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

João Romero do Amaral Santos de Carvalho Rocha[a], Juarez Campolina Machado[b,*], Pedro Crescêncio Souza Carneiro[a], Jailton da Costa Carneiro[b], Marcos Deon Vilela Resende[c], Francisco José da Silva Lédo[b], José Eustáquio de Souza Carneiro[a]

[a] *Universidade Federal de Viçosa, Campus Universitário, Viçosa 36570-000, Brazil*
[b] *Embrapa Gado de Leite, Rua Eugênio do Nascimento 610, Juiz de Fora 36038-330, Brazil*
[c] *Embrapa Florestas, Estrada da Ribeira, Km 111, Colombo 83411-000, Brazil*

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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,

* Corresponding author.
E-mail addresses: joaoascrocha@gmail.com (J.R.d.A.S.d.C. Rocha), juarez.machado@embrapa.br (J.C. Machado), carneiro@ufv.br (P.C.S. Carneiro), jailton.carneiro@embrapa.br (J.d.C. Carneiro), marcos.deon@gmail.com (M.D.V. Resende), francisco.ledo@embrapa.br (F.J.d.S. Lédo), jesc@ufv.br (J.E.d.S. Carneiro).

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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.

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