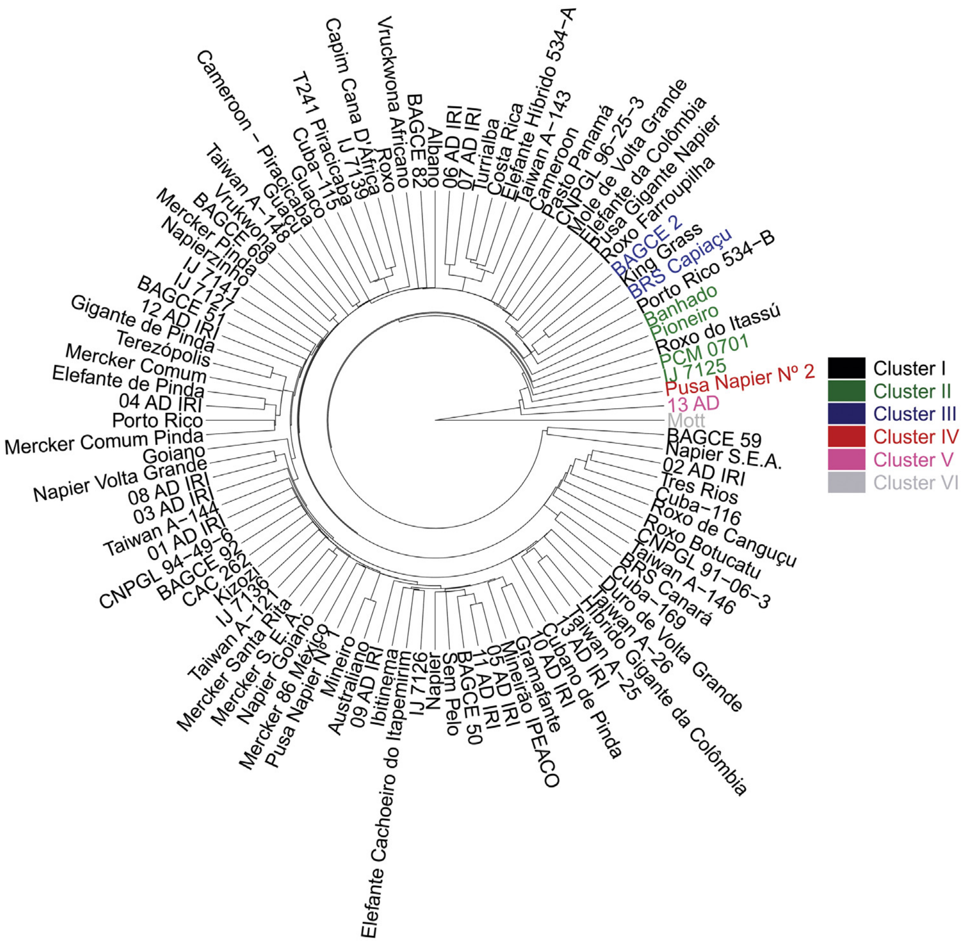

Fig. 3. Dendrogram of 100 accessions of *P. purpureum* of the BAGCE, based on the neighbor joining method, obtained from BLUP means of elephantgrass morpho-agronomic and biomass quality traits. The different colors represent the clusters of similar accessions formed using the Tocher clustering method.

indicate the importance of morpho-agronomic and biomass quality traits in the quantification of the genetic diversity of elephantgrass, since they enable the efficient separation of the genetic similarity clusters. It is worth mentioning that the properties of biomass qualities (moisture content, calorific value, and levels of cellulose, lignin and nitrogen) can influence the entire process of conversion into ethanol and the thermal utilization of the biomass (McKendry, 2002; Gani and Naruse, 2007; Prochnow et al., 2009; Jaradat, 2010; Knoll et al., 2012; Na et al., 2015, 2016a).

The ten most similar accessions in terms of morpho-agronomic and biomass quality traits were: Mineiro, Australiano, 05 AD IRI, 11 AD IRI, Napier, Gramafante, 10 AD IRI, Mineirão IPEACO, BAGCE 50 and Sem Pelo. Azevedo et al. (2012), when using microsatellite markers in the genetic diversity analysis of the 100 accessions used in this work, reported that accessions 05 AD IRI, Cubano de Pinda, 10 AD IRI and BAGCE 50 presented similarity coefficient of 0.98, indicating that these accessions could have the same genetic ancestors, and that some of them could be discarded without loss of genetic diversity.

3.5. Relative importance and discard of traits

The evaluation of the relative importance of the traits in the genetic diversity of the 100 accessions of the BAGCE, using the Singh method (1981), indicated that the five main traits were: flowering, dry matter concentration, stalk diameter, calorific value, and nitrogen level (Table 2). The rest of the traits have lower relative importance, but should not be discarded, since the withdrawal of the less important trait (average height) alters the clustering pattern of the accessions. Thus, all of the traits used in this work were considered for the quantification of the genetic diversity of the accessions. When the relative importance of morpho-agronomic traits was contrasted with the biomass quality traits, it was observed that the latter contributed with 54.10% of the total accessions discrimination. Therefore, it can be inferred that the accessions of the BAGCE presented greater genetic variability in terms of biomass quality traits, when compared with morpho-agronomic traits.

3.6. Choice of the accessions for different final uses

Table 3 presents the genotypic values of elephantgrass morpho-agronomic and biomass quality traits used in the Tocher clustering method. It was possible to identify six clusters, which are described below, based on the five principle traits (flowering, dry matter concentration, stalk diameter, calorific value and nitrogen level), which can direct the accessions to different final uses (e.g. forage, second generation ethanol production, and direct biomass combustion).

Cluster I: Accessions are characterized by presenting intermediate values for the traits: flowering, stalk diameter, dry matter concentration, nitrogen level, and calorific value. It is the cluster that presents the greatest number of accessions and the greatest intracluster diversity. The accessions of this cluster are suitable for both combustion and cellulosic ethanol production, especially those with the greatest potential for biomass production.

Table 2
Relative contribution of elephantgrass morpho-agronomic and biomass quality traits, evaluated in 100 accessions of the BAGCE, based on the Sigh method.

Traits	*S.j*	Relative importance (%)
Flowering (days)	771.83	17.88
Dry matter concentration (g kg^{-1})	538.50	12.47
Stalk diameter (mm)	495.32	11.47
Calorific value (MJ kg^{-1})	430.59	9.97
Nitrogen (g kg^{-1})	324.30	7.51
In vitro digestibility of the dry biomass (g kg^{-1})	298.57	6.92
Phenotypic vigor (1–5)	297.04	6.88
Cellulose (g kg^{-1})	252.65	5.85
Lignin (g kg^{-1})	252.31	5.84
Ash (g kg^{-1})	239.07	5.54
Total dry biomass (Mg ha^{-1})	221.34	5.13
Average height (m)	195.66	4.53

S.j, Singh statistic (1981).

Table 3
Summary of Tocher clustering, mean genotypic values for the morpho-agronomic traits (flowering – days; mean height – m; phenotypic vigor – 1 to 5; stalk diameter – mm; total dry biomass – Mg ha^{-1}) and biomass quality traits (dry matter concentration – g kg^{-1}; cellulose – g kg^{-1}; lignin – g kg^{-1}; hemicellulose – g kg^{-1}; *in vitro* digestibility of the dry biomass – g kg^{-1}; nitrogen – g kg^{-1}; ash – g kg^{-1}; calorific value – MJ kg^{-1}) of elephantgrass in different clusters, and characterization of the clusters for five principal traits.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Flowering[a]	181[g] (223[h]–154[i])	158 (168–152)	204 (210–197)	200 (200–200)	168 (168–168)	217 (217–217)
Average height	3.63 (4.08–3.27)	3.43 (3.61–3.18)	4.06 (4.11–4.01)	4.16 (4.16–4.16)	3.56 (3.56–3.56)	2.54 (2.54–2.54)
Phenotypic vigor	3.20 (3.85–2.76)	2.97 (3.17–2.84)	3.82 (3.94–3.70)	3.22 (3.22–3.22)	3.07 (3.07–3.07)	2.58 (2.58–2.58)
Stalk diameter	18.06 (22.77–14.68)	16.98 (19.99–13.98)	21.96 (22.72–21.20)	20.07 (20.07–20.07)	19.18 (19.18–19.18)	14.29 (14.29–14.29)
Total dry biomass[b]	21.34 (34.60–14.22)	15.72 (19.17–13.26)	37.23 (41.37–33.09)	15.63 (15.63–15.63)	17.38 (17.38–17.38)	11.53 (11.53–11.53)
Dry matter concetration[c]	407.212 (445.500–360.800)	397.525 (416.800–370.300)	406.600 (427.200–386.000)	419.100 (419.100–419.100)	370.700 (370.700–370.700)	391.500 (391.500–391.500)
Cellulose	412.276 (420.059–403.203)	402.262 (404.066–398.420)	414.858 (417.149–412.566)	425.563 (425.563–425.563)	414.123 (414.123–414.123)	402.352 (402.352–402.352)
Lignin	102.598 (109.223–95.623)	97.321 (101.111–93.555)	104.036 (104.958–103.114)	105.179 (105.179–105.179)	112.677 (112.677–112.677)	96.415 (96.415–96.415)
Hemicellulose	270.909 (272.376–268.404)	271.740 (272.441–271.174)	269.990 (270.548–269.432)	271.787 (271.787–271.787)	267.628 (267.628–267.628)	272.508 (272.508–272.508)
In vitro digestibility of the dry biomass	332.564 (349.380–309.004)	344.607 (355.377–332.133)	331.907 (331.995–331.818)	322.772 (322.772–322.772)	308.329 (308.329–308.329)	347.514 (347.514–347.514)
Nitrogen	4.501 (5.244–4.128)	4.799 (4.916–4.674)	4.374 (4.413 4–334)	4.270 (4.270–4.270)	4.253 (4.253–4.253)	5.148 (5.148–5.148)
Ash	66.427 (74.939–59.243)	72.113 (77.756–65.010)	62.332 (63.143–61.521)	60.615 (60.615–60.615)	60.928 (60.928–60.928)	83.407 (83.407–83.407)
Calorific value[d]	18.145 (18.320–17.945)	18.026 (18.099–17.945)	18.225 (18.261–18.189)	18.223 (18.223–18.223)	18.322 (18.322–18.322)	17.938 (17.938–17.938)
Flowering[e]	Intermediate	Early	Late	Late	Premature	Late
Stalk diameter	Intermediate	Thin	Thick	Thick	Intermediate	Very thin
Dry matter concentration	Intermediate	Intermediate	Intermediate	High	Low	Intermediate
Nitrogen	Intermediate	High	Intermediate	Low	Very low	Very high
Calorific value[f]	Intermediate	Low	Intermediate	Intermediate	High	Very low

[aandb] Morpho-agronomic traits.
[c and d] Biomass quality traits.
[e and f] Characterization of the six clusters according to the first five relative important traits.
[g,h,e and i] Average, maximum, and minimum values, respectively, of the trait within the cluster.

Cluster II: Accessions are characterized by presenting early flowering, thin stalk diameter, high nitrogen levels, and low calorific value. It is represented by accessions IJ 7125, Pioneiro, Banhado and PCM 0701. The accessions of this cluster have low aptitude for energetic biomass production, and should be directed, specially for use as forage.

Cluster III: The accessions are characterized by presenting late flowering, thick stalk diameter, and high height. It stands out for the large total dry biomass production. The cluster includes the accessions BAGCE 2 and BRS Capiaçu. As a result of the high potential for biomass production, accessions of this cluster have the ability of direct combustion and cellulosic ethanol production.

Cluster IV: The cluster is composed of only one genotype, Pusa Napier N° 2, and is characterized by late flowering, thick stalk diameter, high dry matter concentration, low nitrogen levels, and intermediate calorific value. Pusa Napier N° 2, can be used for the production of cellulosic ethanol in view of its high cellulose level.

Cluster V: The cluster is composed of one accession – 13 AD, and is characterized by premature flowering, low dry matter concentration, and very low nitrogen level. However, it has the highest calorific value and lignin level, and the lowest *in vitro* digestibility of the dry biomass. The accession of this cluster is specifically adapted to the process of biomass combustion.

Cluster VI: The cluster is formed by one individual – Mott, and is characterized by presenting late flowering, thin stalk diameter, very high nitrogen level. However, the calorific value is the lowest among all of the accessions. Mott is specifically adapted to be used as forage, in pasture, due to the high nutritional value and reduced mean plant height (dwarfing genes).

Late flowering trait is advantageous since it increases the period of vegetative growth; decreases the likelihood of seed set before harvest, which is an important means of spread for elephantgrass, and increases harvest flexibility (Sollenberger et al., 2014). Moreover, it has been reported that early flowering for giant miscanthus is related to the low dry matter production (Fedenko et al., 2013). Plant maturity may cause chemical composition changes, such as the reduction of ash content, nitrogen and dry matter concentration, and the increase of cellulose and lignin; in general, it improves the biomass quality traits for combustion and production of cellulosic ethanol (Na et al., 2016a, 2016b).

The biomass feedstock to use for combustion should present some quality attributes, such as high concentrations of lignin and cellulose (Gani and Naruse, 2007), high calorific value, high carbon/nitrogen ratio, in addition to low levels of moisture, ash, and nitrogen (McKendry, 2002; Long et al., 2006; Jaradat, 2010). When the focus is the production of cellulosic ethanol, it is required high cellulose/lignin ratio and hemicellulose to provide high ethanol production by biomass weight (Porter et al., 2007).

For both purposes (combustion and ethanol), biomass should present low moisture content (high levels of dry matter concentration) at harvest in order to obtain high efficiency of thermal conversion technologies (McKendry, 2002; Jaradat, 2010). In a study which compared energycane, elephantgrass, giant reed and switchgrass as biomass yields under low-input, Knoll et al. (2012) found that elephantgrass and energycane were those with the lowest dry matter concentration. The high moisture is one of the challenges for efficient processing and transportation of the postharvest biomass (Knoll et al., 2012; Na et al., 2015). Results of the current study indicate that there is genetic variability for this dry matter concentration, and therefore it can achieve reduced water content in elephantgrass.

The calorific value is the energy released when the biomass is burned. Therefore, the higher the calorific value, the better is the feedstock for combustion (Jaradat, 2010). Stalk was the major contributor of soluble sugars for total shoot biomass in elephantgrass. However, degradation of free sugars may decrease feedstock quality as they proportionally reduce concentration of structural carbohydrates (Na et al., 2016a). Low requirement of nitrogen is desirable not only because it is a valued constituent in terms of conversion to energy, but for N fertilizer is a costly input (Na et al., 2016b).

The results obtained in this work indicate that it is possible to use and breeding elephantgrass as bioenergetic plant. Elephantgrass accessions present high genetic diversity distributed in different clusters, which can be exploited for the production of second generation ethanol and for direct biomass combustion.

Thus, crosses among divergent individuals belonging to distinct clusters is recommended for the obtainment of populations with high potential for the extraction of superior clones. Therefore, the intercrosses of the accessions of cluster I (accessions: Pusa Gigante Napier, BAGCE 59, Cuba-116 and Pasto Panamá), III and V are promising for the improvement of the potential of elephantgrass for biomass combustion. For second generation ethanol production (cellulosic ethanol), the accessions of clusters I (accessions: Mercker S.E.A., Taiwan A-25, Mole de Volta Grande e CNPGL 96-25-3), III and IV should preferably be intercrossed.

This study is a pioneer in the quantification of genetic diversity aiming at the use of elephantgrass as bioenergetic feedstock, and reinforces the importance of pre-breeding actions to rise elephantgrass cultivation to a relevant level for a sustainable energetic matrix diversification.

4. Conclusions

The Active Elephantgrass Germplasm Bank (BAGCE) presented potential to be used with bioenergy purpose, with greater genetic variability in terms of biomass quality traits, when compared with morpho-agronomic traits.

The 100 accessions of the BAGCE were divided into six genetic dissimilarity clusters, with potential differentiated to be used in the production of second generation ethanol and direct biomass combustion.

It is possible to improve elephantgrass as bioenergetic plant.

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