

Variation of Morpho-Agronomic and Biomass Quality Traits in Elephant Grass for Energy Purposes According to Nitrogen Levels

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Abstract

Elephant grass is a tropical forage plant widely spread in Brazil, used mainly in the livestock sector and in cattle feeding. Because of its high productivity and photosynthetic capacity, this culture has also been considered an alternative source of renewable energy. Six clones of elephant grass (*Pennisetum purpureum* Schum.) were evaluated under five levels of nitrogen fertilization (100, 200, 400, 800, and 1600 kg-N-ha⁻¹), in a randomized-block design with a split-plot arrangement with three replicates, from April 2010 to December 2012, in the city of Campos dos Goytacazes—RJ, Brazil. The objective was to obtain estimates of variation in morpho-agronomic traits and biomass quality. We observed that genotypes Cameroon-Piracicaba and Guaçu I/Z2 have great potential to be used, with maximum dry matter yields of 60.97 and 44.10 t-ha⁻¹ per cut for energy purposes among the studied genotypes.

Keywords

Nitrogen Fertilization, Alternative Energy, Energy Biomass, Dry Matter Yield,
Pennisetum purpureum Schum.

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1. Introduction

Elephant grass (*Pennisetum purpureum* Schum.) is a tropical species of the family Poaceae with a high potential for biomass production. For many years, elephant grass was employed almost exclusively in animal production, in the formation of stocking piles, used in chopped form or as silage. Recently, because of its high biomass production potential, another use for elephant grass has been proposed: its transformation into firewood [1], which may replace the wood extracted in a predatory manner from native forests, thus preventing environmental damage such as ravaging of forests, erosion, siltation, and death of several rivers. The biomass has gained great attention as a new source of sustainable energy alternative to petroleum-based fuels [2], in addition to also working in the carbon sequestration [3].

There appears to be a large genetic variability in this species [4]–[6], so one can assume the existence of an excellent opportunity for success in processes of selection of cultivars and optimization of inputs aiming at a high biomass-production potential [7].

Thus, there has been an intense search for varieties to be used for biomass production that are adapted to the different ecosystems in the state of Rio de Janeiro (Brazil), have faster growth, greater productivity, better energy efficiency, greater efficiency in the use of nutrients, and more equitable distribution of the dry matter production throughout the year, and are resistant to pests and diseases.

Nitrogen plays a key role in plant nutrition, as it is an essential component of proteins and interferes directly with the photosynthetic process, because it participates actively in the molecule of chlorophyll, which thus causes it to be a limiting factor in a system with intensive use of cultivated soils [8].

In the agricultural systems and agribusinesses, nitrogen fertilization is the primary means of adding N to the soil. Nitrogen is one of the inputs of greatest importance because of the increasing response in forage [9] [10] and dry matter production, especially in the generation of energy biomass in the case of elephant grass [11] [12].

Nitrogen management is complex recommendation which is subject, dependent on soil and climatic conditions, occurring losses by leaching, volatilization, denitrification erosion due to multiplicity of chemical and biological reactions [13]. Due to the importance of agriculture to elephant grass, more information about economic and rational use of fertilizers is needed [14]. Because of the importance of agriculture to elephant grass, more information about economic and rational use of fertilizers is needed [15], in addition to actually check for production increases with increasing nitrogen dose or as a dose of production remained stable (maximum point).

The objective of this study was to evaluate six genotypes of elephant grass (Cubano Pinda, Mercker Pinda, Mercker 86 México, Cameroon Piracicaba, Guaçu I/Z2, and Roxo Botucatu) by analyzing morpho-agronomic and biomass-quality traits, subjected to different levels of nitrogen fertilization (100, 200, 400, 800 and 1600 kg·N·ha⁻¹) in the soil-climatic conditions of the north region of Rio de Janeiro State in seeking new alternative sources of sustainable energy.

2. Materials and Methods

The experiment was established on 04/26/2010, conducted in the State Center for Research on Agro-energy and Use of By-products of PESAGRO, located in the municipality of Campos dos Goytacazes-RJ (13 m altitude, -21°45'15" latitude, and 41°19'28" longitude). The climate in the city is hot and humid, with an average annual temperature of 22.7°C. The results of the chemical analysis of the soil sampled at the upper 0 - 20-cm layer on 08/27/2009 were: pH in water = 5.2; organic matter = 16.9 g·cm⁻³; N = 0.8 g·kg⁻¹; H + Al, Ca, Mg, and CEC = 0.7, 1.5, 0.7, and 6.3 cmol·dm⁻³, respectively; P = 5.7 mg·dm⁻³; and K = 104.7 mg·dm⁻³.

The study was developed at the Laboratory of Agricultural Engineering of the Center for Agricultural-Livestock Sciences and Technologies of Universidade Estadual do Norte Fluminense Darcy Ribeiro (LEAG/CCTA/UENF), located in Campos dos Goytacazes-RJ. Planting was carried out by inserting pieces of stems, in a simple row, into 10-cm-deep furrows spaced 1.5 m apart, followed by 100 kg·ha⁻¹ single superphosphate (P₂O₅) incorporated at the bottom of the furrow. Fifty days after planting, topsoil fertilization was applied with 25 kg·ha⁻¹ ammonium sulfide ((NH₄)₂SO₄) and potassium chloride (KCl).

The accessions used in the experiment were selected from the study of [16] for presenting superior traits in terms of biomass generations. Genotypes Cubano Pinda (G1), Mercker Pinda (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6) were used.

The experiment was designed as randomized blocks with three replicates (blocks), in a split-plot arrangement consisting of two factors: Factor 1 (plots): genotypes—six clones; Factor 2 (sub-plots): nitrogen—five levels

(100, 200, 400, 800, and 1600), with fertilization performed on the following days: 11/12/2010, 12/08/2010, 01/17/2011, 03/01/2011 and 03/23/2011. The randomized block design is one in which each block (replicate) receives all treatments at once, and are considered the three principles of experimentation.

The subplots consisted of 3-m-long rows spaced 1.5 m apart. The plot-leveling cut was made on 08/04/2010 (15 weeks after planting), and the cut for experimental purposes was made on 05/23/2011.

The following statistical model was used:

$$Y_{ijl} = \mu + Bl_l + F1_i + \varepsilon_{il} + F2_j + F1F2_{ij} + \varepsilon_{ijl}$$

where: Y_{ijl} = effect of the i -th level of factor F1, j -th level of factor F2 and l -th replicate; μ = overall mean; Bl_l = effect of the k -th block ($l = 1, 2, \dots, L$); $F1_i$ = effect of the i -th factor 1 ($i = 1, 2, \dots, I$); ε_{il} = residual, or random error (I); $F2_j$ = effect of the j -th factor ($j = 1, 2, \dots, J$); $F1F2_{ij}$ = effect of the interaction between the i -th factor 1 ($i = 1, 2, \dots, I$) and the j -th factor 2 ($j = 1, 2, \dots, J$); and ε_{ijl} = residual, or random error (II).

In this study, the morpho-agronomic traits were evaluated, and before the cut, the following traits were evaluated: number of tillers (NT), counted the number of tillers in one linear meter; plant height (HGT), expressed in meters, measured with a graduated ruler, based on the average height of the plants in the plot; stem diameter (SDM), expressed in centimeters, measured using a digital caliper; leaf blade width (LBW), expressed in centimeters, measured with a graduated ruler in the center of the leaf blade.

The material was fractionated as follows: for the leaf/stem ratio, it was separated into leaf + sheath, then weighted and placed separately into paper bags, which were immediately dried in a forced-circulation oven at 65°C for 72 h. Later, the samples were weighed, obtained the percentage of stem (%STEM) and ground in a Wiley mill with 1-mm sieve and conditioned in an air-tight container for subsequent analyses. The same procedure was adopted for the analysis of the whole plant, but without the separation of stem and leaf. The experiment was conducted under natural conditions, without the use of irrigation.

The material was evaluated in the Laboratory of Animal Science and Animal Nutrition of Universidade Estadual do Norte Fluminense Darcy Ribeiro (LZNA/UENF), where the following traits were calculated: plant dry matter yield (DMY), stem dry matter yield (SDMY) and leaf blade dry matter yield (LBDMY) estimated by multiplying the fresh matter production of the whole plant by the percentage of dry matter of the whole plant (%DM), and then converting the obtained values to $t \cdot ha^{-1}$, in accordance with the methodology described by [17].

The next evaluation stage was held at the Food Analysis Laboratory of the Embrapa Gado de Leite, Coronel Pacheco (MG), by reflectance of near infrared method (NIRS) in a spectrometer Perstorp analytical, Silver Spring, MD, model 5000, coupled to a microcomputer equipped with software ISI version 4.1 (Infrasoft International, University, Park, PA). The reading was performed using the wavelength 1100 to 2500 nanometers. For this methodology the following traits were analyzed: percentage of neutral detergent fiber (%NDF); percentage of ash (%ASH); percentage of acid detergent fiber (%ADF); percentage of lignin (%LIG); percentage of cellulose (%CEL); percentage of hemicellulose based on NDF, lignin and cellulose (%HEMI_{NDFLIGCEL}); percentage of hemicellulose based on NDF and ADF (%HEMI_{NDFADF}).

Tukey's multiple comparison test at 5% significance was used for situations in which there was a significant effect involving the source of variation genotype, and for the case of a significant effect involving the source of variation nitrogen levels, combined polynomial regression was used for the 1st- and 2nd-degree polynomial models, with the respective analysis of variance of regression, testing the significances of the sources of variation due to regression and due to deviations of regression. Statistical analyses were performed using the GENES software [18], and the analysis of variance of regression was obtained by the F test ($P < 0.05$).

3. Results and Discussion

3.1. Morpho-Agronomic Traits

For the morpho-agronomic traits shown in **Table 1**—number of plants per meter (NT), plant height (HGT), stem diameter (SDM), percentage of stem (%STEM), and percentage of dry matter (%DM)—there was no genotype \times nitrogen interaction effect ($P > 0.05$), indicating independence among the factors. In contrast, the interaction had a significant effect ($P < 0.05$) on all other traits, but effect at significance level of 1% was only seen on dry matter yield (DMY).

Table 1. Estimate of the mean squares (MS) and their significance levels for morpho-agronomic traits in six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

MS	SV					
	G	N	G × N	MEAN	CV (G) (%)	CV (N) (%)
DMY	573.10**	403.52**	176.53**	35.03	16.44	20.56
SDMY	228.10*	178.74**	62.94*	22.77	32.24	25.56
LBDMY	127.81**	47.01*	32.71*	12.26	35.43	32.76
NT	616.03**	268.60**	62.84 ^{ns}	27.55	31.81	27.01
HGT	0.65*	2.56**	0.04 ^{ns}	3.54	12.09	6.91
SDM	0.12**	0.39**	0.01 ^{ns}	1.60	8.90	7.35
LBW	3.01*	5.87**	0.28*	4.71	16.55	8.05
%STEM	249.63**	13.07 ^{ns}	15.57 ^{ns}	65.19	9.98	5.88
%DM	51.72**	22.80**	7.13 ^{ns}	32.24	8.73	6.28

DMY = dry matter yield, in $\text{t}\cdot\text{ha}^{-1}$; SDMY = stem dry matter yield in $\text{t}\cdot\text{ha}^{-1}$; LBDMY = leaf blade dry matter yield, in $\text{t}\cdot\text{ha}^{-1}$; NT = number of tillers per meter; HGT = plant height, in meters; SDM = stem diameter, in millimeters; LBW = leaf blade width, in centimeters; %STEM = percentage of stem; %DM = percentage of dry matter. ** = significant at 1% probability by the F test; * = significant at 5% probability by the F test; ns = not significant.

Considering the main effects of genotype and nitrogen, only %STEM was not affected significantly by nitrogen ($P > 0.05$), *i.e.*, fertilization had no effect, while nitrogen had a significant effect ($P < 0.05$) on all other traits except leaf blade dry matter yield (LBDMY), with a significance level of 5%. Thus, it is observed that the fertilization effect does not influence ($P > 0.05$) the %STEM of elephant grass and that the variation originates from the genetic value only. For the genotypes variable, all traits were affected significantly ($P < 0.01$), except stem dry matter yield (SDMY), HGT and leaf blade width (LBW), which had an effect of ($P < 0.05$).

The coefficients of variation give an idea of precision in the experiment, and when found in agricultural field trials, they may be considered low if lower than 10%; medium from 10 to 20%; high from 20% to 30%; and very high above 30% [19].

For the morpho-agronomic traits, except DMY, the genetic coefficient of variation was always higher than the experimental coefficient in all evaluated characters, which demonstrates a greater magnitude in the variations involving levels of nitrogen, indicating a large variability among genotypes. The coefficient of variation in the morpho-agronomic traits SDM, %STEM, and %DM was lower than 10% in both the genetic and experimental factors, and in the HGT and LBW traits only in the experimental factor.

Regarding the DMY the genotype G4 showed highest value ($P < 0.05$) at levels N1 ($36.70 \text{ t}\cdot\text{ha}^{-1}$), N4 ($52.81 \text{ t}\cdot\text{ha}^{-1}$) and N5 ($57.95 \text{ t}\cdot\text{ha}^{-1}$) compared with other genotypes. At levels N2 and N3 the genotype G5 showed the highest values 53.21 and $50.04 \text{ t}\cdot\text{ha}^{-1}$, respectively (Figure 1(a)). For SDMY, at levels N1, N3, and N5, the means between the genotypes did not differ statistically ($P > 0.05$). At level N2 at level G5 presented the highest value ($P < 0.05$) of $32.46 \text{ t}\cdot\text{ha}^{-1}$ and, at level N4 genotypes were G1 and G4 with values of 32.10 and $33.08 \text{ t}\cdot\text{ha}^{-1}$, respectively (Figure 1(b)). For the trait LBDMY, there were no significant differences ($P > 0.05$) between genotypes at level N1. At levels N4 and N5 the genotype G4 showed higher LBDMY of 19.72 and $24.93 \text{ t}\cdot\text{ha}^{-1}$, and levels N2 e N3 the genotype G5 showed higher values of 20.74 and $19.33 \text{ t}\cdot\text{ha}^{-1}$ ($P < 0.05$), respectively (Figure 1(c)).

The trait NT showed the same response previously achieved, *i.e.* the genotype showed the highest values in levels N4 and N5 it was G4 ($P < 0.05$). At level N1 the average between genotypes was not statistically different ($P > 0.05$). However, G5 obtained the largest NT at levels N2 and N3 ($P < 0.05$). At level N5 the genotypes G1, G2, G3 and G6 showed the highest values of 26, 29.78, 27.11 and 26, respectively (Figure 1(d)).

The HGT high homogeneity at levels N1, N3 and N5, with which there were no significant differences between the means ($P > 0.05$). At N2, however, the genotype that had the highest HGT was G6, with classification “a”; genotype G5 obtained “b”; and the others, G1, G2, G3, and G4, were classified as “ab”. For level N4, the

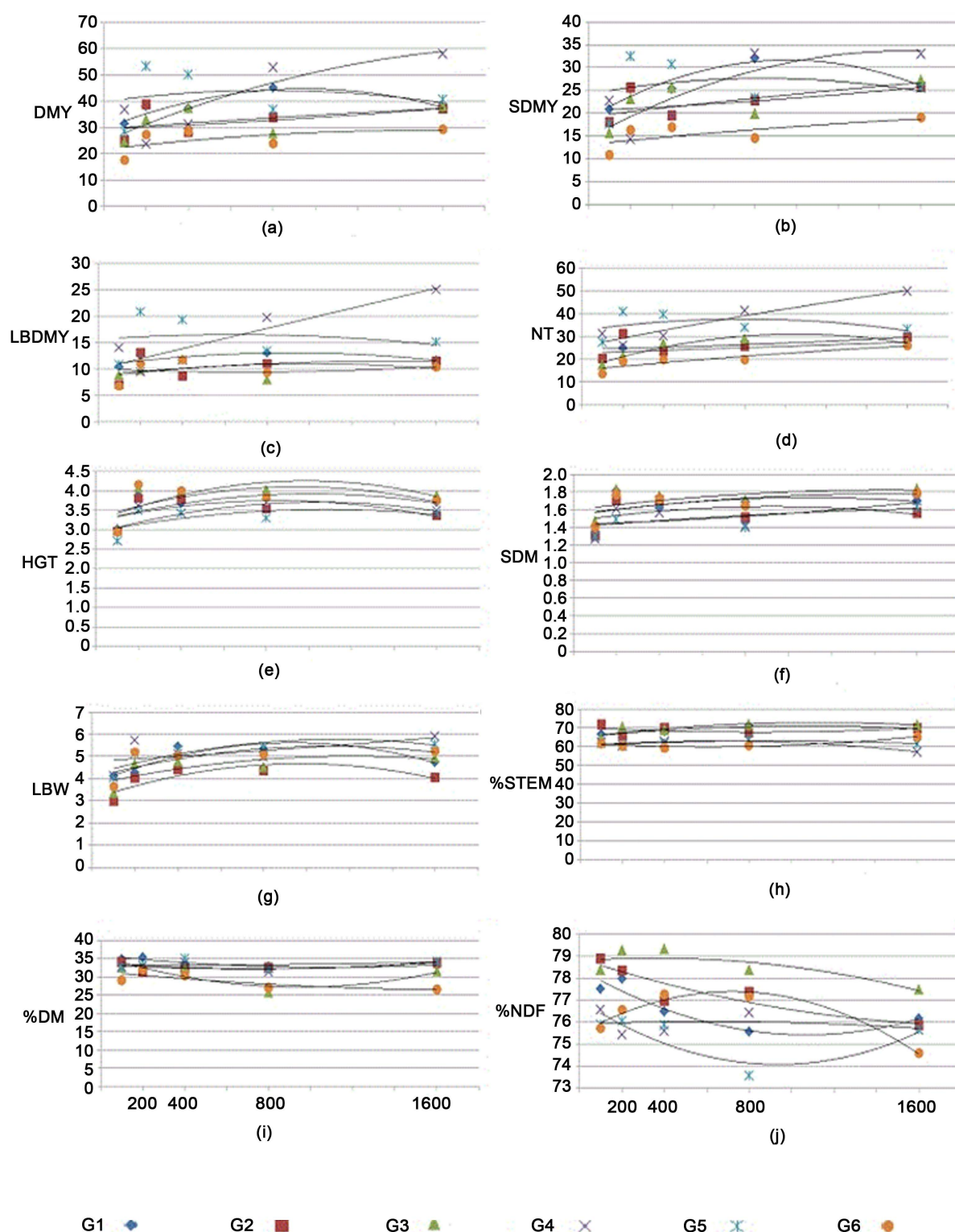


Figure 1. Regression curves of traits dry matter yield (DMY-A), in t-ha⁻¹; stem dry matter yield (SDMY-B), t-ha⁻¹; leaf blade dry matter yield (LBDMY-C), t-ha⁻¹; number of tillers (NT-D), plant height (HGT-E), in m; stem diameter (SDM-F), in cm; leaf blade width (LBW-G), in cm; percentage of stem (%STEM-H), percentage of dry matter (%DM-I) and of hemi-cellulose based on NDF (%NDF-J) of genotypes Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 6 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5) and Roxo Botucatu (G6).

genotype with highest HGT was G3, classified as “a”. Genotypes G1, G2, G4 and G6 obtained classification “ab”, while G5 was classified as “b” ($P < 0.05$).

Regarding SDM, at levels N1, N3 and N5 there was no statistical difference between the means ($P > 0.05$). For level N2, though, genotypes G3, G1 and G6 obtained classification “a”; genotypes G2 and G4, “ab”; and G5 was classified as “b”. At level N4, genotypes G3 and G1 obtained classification “a”; G6, G2, and G4 were classified as “ab”; and G5 was classified as “b” ($P < 0.05$).

The genotype with the highest LBW was G4 at levels N1, N2, and N5, but at level N1 those with the genotypes classified as “a” were G4, G1, and G5. At level N3, the best genotype was G1, with classification “a”; the second best mean was G2; and the others did not differ statistically. At N4, the highest LBW was found with genotypes G5 and G1, which were both classified as “a” ($P < 0.05$).

Analyzing %STEM, at level N1, the genotype that showed the highest value was G2. At levels N2 and N5, the genotype that stood out the most was G3. At N3, those with the highest %STEM were G2 and G3. At level N4, the highest value was found with G3 and G1 ($P < 0.05$).

With regard to %DM, the genotypes that had highest values were G1 and G2 for level N1. For levels N2 and N3 there was no significant difference between the means. For level N4, however, genotypes G1, G2 and G5 obtained the highest mean. For level N5, though, genotypes G5, G4, G2 and G1 achieved the highest %DM, was classified as “a” ($P < 0.05$).

In terms of maximum productivity achieved by adding increasing levels of nitrogen, and regarding the morpho-agronomic traits evaluated in the six genotypes studied here, three of them were noteworthy: Guaçu I/Z2 (G5), Cameroon Piracicaba (G4), and Cubano Pinda (G1).

Genotype Guaçu I/Z2 (G5) was classified as “a” at all levels for the following morpho-agronomic traits: DMY, SDMY, NT, LBW and %DM. Its maximum production, however, concerning all evaluated traits, is between the levels of 200 and 400 kg·N·ha⁻¹, except for LBW, which showed increasing gain in production as the N level was increased. Yet, this was observed that in traits such as SDM, %STEM and %DM, this genotype achieved the highest mean with levels of approximately 400 kg·N·ha⁻¹ ($P < 0.05$). Thus, overall, this genotype has a great potential for production with lower levels of nitrogen fertilization. In the study of [20], high yields were found with genotype Guaçu I/Z, which produced over 50 t·ha⁻¹ of dry matter.

As for genotype Cameroon Piracicaba (G4), classification “a” was obtained at all levels in the following morpho-agronomic traits: NT, HGT, SDM, LBW and %DM. Overall, the addition of increasing levels of nitrogen influenced this genotype in a linear manner, *i.e.*, there was gain in productivity as the fertilization levels were increased, wherein variables DMY, LBDY, NT, SDM, LBW and SDMY stood out ($P < 0.01$). This genotype reached better gains in productivity than all others evaluated in this study at the N level of 1600 kg·ha⁻¹ ($P < 0.05$). In [16], genotype Cameroon is known for its excellent performance in the field and, also in that study, its production was similar to that found by [20]: 32 t·ha⁻¹ of dry matter.

Genotype Cubano Pinda (G1) was classified as “a” in the following morpho-agronomic traits: SDMY, HGT, SDM, %STEM, %DM. This genotype had its greatest productivity at the N level of 800 kg·ha⁻¹ in traits DMY, SDMY, LBDY, NT, LBW, and %STEM. For the remaining traits—HGT, SDM and %DM—it the highest values were achieved at the N level of 200 kg·ha⁻¹ ($P < 0.05$).

With the analysis of regression of the traits DMY, SDMY, LBDY and NT was observed that the only genotype to show regression was Cameroon Piracicaba (G4), and the model with best fit was the 1st-degree model in all these traits, indicating that there is a need for greater nitrogen fertilization, which would generate a better response (Figure 1). Genotype Cameroon also has stood out for its high biomass production, especially in the dry season, besides its high fiber contents. It is a widely cultivated species due to its high production of forage with considerable nutritive value when managed properly [21]–[23].

The regression analysis for HGT determined that the only genotype that did not obtain regression was Guaçu I/Z2 (G5), while all others did, wherein the model with best fit was the 2nd-degree ($P < 0.01$; $P < 0.05$) (Figure 1(e)). If fertilized with greater amounts of nitrogen, the genotypes that showed a significant regression have a growth potential and consequently generate more dry matter. It should be noted that HGT is a variable related to forage DMY, and thus its importance to the culture [24].

Concerning the factor SDM, the analysis showed lack of regression for genotype Mercker Pinda México (G2); 2nd-degree model ($P < 0.05$) for genotype Cubano Pinda (G1); and 1st-degree model for all others ($P < 0.01$; $P < 0.05$) (Figure 1(f)). Therefore, it is observed that SDM can be influenced by nitrogen fertilization, insofar as four out of the six genotypes obtained regression of first degree, indicating that as the fertilization levels increase, the

SDM will also increase proportionally.

With regard to the LBW, all genotypes obtained regression. Genotypes Cubano Pinda (G1), Mercker Pinda México (G2), and Guaçu I/Z2 (G5) obtained better fit with the 2nd degree model, while genotypes Cameroon Piracicaba (G4), Mercker 86 México (G3), and Roxo Botucatu (G6) obtained the best fit with the 1st degree model (**Figure 1(g)**).

Thus, it can be observed that all genotypes show potential for forage production; however, it is noteworthy that because their most representative equations have a linear aspect, genotypes G4, G6, and G3 show a great capacity for more absorption of nitrogen, indicating potential for even larger levels, as well as increase in leaf production. Analyzing a clone of elephant grass in Pernambuco State (Brazil), [25] found that LBW production has a positive relationship with HGT, demonstrating that plants with greater leaf production usually have higher DMY and HGT.

Regarding the %STEM, all genotypes had lack of regression, requiring better levels of fertilization, such that the ratio between the stem and the plant as a whole has representativeness (**Figure 1(h)**). Studying the influence of the time of cutting of elephant grass, [26] observed a positive linear response for the stem/leaf ratio according to the cutting age, which corroborates the results reported by [27], who worked with genotypes of elephant grass and also detected an increase in the stem/leaf ratio as the plant aged.

In [26], an increase in stem/leaf ratio was also observed as the cutting age of elephant grass genotype Cameroon was increased. These results are important, since the desired management for production of elephant grass with energy purposes is aimed at a greater stem/leaf ratio, as this is a parameter that can indicate the quality of the produced material, because the stem is a compartment with better desirable characteristics for energy production than leaves, which, in general, have greater protein contents.

Only two of the six genotypes had regression in the factor %DM: Mercker 86 México (G3), and Roxo Botucatu (G6), wherein the model with the best fit for both was the 2nd-degree one ($P < 0.01$) (**Figure 1(i)**). The estimates of the regression models that best fitted the morpho-agronomic traits of the elephant grass genotypes are shown in **Table 2**.

Table 3 specifies the obtained coefficients of determination of the regression and the significance levels by the F test for the morpho-agronomic traits of six genotypes of elephant grass. Among many studies conducted to evaluate the elephant grass' response to nitrogen fertilization, these show an increase in DMY as the N level was increased [9]-[12] [29]-[31].

The fertilization factor was highly effective in terms of changing the morpho-agronomic traits of the elephant grass genotypes for energy purposes. The genotype that displayed the best performance for most traits was G4 (Cameroon Piracicaba), which had considerable relevance in the analysis of regression, in the test of comparison of means (Tukey), and in the analysis of variance, especially for the stem/leaf ratio. For instance, the DMY ob-

Table 2. Estimate of the regression models for the morpho-agronomic traits of six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

VARIABLE	G1	G2	G3	G4	G5	G6
DMY	NO	NO	NO	1 st DEGREE	NO	NO
SDMY	NO	NO	NO	1 st DEGREE	NO	NO
LBDMY	NO	NO	NO	1 st DEGREE	NO	NO
NT	NO	NO	NO	1 st DEGREE	NO	NO
HGT	2 nd DEGREE	2 nd DEGREE	2 nd DEGREE	2 nd DEGREE	NO	2 nd DEGREE
SDM	2 nd DEGREE	NO	1 st DEGREE	1 st DEGREE	1 st DEGREE	1 st DEGREE
LBW	2 nd DEGREE	2 nd DEGREE	1 st DEGREE	1 st DEGREE	2 nd DEGREE	1 st DEGREE
%STEM	NO	NO	NO	NO	NO	NO
%DM	NO	NO	2 nd DEGREE	NO	NO	1 st DEGREE

DMY = dry matter yield, in $t \cdot ha^{-1}$; SDMY = stem dry matter yield, in $t \cdot ha^{-1}$; LBDMY = leaf blade dry matter yield, in $t \cdot ha^{-1}$; NT = number of tillers per meter; HGT = plant height, in meters; SDM = stem diameter, in millimeters; LBW = leaf blade width, in centimeters; %STEM = percentage of stem; %DM = percentage of dry matter.

Table 3. Estimate of the coefficients of determination (R^2) of regression and significance levels by the F test for the morpho-agronomic traits of six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

VARIABLE	G1	G2	G3	G4	G5	G6
DMY	ns	ns	ns	(73.89%)**	ns	ns
SDMY	ns	ns	ns	(63.27%)**	ns	ns
LBDMY	ns	ns	ns	(84.63%)**	ns	ns
NT	ns	ns	ns	(90.36%)**	ns	ns
HGT	(37.04%)*	(26.69%)*	(45.45%)**	(41.93%)**	ns	(24.56%)*
SDM	(18.62%)*	ns	(26.79%)*	(33.82%)**	(24.03%)*	(25.18%)*
LBW	(79.65%)**	(66.88%)**	(33.72%)*	(35.23%)**	(69.55%)**	(27.29%)**
%STEM	ns	ns	ns	ns	ns	ns
%DM	ns	ns	(70.57%)**	ns	ns	(60.93%)**

DMY = dry matter yield, in $\text{t}\cdot\text{ha}^{-1}$; SDMY = stem dry matter yield, in $\text{t}\cdot\text{ha}^{-1}$; LBDMY = leaf blade dry matter yield, in $\text{t}\cdot\text{ha}^{-1}$; NT = number of tillers per meter; HGT = plant height, in meters; SDM = stem diameter, in millimeters; LBW = leaf blade width, in centimeters; %STEM = percentage of stem; %DM = percentage of dry matter. ** = significant at 1% probability by the F test; * = significant at 5% probability by the F test; ns = not significant.

tained at the N level of $800 \text{ kg}\cdot\text{ha}^{-1}$ in a 10-month cut was $52.8 \text{ t}\cdot\text{ha}^{-1}$, which was higher than the $38.7 \text{ t}\cdot\text{ha}^{-1}$ obtained by [32] at a N level of $1000 \text{ kg}\cdot\text{ha}^{-1}$ in 300 days, and also higher than the total $27.25 \text{ t}\cdot\text{ha}^{-1}$ obtained by [33] with two cuts, in the city of Campos dos Goytacazes.

The DMY of cv. “Guaçu/I.Z.2” was $45.2 \text{ t}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$, referring to cuts 1 and 3, differed from the results obtained by [11] in experiments in the cities of Brotas ($49.5 \text{ t}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) and Nova Odessa ($30.9 \text{ t}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) using lower levels of nitrogen. The DMY obtained in cut 2, $34.2 \text{ t}\cdot\text{ha}^{-1}$, in a 10-month period, diverges from [11] and [16], who obtained high yields for cv. “Guaçu/I.Z.2”, which produced over $50 \text{ t}\cdot\text{ha}^{-1}$ in the same period of time, at a lower level of nitrogen. High yields for genotype Guaçu I/Z were found in the study of [20], with over $50 \text{ t}\cdot\text{ha}^{-1}$ dry matter. A DMY of $49.48 \text{ t}\cdot\text{ha}^{-1}$ was obtained by [11] in a cutting interval of 6 months in São Paulo State.

Through this experiment, we could observe that genotypes Cameroon Piracicaba (G4), with maximum DMY of 60.97 t with optimal fertilization of $1995.64 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$, and Guaçu I/Z2 (G5), with 44.10 t and optimal fertilization of $662.18 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$, have great potential for use with energy purposes among the studied genotypes. Genotype Guaçu I/Z2 (G5) obtained the equivalent of 72.33% of the productivity of genotype Cameroon Piracicaba G4, requiring only 33.18% of the total fertilizer used by the latter. Therefore, it can be concluded that genotype Guaçu I/Z2 (G5) has great potential, since with twice the area planted with genotype G5, the optimal fertilization (X-Optimal) would increase from $662.18 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$ to $1324.36 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$, which is also lower than the X-Optimal of genotype G4 in a hectare with $1995.64 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}$ (Figure 1(a)).

Thus, elephant grass is able to capture and absorb nitrogen, which can represent a great gain to its morpho-agronomic traits. Because it is a species of fast growth and high yields, the burning of elephant grass shows great potential to be an alternative source of energy, and the search for varieties for biomass production adapted to different regions will soon intensify. According to [34], the increase in global prices of fossil fuels lead to an escalation in the price of products and the development of alternative energy sources should solve this problem because bioenergy production is aimed at obtaining maximum yield, with proper quality.

However, as shown in this study, these genotypes have great potential to be used for energy purposes, e.g., Cameroon Piracicaba (G4) and Guaçu I/Z2 (G5), and some of these have their own features that make them more likely to other purposes, as is the case of genotype G6 (Roxo Botucatu), whose use in animal production and ruminant feeding is widespread.

3.2. Biomass Quality Traits

For the biomass quality traits shown in Table 4—percentage of neutral detergent fiber (%NDF), percentage of

Table 4. Estimate of the mean squares (MS) and their significance levels for the biomass quality traits of six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

MS	SV					
	G	N	G × N	MEAN	CV (G) (%)	CV (N) (%)
%NDF	19.63**	5.83**	2.15 ^{ns}	76.73	2.02	1.45
%ASH	2.05*	0.38 ^{ns}	0.30 ^{ns}	4.19	18.58	12.83
%ADF	62.07**	7.75**	3.07 ^{ns}	48.07	4.21	2.93
%LIG	18.17**	0.13 ^{ns}	0.56 ^{ns}	8.71	10.93	7.19
%CEL	21.24**	13.83**	1.70*	38.93	3.35	2.34
%HEM _{NDFLIGCEL}	12.22**	1.75*	0.94*	29.09	2.46	2.47
%HEM _{NDFADF}	13.00**	0.26 ^{ns}	0.88 ^{ns}	28.66	2.80	2.58

%NDF = percentage of neutral detergent fiber; %ASH = percentage of ash; %ADF = percentage of acid detergent fiber; %LIG = percentage of lignin; %CEL = percentage of cellulose; %HEM_{NDFLIGCEL} = percentage of hemicellulose based on NDF, lignin, and cellulose; %HEM_{NDFADF} = percentage of cellulose based on NDF and ADF. ** = significant at 1% probability by the F test; * = significant at 5% probability by the F test; ns = not significant.

ash (%ASH), percentage of acid detergent fiber (%ADF), percentage of lignin (%LIG) and percentage of cellulose based on NDF and ADF (%HEM_{NDFADF})—the genotype × nitrogen interaction had no effect ($P > 0.05$), indicating interdependence among the factors. For the remaining traits—percentage of cellulose (%CEL) and percentage of hemicelluloses based on NDF, lignin and cellulose (%HEM_{NDFLIGCEL})—however, the interaction had a significant effect ($P < 0.05$). For main effects of the biomass quality traits genotype and nitrogen, however, fertilization had no significant effect ($P > 0.05$) on %ASH, %LIG and %HEM_{NDFADF}, but it had a significant impact ($P < 0.01$), on all other traits except %HEM_{NDFLIGCEL}, on which the effect was ($P < 0.05$).

With regard to the genotypes variable, all biomass-quality traits were affected significantly ($P < 0.01$) except %ASH, on which the effect was ($P < 0.05$).

With the exception of %HEM_{NDFLIGCEL}, the genetic coefficient of variation was always higher than the experimental coefficient of variation in all biomass-quality traits. As in the morpho-agronomic characters, this results shows that variations involving genotype have a greater magnitude than those involving nitrogen levels.

Regarding the regression analysis, analyzing the %NDF, only genotypes Mercker 86 México (G3) and Cameroon Piracicaba (G4) showed no regression. The others displayed the following ranking according to their coefficients of determination: Roxo Botucatu (G6) with a significance level of 1% by the F test, 2nd degree model, and $R^2 = 96.06\%$; Cubano Pinda (G1) with a significance level of 5% by the F test, 2nd degree model, and $R^2 = 88.17\%$; Mercker Pinda México (G2) at a significance level of 1% by the F test, 1st degree model, and $R^2 = 82.94\%$; and Guaçu I/Z2 (G5) with a significance level of 5% by the F test, 2nd degree model, and $R^2 = 69.02\%$.

According to [24], the analysis of NDF estimates the total concentration of cellulose, hemicellulose, and lignin of the cell wall. According to [28], the NDF content is inversely related to the dry matter intake capacity, which means that the as this estimated value is reduced, the expected intake increases. With these data, we note that genotype Roxo Botucatu (G6) is the most promising for animal breeding programs and ruminant feeding, as it has a large amount of fibers described by the NDF content.

Table 5 and **Table 6** and **Figure 1(j)**, below, show the regression analysis of the %NDF factor as well as the significance levels for the genotypes evaluated in this study:

In %NDF for level N1, the genotype with higher average was G2, and at levels N2, N3, N4, and N5, the genotype with higher %NDF was G3 ($P < 0.05$). For %ASH, at levels N1, N2 and N3, there was no significant difference between the means ($P > 0.05$). At level N4, however, the genotypes that obtained the highest mean were G4 and G6. At N5, however, the genotype with higher %ASH was achieved by genotype G6 ($P < 0.05$).

For %ADF, at N1, the genotype that showed the highest mean was G2, and for the other levels—N2, N3, N4, and N5—the genotype with higher %ADF was G3 ($P < 0.05$).

The genotypes that best stood out for %LIG at level N1 were G2 and G1, while the worst result was found for

Table 5. Estimate of the regression models for the biomass-quality trait %NDF in six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

VARIABLE	G1	G2	G3	G4	G5	G6
%NDF	2 nd DEGREE	1 st DEGREE	NO	NO	2 nd DEGREE	2 nd DEGREE

%NDF = percentage of neutral detergent fiber.

Table 6. Estimate of the coefficients of determination (R^2) of regression and significance levels by the F test for the biomass-quality variable %NDF in six genotypes of elephant grass: Cubano Pinda (G1), Mercker Pinda México (G2), Mercker 86 México (G3), Cameroon Piracicaba (G4), Guaçu I/Z2 (G5), and Roxo Botucatu (G6).

VARIABLE	R ² VALUES AND F TEST					
	G1	G2	G3	G4	G5	G6
%NDF	(88.17)*	(80.21)**	ns	ns	(69.02)*	(96.06)**

** = significant at 1% probability by the F test; * = significant at 5% probability by the F test; ns = not significant.

G5, classified as “c”. At level N2, the highest mean were achieved with genotypes G3 and G2 and the worst average results were obtained by G5 and G4. For the N3 level, the highest value was shown by G3, classified as “a”, and the worst, by G4, classified as “d”. With N4, the highest %LIG was found in genotypes G6, G3 and G2, while the worst result was shown by G5, which was classified as “c”. And for level N5, the highest mean were found with G3 and G2, classified as “a”, and the worst with G4, classified as “c” ($P < 0.05$).

At level N1, the genotype with highest value for %CEL was G2, classified as “a”, whereas the worst mean value at this level was found with G6, which was classified as “c”. For all other levels—N2, N3, N4 and N5—the genotype that obtained the highest value of all was G3. The worst mean values were: for N2, genotype G6; for N3, G4 and G6; for N4, G5; and for N5, genotype G6 ($P < 0.05$).

Concerning %HEM_{NDFLIGCEL} at levels N1 and N4, the genotype that best stood out was G5. At N2, the best was G6; at N3, genotypes G6 and G4 obtained the highest %HEM_{NDFLIGCEL}; and for level N5, G4 ($P < 0.05$). By the other forms of calculating the percentage of hemicellulose (%HEM_{NDFADF}), at levels N1 and N2, the mean values of the genotypes did not differ statistically ($P > 0.05$). At levels N3, N4 and N5, the genotypes with highest %HEM_{NDFADF} were G4 and G5, together ($P < 0.05$).

In terms of maximum productivity achieved by addition of increasing levels of nitrogen and regarding the biomass quality traits evaluated in the six genotypes studied here, the following three genotypes are noteworthy: Guaçu I/Z2 (G5), Cameroon Piracicaba (G4) and Cubano Pinda (G1). Genotype Guaçu I/Z2 (G5) achieved classification “a” at all levels, in the following biomass quality traits: %ASH, %HEM_{NDFLIGCEL} and %HEM_{NDFADF}. Its maximum production, however, regarding the evaluated biomass quality traits %NDF, %ADF, %CEL, was achieved with a N level of kg·ha⁻¹. Trait %ASH was optimal at the N level of 800 kg·ha⁻¹, whereas the traits %LIG, %HEM_{NDFLIGCEL}, %HEM_{NDFADF} responded best at the N level of 1600 kg·ha⁻¹.

Genotype Cameroon Piracicaba (G4) obtained maximum production in the following biomass quality traits with the respective levels: %NDF, %ADF, %LIG, and %CEL with 100 kg·N·ha⁻¹; %ASH with 800 kg·N·ha⁻¹; and %HEM_{NDFLIGCEL} and %HEM_{NDFADF} with 1800 kg·N·ha⁻¹. This genotype was classified as “a” at all levels for the following traits: %ASH, %HEM_{NDFLIGCEL} and %HEM_{NDFADF}.

Genotype Cubano Pinda (G1) obtained maximum production in the following biomass traits with the respective nitrogen levels: %NDF, %CEL, %HEM_{NDFLIGCEL}, and %HEM_{NDFADF} with 200 kg·ha⁻¹; %ASH with 1800 kg·ha⁻¹; and %ADF and %LIG with 100 kg·ha⁻¹. This genotype did not achieve classification “a” at all levels for the evaluated biomass quality traits.

4. Conclusion

The results of the present study show the high potential of the elephant grass cultivars for biomass production according to the nitrogen fertilization aiming to meet the increasing demand for energy. Of the tested cultivars, genotypes Cameroon Piracicaba (G4) and Guaçu I/Z2 (G5) are the most promising for biomass production. We found that for the biomass quality traits, except %HEM_{NDFLIGCEL}, the genetic coefficient of variation was always greater than the experimental coefficient of variation. This outcome shows that variations involving genotype

have a greater magnitude than those involving nitrogen in morpho-agronomic traits. Elephant grass is a tropical grass with great potential, and these studies can be applied to other countries in the tropical region as well. As a proposal for future lines of research, it is suggested that elephant grass genotypes should be evaluated according to fertilization with NPK as well as its fractioning into tip-stem with topsoil fertilization and presence of irrigation.

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