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Canonical correlations in elephant grass for energy purposes

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Elephant grass has the potential to be used as a source for energy production. Besides dry matter yield, other characteristics related to biomass quality are important. The canonic correlation analysis is a multivariate statistical procedure that allows for discovering characteristic associations among groups. The objective of this study was to evaluate the canonic correlation existing between the groups of agronomic traits and biomass traits in elephant-grass genotypes aiming to identify possible associations between the groups of variables in order to use this information in breeding programs of elephant grass for energy. The experiment was conducted at Colégio Estadual Agrícola Antônio Sarlo, located in Campos dos Goytacazes, RJ, Brazil. The evaluation cuts were made in June 2009 and February 2011; the first and third evaluation cuts, respectively. In the first cut, the following morpho-agronomic traits were evaluated: dry matter yield, percentage of dry matter, number of tillers, plant height, stem diameter, percentages of ash, cellulose, lignin, acid detergent fiber and crude protein and calorific value. In the third cut, in addition to the morpho-agronomic traits assessed in the first cut, the leaf-blade width and percentages of ash, cellulose, lignin, acid detergent fiber and neutral detergent fiber were also evaluated. The experimental design was of randomized blocks with 40 genotypes. Variance analysis and canonic correlation were performed between the morpho-agronomic and biomass-quality traits for the cuts isolated. A positive correlation was found between the percentage of dry matter and the fiber content and the plant height and number of tillers and the percentage of crude protein.

Key words: Bioenergy, multivariate, *Pennisetum purpureum.*

INTRODUCTION

There is great expectation on the possible economic benefits from the clean development mechanism projects coming from the use of renewable sources in the agricultural sector (Boddey et al., 2008). Elephant grass (*Pennisetum*

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purpureum Schum.) has been used for decades as a source of nutrients in the animal diet, and millions of tons of this material are produced every year (Cleef et al., 2013). In addition to this purpose, the biomass originated from elephant grass has the potential to be used in the production of biofuels in the form of charcoal, alcohol, methane, or even for direct combustion (Lee et al., 2010).

Elephant grass is an abundant, fast growing plant with significant potential as a renewable energy source and for conversion to higher calorific value fuels (Strezov et al., 2008). According to Morais et al. (2009), this culture is highly efficient in the fixation of atmospheric CO_2, and it can produce over 60 mg ha per year. There is only a small response of biomass production to the additions of urea fertilizer (Morais et al., 2013) and accumulates N derived from Biological nitrogen fixation (BNF) (Videira et al., 2012). With this, large amounts of dry matter can be produced with a small amount of fertilizer N.

Besides the production of dry matter, biomass quality characteristics are important to evaluate. The energy capacity of wood in Eucalyptus can be influenced both by its chemical composition and lignin and extractives contents (Santos et al., 2011). According to Paterlini et al. (2013), elephant grass has low fiber content and high extractives contents in the leaves, having good properties for its energy use.

The analysis of canonic correlation is a multivariate statistical procedure that allows for verifying the linear multidimensional relationships between two sets of variables (Costa et al., 2011). The association between traits is of great importance for plant breeding, because the selection practiced in a certain trait may cause changes in another (Marzban et al., 2014). This technique has been used in elephant grass (Cunha et al., 2011) to analyse the basal tillers density and plant height which are responsible for production of dry matter of evaluated clones.

Based on the above information, the objective of this study was to evaluate the canonic correlation between the groups of agronomic traits and biomass traits in elephant-grass (*Pennisetum purpureum* Schum.) genotypes aiming to identify the associations and the interdependence between the studied sets of variables order to use this information in breeding programs of elephant grass for energy.

MATERIALS AND METHODS

Cultivation conditions and genetic materials

The experiment was conducted at Colégio Estadual Agrícola Antônio Sarlo, in Campos dos Goytacazes/RJ, Brazil, located at 21°44' S latitude, 41°18' W longitude and 12 m elevation. The soil is characterized as a terrace soil, classified as a Dystrophic Ultisol (FAO, 1997). Planting was performed with stems positioned with their base in contact with the apex of the next plant, in 10 cm deep furrows, in March 2008. The plot-leveling cut was made 90 days after planting. The evaluation periods were considered from June 2008 to June 2009 and April 2010 to February 2011 for the first and third evaluation cuts, respectively.

During planting, 100 kg ha^{-1} P_2O_5 (Single superphosphate) were used. After plot leveling, topdressing was performed again, with 25 kg ha^{-1} N (ammonium sulfate) and 25 kg ha^{-1} K_2O (potassium chloride). The experimental design was of randomized blocks with 40 treatments (genotypes). Each 4.5 m^2 plot was composed of a 3.0 m row, with 1.5 m spacing between rows. During the cut, 1.5 m was considered in each plot for evaluation and 0.75 m of each side of the row was considered the border, totaling a floor area of 2.25 m^2

The elephant-grass genotypes utilized were: Elefante da Colômbia, Mercker, Três Rios, Mercker Santa Rita, Pusa Napier N 2, Gigante de Pinda, Napier N 2, Mercker S. E. A, Taiwan A-148, Porto Rico 534-B, Albano, HíbridoGigante Colômbia, Pusa Gigante Napier, Costa Rica, Cubano Pinda, Mercker Pinda, Mercker Pinda México, Mercker 86 México, Taiwan A-144, Napier S.E.A., Taiwan A-143, Elefante de Pinda, Mineiro, Mole de Volta Grande, Napier, Teresopólis, Taiwan A-46, Duro de Volta Grande, Mercker Comum Pinda, Cameroon - Piracicaba, Taiwan A-121, P241 Piracicaba, IAC-Campinas, Elefante Cachoeira Itapemirim, Roxo, Guaçu/I.Z.2, Cuba-116, King Grass, Roxo Botucatu, and Vruckwona Africano.

Evaluated traits

In the first cut, the following morpho-agronomic traits were evaluated: dry matter yield, percentage of dry matter, number of tillers per linear meter, plant height and stem diameter. The biomass-quality traits assessed in the first cut were: percentages of ash, cellulose, lignin, acid detergent fiber and crude protein, and calorific value. In the third cut, in addition to the morpho-agronomic traits assessed in the first cut, the leaf blade width was also evaluated. The biomass-quality traits evaluated in the first cut were: percentages of ash, cellulose, lignin, acid detergent fiber and neutral detergent fiber.

The variable dry matter yield (DMY), in mg. ha^{-1}, was obtained from the conversion of fresh matter production by the percentage of dry matter. To obtain the percentage of dry matter (%DM), a tiller (leaves and stem) was collected for drying in an oven at 65°C for 72 h until constant weight was reached (ADS - air-dried sample); the dried material was ground in a Wiley mill (1 mm sieve) and conditioned in a plastic bottle. Next, the samples were once again dried in an oven at 105°C for 12 h (ODS - oven-dried sample). Soon after, the %DM was estimated as the product between ADS and ODS. The plant height (HGT) was measured from the soil until the curvature of the last expanded leaf, expressed in m; stem diameter (SD), expressed in cm, was measured at 10 cm above the soil, with a caliper ruler; the number of tillers was measured per linear meter (NT); and the leaf blade width (LW), at the first fully expanded leaf, expressed in cm.

The analyses of the percentages of crude protein (CP), acid detergent fiber (%ADF) and neutral detergent fiber (%NDF) were carried out as described by Pomeranz and Meloan (1994). The percentage of lignin was determined from the ADF (cellulose, lignin, cutin, minerals, silica and ADIN) using the reagent sulfuric acid (72%); the percentage of ash (%ASH) was determined as the difference in weight after analysis of determination of the %LIG; the percentage of cellulose (%CEL) was determined as the difference in weight between %ADF and %LIG, as described by Pomeranz and Meloan (1994); and the calorific value (CFV), expressed as kcal. g^{-1}, by using 1 g of sample, determined using the adiabatic calorimeter IKA model C-200.

Statistical analysis

Variance analysis and canonic correlation were performed for the

Table 1. Estimates of the mean squares of genotype and residue mean and coefficient of variation of 11 traits of 40 genotypes of elephant grass in the first evaluation cut.

Variable	Mean square		Mean	CV(%)
	Genotype	Residue		
DMY	66.70*	21.90	20.37	22.96
%DM	64.89*	9.56	37.15	8.32
NT	617.40*	59.22	44.14	17.43
HGT	0.24*	0.11	3.33	10.26
SD	9.38*	2.62	11.80	13.73
%ASH	1.86*	0.71	3.11	27.21
%CEL	4.95^{ns}	3.56	34.86	5.41
%LIG	29.30^{ns}	20.52	47.38	9.56
%ADF	4.92*	1.35	8.92	13.02
%CP	0.83^{ns}	0.65	3.65	22.16
CFV	3848.38^{ns}	2649.56	3898.24	1.32

DMY, dry matter yield (t. ha^{-1}); %DM, percentage of dry matter; NT, number of tillers per linear meter; HGT, plant height (m); SD, stem diameter (mm); %ASH, percentage of ash; %CEL, percentage of cellulose; %LIG, percentage of lignin; %ADF, percentage of acid detergent fiber; %CP, percentage of crude protein; CFV, calorific value (kcal. g^{-1}); CV, coefficient of variation. *significant at 1% probability; ^{ns} = not significant at 1% by the F test.

cuts isolated. Canonic correlation analyses were conducted to check the existing associations between a first group of morphological traits (dependent variables Y) with the second group formed by the biomass-quality traits (according to the independent variables X). All statistical analyses were conducted on the Genes computer software (Cruz, 2013).

RESULTS AND DISCUSSION

Variance analysis

In the first evaluation cut, there were significant differences at 1% probability among the genotypes of elephant grass with regard to the variables dry matter yield (DMY), percentage of dry matter (%DM), number of tillers per linear meter (NT), plant height (PHT), stem diameter (SD), percentage of ash (%ASH) and percentage of acid detergent fiber (%ADF). In the case of percentage of cellulose (%CEL), percentage of lignin (%LIG), percentage of crude protein (%CP) and calorific value (CFV), there were no significant differences at 5% probability among the evaluated genotypes. The observed dry matter yield was 20.37 t. ha^{-1} (Table 1). Morais et al. (2009), evaluating genotypes of elephant grass during the growth period of seven months, observed mean values of 18.9 t. ha^{-1}, which is close to that found in the first evaluation cut of the present study. The average number of tillers was 11.8 mm (Table 1).

At the third cut, there was significance for all the evaluated traits, except for plant height (HGT) and percentage of ash (%ASH). The traits dry matter yield (DMY), percentage of dry matter (%DM), number of tillers per linear meter (NT), stem diameter (SD), leaf blade width (LW), percentage of cellulose (%CEL), percentage of neutral detergent fiber (%NDF) and percentage of lignin (%LIG) presented significance at 1% probability. The average stem diameter found in the third cut (17.29 mm) was higher than that found by Ferreira et al. (2013) whose highest value was 15.40 mm in the genotype Itambé I-1.20, not used in this experiment (Table 2).

The percentage of NDF corresponds to the sum of the lignin, cellulose and hemicellulose contents in the plant tissue. As the %NDF is increased, the biomass for energy purposes also increases. The average %NDF observed was 76.38 (Table 2). These values are above the 57.1 and 59% found by Flores et al. (2012) in groups of unfertilized elephant grass and the same grass fertilized with 100 kg. ha^{-1} of nitrogen, respectively. Another advantageous result found in this study concerned %ASH, because low ash contents are desirable for energy uses. Paterlini et al. (2013) found an average %ASH of 6.15%. In another study, conducted by Flores et al. (2013), the lowest value found for this trait was 4.9% in plants at 180 days after sprouting, which is higher than 4.38% (Table 2).

Canonical correlation

The first, second and third canonic correlations were, at the level of 5% probability, significantly different from zero at the first evaluation cut (Table 3). Thus, it is concluded that the groups are not independent and that the intergroup associations can be established. Hence, only these three correlations are of interest in this study, because these were the canonic pairs that maximized the relationship between the primary components of the morpho-agronomic and biomass-quality traits in the

Table 2. Estimates of the mean squares of the genotype and residue mean and coefficient of variation of eleven traits of forty genotypes of elephant grass in the third evaluation cut.

Variable[1/]	Mean square		Mean	CV (%)
	Genotype	Residue		
DMY	196.49*	62.63	36.96	21.41
%DM	26.90*	7.28	34.28	7.87
NT	187.71*	70.08	32.30	25.91
HGT	0.073**	0.03	2.70	7.06
SD	15.87*	5.07	17.29	13.03
LW	71.99*	17.12	39.54	10.46
%ASH	0.78**	0.44	4.38	15.19
%CEL	4.83*	1.42	38.50	3.10
%ADF	10.07*	3.51	47.37	3.95
%NDF	8.49*	2.23	76.38	1.95
%LIG	2.15*	0.47	7.79	8.82

DMY, dry matter yield (t. ha^{-1}); %DM, percentage of dry matter; NT, number of tillers per linear meter; HGT, plant height (m); SD, stem diameter (mm); %ASH, percentage of ash; %CEL, percentage of cellulose; %LIG, percentage of lignin; %ADF, percentage of acid detergent fiber; %CP, percentage of crude protein; CFV, calorific value (kcal. g^{-1}); CV, coefficient of variation. *significant at 1% probability; ^{ns} = not significant at 1% by the F test.

Table 3. Canonic correlations estimated between agronomic traits (group I) and biomass quality (group II) in elephant grass in the first evaluation cut.

Variable	Canonical pairs				
	1º	2º	3º	4º	5º
DMY	0.17426	0.00121	0.12068	0.21066	0.95430
%DM	-0.45237	-0.71747	0.20645	0.31041	0.37632
NT	0.78062	-0.55633	0.23500	-0.02711	0.15866
HGT	-0.50184	0.34644	0.65748	0.07227	0.43661
SD	-0.17376	0.73871	0.04741	0.57699	0.29825
%ASH	-0.00652	-0.17461	-0.31509	0.44914	0.53672
%CEL	0.10556	-0.53751	0.64803	-0.48569	0.19730
%LIG	-0.10467	-0.40174	-0.12661	-0.47119	0.74066
%ADF	-0.33047	-0.56879	-0.45071	0.00103	-0.13921
%CP	0.91105	0.13390	-0.28606	-0.06577	-0.23554
CFV	-0.20598	-0.30631	-0.16856	-0.84628	-0.33063
R	0.69813	0.5614	0.1338	0.2936	0.3731
α (%)	0.0	0.0	1.47	10.53	41.65

DMY, dry matter yield (t. ha^{-1}); %DM, percentage of dry matter; NT, number of tillers per linear meter; HGT, plant height (m); SD, stem diameter (mm); %ASH, percentage of ash; %CEL, percentage of cellulose; %LIG, percentage of lignin; %ADF, percentage of acid detergent fiber; %CP percentage of crude protein; CFV, calorific value (kcal. g^{-1}); R, correlation; α, significance level.

elephant grass.

The association made in the first canonic pair is between the traits HGT and %CP. The results indicate that %CP decreases as HGT increased. For the same canonic pair it was observed that between the traits NT and %CP, as the NT increased, %CP elevated. Regarding the third canonic pair, it can be observed that as the %DM decreases, the %CEL and %ADF also reduced. Observing the associations of the third pair, as the HGT increased, %CEL also increased (Table 3), because as the plant ages, with an increase in HGT and %DM, the plant cell wall thickens and lignifies, especially due to the proportion of stems in the harvested material (Xie et al., 2011). According to Braga et al. (2014), elephant grass genotypes due to having lower ash requires less energy to thermal conversion process to break links hemicelusose and cellulose than rice husk for the production of bio-oil by pyrolysis.

Table 4. Canonic correlations estimated between agronomic components (group I) and biomass quality (group II) in elephant grass at the third evaluation cut.

Variable	Canonical pairs				
	1º	2º	3º	4º	5º
DMY	0.1842	-0.18283	0.73418	0.40409	-0.17855
%DM	0.60212	0.68645	0.16150	-0.08567	0.17938
NT	0.67795	0.31069	-0.00244	0.59179	-0.28928
HGT	0.19719	0.01362	0.04583	-0.18788	-0.18954
SD	-0.35403	-0.35300	-0.50891	0.25258	-0.64921
LW	-0.78153	0.29916	0.34309	0.05606	-0.30385
%ASH	-0.13972	0.08914	0.68986	0.00338	0.70471
%CEL	0.67303	0.44269	0.29361	0.20102	-0.47375
%ADF	0.86729	0.2622	-0.19036	0.27878	-0.25516
%NDF	0.68488	0.64475	0.07453	0.18679	-0.27349
%LIG	0.84655	0.20363	-0.31169	-0.33215	-0.18551
R	0.6989	0.5588	0.4647	0.0699	0.3885
α (%)	0.0	0.0	0.27	5.55	83.59

DMY, dry matter yield (t. ha^{-1}); %DM, percentage of dry matter; NT, number of tillers per linear meter; HGT, plant height (m); SD, stem diameter (mm); LW, leaf blade width (mm); %ASH, percentage of ash; %CEL, percentage of cellulose; %ADF, percentage of acid detergent fiber; %NDF, percentage of neutral detergent fiber; %LIG, percentage of lignin; R, correlation; α , significance level.

In the third cut, the first, second and third canonic pairs were not independent; that is, they obtained significance at 1% probability. The associations that can be established in the first canonic pair are between the morpho-agronomic traits %DM, NT and LW and the biomass-quality traits %CEL, %ADF, %NDF and %LIG. Based on the obtained results, when %DM and NT increase, there was an elevation in %CEL, %ADF, %NDF and %LIG. With regards to LW and the same biomass-quality traits, the effect is inversely correlated: when LW is reduced, %CEL, %ADF, %NDF and %LIG increased (Table 4).

Similarly to what was observed in the first canonic pair, in the second, the increase in %DM leads to augmenttations in %CEL and %NDF (Table 4). Evaluating the energy value of elephant grass at different regrowth ages, Silva et al. (2007) observed an increase in %DM and %NDF as the regrowth days advanced. This increase in %NDF is important for the elephant grass, because it indicates potential for energy production.

With regard to the third canonic pair, an association can be observed between the morpho-agronomic traits DMY and SD and %ASH. As the DMY increases and SD decreases, %ASH increased (Table 4). Flores et al. (2013), evaluating elephant-grass genotypes under different nitrogen doses and cut ages on a Latosol, observed that, both in leaf and stem, the cut age (greater accumulation of dry matter) decreased the ash contents in the elephant grass that is an opposite result to that found herein. According to Flores et al. (2013), the ash contents are considered high, reaching 10.3% at the dose of 77.5 kg. ha^{-1} of N, and according to the authors of that study this can be due to the good availability of nutrients of the soil utilized, promoting the better nutrition of the elephant grass.

Conclusions

The percentage of dry matter in elephant-grass genotypes is positively correlated with the fiber content (%ADF and %NDF). The association between these morpho-agronomic and biomass-quality traits is a criterion of paramount importance in the selection of elephant-grass genotypes for energy production.

Plant height and number of tillers are positively correlated with the percentage of crude protein. The percentage of ash is negatively correlated with the stem diameter and positively correlated with the dry matter yield.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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