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Quantitative and qualitative characteristics of elephant grass (*Pennisetum purpureum* Schum) clones in the semi-arid lands of Pernambuco (Brazil)¤

Características cuantitativas y cualitativas de clones pasto elefante (Pennisetum purpureum Schum) en las tierras semi-áridas de Pernambuco (Brasil)

Características quantitativas e qualitativas de clones de capim-elefante (Pennisetum purpureum Schum) no semi-árido pernambucano (Brasil)

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Summary

Background: the efficient use of good quality forage represents one of many ways to improve animal productivity and, consequently, reduce the feed costs of dairy farming. Between the wide variety of studies aiming to improve the nutritional value of forage, histological studies, allow for both the comparison of species or cultivars and the monitoring of tissue aging within the plant. **Objective:** the present work aimed to characterize the stem morphology of *Pennisetum* clones (Itambé IV-46, Itambé I-1.20, Itambé I-1.4, Milheto x Buaçu/112-23.4, Cuba-116-29.3, CAC-262-12.102, Roxo of Botucatu x CAC-282-18.29, Taiwan-146-2.6, Itambé I-1.5, Pusa Napier or 419-76 x Buaçu/122-11.2, Taiwan-146-2.03, Taiwan-146-2.85, Itambé II-2.46, Pusa Napier or 419-76 x Cuba-116-12.3 and Pusa Napier or 412-76 x Buaçu/122-8.22) into three strata (basal, medium and apical) and three tillers of the plant using histological sections. **Methods:** the material was collected in a previously established area at the Experimental Station of São Bento do Una at the Agronomic Institute of Pernambuco. The materials were distributed in a completely randomized 15 x 3 x 3 factorial design (14 clones and one hybrid, three layers of stem and three tillers). The samples were collected during

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the dry season beginning in August 2008. **Results:** there were significant differences (p<0.05) among the clones evaluated, and the average values for the lignified cells in the cortex region ranged from 2.21 to 4.21 for the Taiwan-146-2.6 and Roxo of Botucatu x CAC-282-18.29 clones; however, this was not different from the other clones in the medullary region. The Itambé II-2.46 clone showed the highest absolute value in the percentage of phloem in the cortex region (2.32%) and a high value, with significant differences, in the medullary region (1.59%) compared to the other clones. **Conclusion:** the highest values of cellulose in the medium and apical regions of the studied stems represent a benefit to grazing animals.

Key words: *cellulose, hemicellulose, histological, lignin.*

Resumen

Antecedentes: el uso eficiente de forraje de buena calidad es una de las muchas maneras de mejorar la productividad animal y por lo tanto reducir el costo de la alimentación del ganado lechero. Entre la variedad de estudios que permiten mejorar el valor nutritivo del forraje, los estudios histológicos se destacan, porque permiten tanto la comparación de especies o cultivares y el seguimiento del envejecimiento de los tejidos con la madurez de la planta. **Objetivo:** el presente trabajo tuvo como objetivo caracterizar la morfología del tallo de los clones de *Pennisetum* (Itambé IV-46, I-Itambé 1.20, Itambé I-1.4, Milheto x Buaçu/112-23.4, Cuba-116-29.3, el CAC-262-12.102, Roxo de Botucatu x CAC-282-18.29, Taiwán-146-2.6, Itambé I-1.5, Pusa Napier o 419-76 x Buaçu/122-11.2, Taiwan-146-2.03, Taiwán-146-2.85, Itambé II-2.46 , Pusa Napier 419-76 x Cuba-116-12.3 y Napier Pusa o 412-76 x Buaçu/122-8.22) en tres estratos (basal, medio y apical) y tres tallos de la planta con los cortes histológicos. **Métodos:** el material se recogió en una zona ya establecida en la Estación Experimental de São Bento do Una en el Instituto Agronómico de Pernambuco. Los materiales se distribuyeron en un diseño factorial completamente al azar de 15 x 3 x 3 (14 clones y un híbrido, tres capas de la madre y los tallos de tres). Las muestras fueron recolectadas durante la estación seca comenzando en agosto de 2008. **Resultados**: hubo diferencias significativas (p<0,05) entre los clones evaluados, y los valores promedio de las células lignificadas en la región de la corteza variaron desde 2,21 hasta 4,21 para los clones Taiwán-146-2.6 y Roxo de Botucatu X CAC-282-18.29, sin embargo, esto no fue diferente de los otros clones en la región medular. El clon Itambé II-2.46 mostró el mayor valor absoluto en el porcentaje de floema en la región de la corteza (2,32%) y un alto valor, con diferencias significativas, en la región medular (1,59%) en comparación con los otros clones. **Conclusión:** los valores más altos de celulosa en las regiones media y apical de los tallos estudiados representa un beneficio para los animales de pastoreo.

Palabras clave: *celulosa, hemicelulosa, histológico, lignina.*

Resumo

Antecedentes: o uso eficiente de forragem de boa qualidade representa uma das muitas maneiras de melhorar a produtividade animal e, consequentemente, reduzir os custos de alimentação da pecuária leiteira. Entre a variedade de estudos com o objetivo de melhorar o valor nutritivo da forragem, os estudos histológicos destacam-se, o que permite tanto a comparação de espécies ou cultivares e acompanhamento do envelhecimento dos tecidos com a maturidade da planta. **Objetivo:** o presente trabalho teve como objetivo caracterizar a morfologia do caule de *Pennisetum* clones (IV-46 Itambé, Itambé I-1.20, Itambé I-1.4, Milheto x Buaçu/112-23.4, Cuba-116-29.3, CAC-262-12,102, Roxo de Botucatu x CAC-282-18,29, Taiwan-146-2.6, Itambé I-1.5, Pusa Napier ou 419-76 x Buaçu/122-11.2, Taiwan-146-2.03, Taiwan-146-2.85, Itambé II-2,46 , Pusa Napier ou 419-76 x Cuba-116-12.3 e Pusa Napier ou 412-76 x Buaçu/122-8.22) em três estratos (basal, média e apical) e três perfilhos da planta, utilizando cortes histológicos. **Métodos:** o material foi coletado em uma área já estabelecida na Estação Experimental de São Bento do Una, no Instituto Agronômico de Pernambuco). Os materiais foram distribuídos em um inteiramente casualizado 15 x 3 x fatorial 3 (14 clones e um híbrido, três camadas de tronco e três perfilhos). As amostras foram coletadas durante a estação seca início em agosto de 2008. **Resultados:** houve diferenças significativas (p<0,05) entre os clones avaliados, e os valores médios para as células lignificadas na região do córtex variou 2,21-4,21 para o Taiwan-146-2,6 e Roxo de Botucatu X CAC-282-18,29 clones, no entanto, isto não era diferente dos outros clones da região medular. O clone II-Itambé 2,46 apresentaram o maior valor absoluto da percentagem de floema na região córtex (2,32%) e um valor elevado, com diferenças significativas, na região medular (1,59%) em comparação com os outros clones. **Conclusão:** os valores mais elevados de celulose nas regiões média e apical das hastes estudadas contribuir para características positivas para animais em pastejo.

Palavras chave: *cellulose, hemicellulose, histológico, lignina.*

Introduction

The efficient use of good quality forage represents one of many ways to improve animal productivity and, consequently, reduce feed costs of dairy farming. Among the diversity of forage species, elephant grass (*Pennisetum purpureum* Schum) is noteworthy due to its high productive potential, for presenting various kinds of genetic materials such as varieties and clones, and for expanding its adaptability to very diverse climate conditions (Cóser *et al.*, 1989). Between the variety of studies aiming to improve the nutritional value of forage and to understand the factors limiting their digestibility, histological studies, allow for both the comparison of species or cultivars and the monitoring of tissue aging with plant maturity, are noteworthy. Currently, plant anatomy has become an important tool for various grass studies, allowing the correlation of structures with chemical composition and utilization by the animal (Ferreira *et al.*, 2007, 2010). The cell wall constituents, cellulose and hemicellulose, represent most of the available substrates for rumen fermentation and are the main energy source for ruminants. However, the presence of lignin influences the digestibility of these compounds (Alves de Brito *et al.,* 2002).

Qualitative and quantitative anatomy studies of the forage can contribute to a better understanding of the factors involved in ruminant tissue digestion. Generally, grasses have great genetic variability regarding anatomical configuration and tissue degeneration (Grabber *et al.,* 2004). According to Mello *et al.* (2002), the proportion of sclerenchyma is the anatomical characteristic most correlated with dry matter digestibility. Positive and significant correlations were observed between the proportion of parenchyma sheath in the vascular bundles, lignified vascular tissue and sclerenchyma to neutral detergent fiber, acid detergent fiber, and lignin, while the proportions of mesophyll and epidermis correlated negatively with these components. In this way, anatomical characterization of a forage species and its organs complement previous knowledge and contributes to a better understanding of the factors involved in their digestion by ruminants.

The objective of this study was to structurally characterize the stem morphology of elephant grass clones in three layers (basal, medium, and apical region) and three tillers of the plant.

Materials and methods

The experiment was conducted at the experimental station of São Bento de Una, at the Agronomic Institute of Pernambuco (AIP), located in the São Bento do Una municipality (latitude -8.52º, longitude 36.46º and an altitude of 631 m). The histological analysis was performed at the Animal Nutrition Laboratory, Federal Rural University of Pernambuco/Academic Unit of Garanhuns (UFRPE/UAG) in Garanhuns, PE (Brazil).

The rainfall data observed during the experimental period were collected at the Meteorological Station of PCD (LAMEPE/ITEP), São Bento do Una (Table 1).

Table 1. Average monthly rainfall in São Bento do Una during clone growth.

	Months (2008)			
	May	June	July	August
Rainfall (mm)	141.1	33.8	62.8	42.8

The sectors consisted of a single clump with 1 m x 1 m spacing, and fertilization was performed with 100 kg P2O5, according to soil analysis. The vegetable material was collected using the method of direct cut in August 2008, at the beginning of the dry season in the region. The samples constituted of fresh stems –corresponding to the basal tillers– and were collected at random. Each tiller was divided into three strata (basal, medium, and apical region) considering the first (basal) and the last (apical) visible knots on the tiller. Samples were identified and stored for 48 hours in polyethylene jars containing fixation solution composed of 90% alcohol (50%), 5% acetic acid, 5% formaldehyde. Next, each sample was removed from the solution, washed in distilled water, and reimmersed in 70% alcohol for future histological evaluations.

The stem dehydration began by gradually removing water with the purpose of avoiding cellular plasmolysis. For each sample, cuts were made at approximately 7 µm on a manual microtome and the sections were incubated in a Pisgah solution diluted to 1:8 (Tolivia and Tolivia, 1987) and individually placed between a slide and a coverslip for future measurements. The images were captured by a video camera attached to the microscope and used to determine the amount of lignin and cellulose in the cortex and medulla regions of the stem. The number of red and blue cells of the general view from the cortex and from the medullar region was evaluated through a score system from 1 to 5 (Ferreira *et al.*, 2007), with 1 indicating regions completely covered by red blood cells (rich in lignin) and 5 indicating regions fully covered by blue cells (rich in cellulose) between vascular bundles with the help of three trained raters. The Sigma Scan Pro 5 program was used to measure the proportions of each tissue found in stem (% phloem in the cortex and medullar region, % of xylem and sclerenchyma in the cortex and medullar region, and % of parenchyma in the cortex and medullar region).

Fourteen clones of elephant grass (Itambé IV-46, Itambé I-1.20, Itambé I-1.4, Cuba-116-29.3, CAC-262-12.102, Roxo of Botucatu x CAC-282-18.29, Taiwan-146-2.6, Itambé I-1.5, Pusa Napier or 419-76 x Buaçu/122-11.2, Taiwan-146-2.03, Taiwan-146-2.85, Itambé II-2.46, Pusa Napier or 419-76 x Cuba-116-12.3 and Pusa Napier or 412-76 x Buaçu/122-8.22), and one hybrid (Milheto x Buaçu/112-23.4) were distributed in a completely randomized 15 x 3 x 3 factorial design (14 clones and one hybrid, three layers of stem, and three tillers) according to the model:

$$Y_{ijk} = \mu + C_i + P_j + E_k + C_i{*}P_j + e_{ijklm}$$

where:

Y_{ijk} = all observations effect.

μ = general constant.

C_i = clone and hybrid i effect.

i = 1, 2, 3…15.

P_j = random variable effect – tiller j.

j = 1, 2 and 3.

E_k = stratum k effect.

k = 1, 2 and 3.

$C_i{*}E_k$ = effect of the interaction between clone i and stratum k.

e_{ijkl} = random error associated with each observation Y_{ijk}.

The data were submitted a Tukey test yielding 5% probability when the T test was significant.

Results

The clone factor presented a significant effect ($p<0.05$) over most of the evaluated histological characteristics (Table 2). The average values for the lignified cells in the cortex region ranged from 2.21 to 4.21 for clones Taiwan-146-2.6 and Roxo of Botucatu CAC-282-X 18.29, respectively. There was no difference ($p>0.05$) for the values of lignified cells in the medullary region (Table 2). It was observed that clone Taiwan-146-2.85 cells showed virtually no lignin in the cell wall.

Table 2. Morpho-anatomical evaluation of the elephant-grass clone stems.

Clones	Variables									
	CRC[1]	RCMR[2]	PHLOC (%)[3]	PHLOM (%)[4]	XSFC (%)[5]	XSFM (%)[6]	PARCR+ SCL(%)[7]	PARMR + SCL(%)[8]	STEM DIAM[9]	RADIUS (mm)
Itambé IV-46	3.39^{ab}	4.70^{a}	2.07^{a}	1.72^{ab}	16.19^{ab}	9.77^{abc}	82.19^{a}	88.50^{ab}	12.50^{ab}	6.25^{ab}
Itambé I-1.20	3.44^{ab}	4.65^{a}	2.29^{a}	1.85^{ab}	15.25^{b}	10.07^{abc}	82.44^{a}	76.96^{ab}	13.74^{a}	6.87^{a}
Itambé I-1.4	3.44^{ab}	4.77^{a}	1.92^{a}	1.29^{ab}	15.13^{b}	7.97^{abc}	83.90^{a}	90.27^{ab}	11.49^{abc}	5.74^{abc}
Milheto X Buaçu/112-23.4	3.55^{ab}	4.33^{a}	1.93^{a}	1.80^{ab}	29.23^{a}	11.59^{ab}	78.28^{a}	86.59^{ab}	10.23^{bcd}	5.11^{bcd}
Cuba-116-29.3	3.80^{ab}	4.94^{a}	1.76^{a}	1.73^{ab}	20.74^{ab}	10.35^{abc}	77.93^{a}	87.91^{ab}	13.54^{a}	6.77^{a}
CAC-262-12.102	2.77^{ab}	4.81^{a}	1.81^{a}	1.33^{ab}	19.67^{ab}	8.06^{abc}	78.44^{a}	90.58^{ab}	12.92^{ab}	6.46^{ab}
Roxo de Botucatu	4.21^{a}	4.93^{a}	1.68^{a}	2.11^{a}	12.36^{b}	13.60^{a}	85.96^{a}	84.28^{ab}	12.25^{ab}	6.12^{ab}
Taiwan-146-2.6	2.21^{b}	4.62^{a}	1.62^{a}	1.24^{ab}	14.69^{b}	6.83^{bc}	83.68^{a}	69.74^{b}	6.78^{ef}	3.39^{ef}
Itambé I-1.5	2.64^{ab}	4.77^{a}	1.44^{a}	1.26^{ab}	10.63^{b}	5.94^{bc}	76.80^{a}	92.79^{a}	6.31^{ef}	3.15^{ef}
Pusa Napier or 419-76 X Buaçu/122-11.2	3.72^{ab}	4.66^{a}	1.52^{a}	1.40^{ab}	9.94^{b}	8.51^{abc}	88.52^{a}	90.08^{ab}	7.75^{def}	3.87^{def}
Taiwan-146-2.03	2.72^{ab}	4.16^{a}	1.71^{a}	1.17^{ab}	13.96^{b}	5.42^{c}	84.05^{a}	91.40^{ab}	5.80^{f}	2.90^{f}
Taiwan-146-2.85	3.96^{a}	4.92^{a}	1.81^{a}	0.94^{b}	14.66^{b}	7.42^{bc}	83.35^{a}	93.07^{a}	7.79^{def}	3.89^{def}
Itambé II-2.46	2.35^{a}	4.12^{a}	2.32^{a}	1.59^{ab}	15.40^{b}	9.64^{abc}	82.26^{a}	88.75^{ab}	6.81^{ef}	3.40^{ef}
Pusa Napier or 419-76 X Cuba-116-12.3	3.12^{ab}	4.14^{a}	1.4030^{a}	1.12^{ab}	7.66^{b}	6.90^{bc}	89.36^{a}	91.97^{a}	7.89^{def}	3.94^{def}
Pusa Napier or 412-76 X Buaçu/122-8.22	3.42^{ab}	4.71^{a}	1.46^{a}	1.32^{ab}	16.39^{ab}	10.87^{abc}	82.66^{a}	87.76^{ab}	8.82^{cde}	4.41^{cde}
Effects*										
Clones	0.00022	0.18059	0.04769	0.02455	0.00010	0.00005	0.10259	0.02478	0.00000	0.00000
Tiller	******	******	0.16774	*******	*******	*******	*******	0.04257	0.20538	0.20538
Region	0.0000	******	0.00000	0.09540	*******	0.12398	0.06609	*******	0.00000	0.00000
Clone*Region	0.0000	******	0.16774	*******	*******	*******	0.43540	*******	*******	*******

[1]CRC= Cortex red cells; [2]RCMR= red cells in the medullary region; [3]PHLOC= % of phloem in the cortex region; [4]PHLOM= % of phloem in the medullary region; [5]XSFC= % of xylem plus sclerenchyma fibers in the cortex region; [6]XSFM= % of xylem plus sclerenchyma fibers in the medullary region; [7]PARCR + SCL= % of parenchyma plus sclerenchyma in the cortex region; [8]PARMR + SCL= % of parenchyma plus sclerenchyma in the medullary region; [9]STEM DIAM= stem diameter (mm). Same superscripts in a row indicate no difference in Tukey test (P>0.05).

Even though it was not significant, the clone Itambé II-2.46 presented a higher concentration of phloem in the cortex region (2.32%) and significantly higher values ($p<0.05$) in the medullar region (1.59%). The Itambé II-2.46 clone (Table 2) also showed a low percentage of xylem and sclerenchyma fibers, both in the region of the cortex (15.40%) and in the medullary region (9.64%) in relation to the hybrid Milheto X Buaçu/112-23.4 (29.23%) for the cortex region and no significant difference ($p>0.05$) either in the medullary region (11.59%). The Itambé II-2.46 clone also showed high percentages of parenchyma and sclerenchyma tissues, both in the region of the cortex and in the medullar region (Table 2), even though the same clone showed low values for stem diameter and length of the radius.

Average values showed (Table 3) that all clones had a higher cellulose concentration ($p<0.05$) in the apical and medium strata, associated with higher levels of lignin in the basal layer. The Itambé II-2.46 and Pusa Napier or 412-76 X Buaçu/122-8.22 clones presented higher values ($p<0.05$) of phloem in the apical stratum in relation to the other strata, and at the same time did not differ in all strata of the medullary region (Table 3).

There were few significant differences ($p<0.05$) between tillers in the studied variables (Table 4).

Table 3. Morpho-anatomical evaluation of the stem strata from the elephant-grass clones.

Stratum	Variables									
	CRC[1]	RCMR[2]	PHLOC[3]	PHLOM[4]	XSFC[5]	XSFM[6]	PARCR[7]	PARMR[8]	STEM DIAM[9]	RADIUS (mm)
Itambé IV-46										
Apical	4.75[a]	4.80[a]	2.66[a]	2.11[a]	17.73[a]	11.36[a]	83.19[a]	89.90[a]	12.99[a]	6.49[a]
Medium	3.93[a]	4.74[a]	1.85[a]	1.68[a]	16.33[a]	9.54[a]	82.83[a]	89.07[a]	12.60[a]	6.30[a]
Basal	1.50[b]	4.58[a]	1.70[a]	1.37[a]	14.50[a]	8.40[a]	80.55[a]	86.52[a]	11.93[a]	5.96[a]
Itambé I-1.20										
Apical	5.00[a]	3.97[a]	3.49[a]	2.05[a]	14.66[a]	5.82[b]	81.83[a]	58.78[b]	12.14[a]	6.07[a]
Medium	4.33[a]	5.00[a]	1.74[b]	2.01[a]	14.86[a]	12.74[a]	83.48[a]	85.23[a]	13.68[a]	6.84[a]
Basal	1.00[b]	5.00[a]	1.64[b]	1.48[a]	16.23[a]	11.64a[b]	82.02[a]	86.86[a]	15.40[a]	7.70[a]
Itambé I-1.4										
Apical	5.00[a]	5.00[a]	3.03[a]	0.98[a]	16.61[a]	4.67[b]	82.99[a]	94.33[a]	10.89[a]	5.44[a]
Medium	4.00[a]	5.00[a]	1.69[b]	1.61[a]	14.72[a]	10.00[a]	83.82[a]	88.44[a]	10.59[a]	5.29[a]
Basal	1.33[b]	4.33[a]	1.04[b]	1.29[a]	14.06[a]	9.22[a]	84.91[a]	88.05[a]	12.99[a]	6.49[a]
Milheto X Buaçu/112-23.4										
Apical	4.33[a]	4.33[a]	2.48[a]	1.72[a]	24.32[b]	10.23[a]	73.19[a]	88.03[a]	9.53[a]	4.76[a]
Medium	3.66[a]	4.66[a]	1.63[a]	1.82[a]	43.58[a]	11.40[a]	83.15[a]	86.77[a]	9.07[a]	4.53[a]
Basal	2.66[a]	4.00[a]	1.67[a]	1.87[a]	19.80[b]	13.14[a]	78.52[a]	84.97[a]	12.09[a]	6.04[a]
Cuba-116-29.3										
Apical	4.97[a]	4.99[a]	1.57[a]	1.91[a]	23.01[a]	11.09[a]	75.41[a]	86.98[a]	12.73[a]	6.36[a]
Medium	4.00[ab]	4.99[a]	1.76[a]	1.46[a]	21.13[a]	8.86[a]	77.09[a]	89.67[a]	12.62[a]	6.31[a]
Basal	2.43[b]	4.85[a]	1.96[a]	1.82[a]	18.09[a]	11.09[a]	81.30[a]	87.07[a]	15.26[a]	7.63[a]
CAC-262-12.102										
Apical	3.66[a]	4.49[a]	2.14[a]	1.13[a]	17.59[a]	5.76[a]	80.25[a]	93.09[a]	11.43[a]	5.71[a]
Medium	3.61[a]	5.00[a]	2.00[a]	1.66[a]	19.92[a]	8.59[a]	77.86[a]	89.74[a]	13.48[a]	6.74[a]
Basal	1.04[b]	4.95[a]	1.30[a]	1.21[a]	21.48[a]	9.85[a]	77.22[a]	88.92[a]	13.86[a]	6.93[a]
Roxo de Botucatu X CAC-282-18.29										
Apical	4.91[a]	4.91[a]	2.24[a]	2.56[a]	10.84[a]	13.89[a]	86.91[a]	83.53[a]	11.39[a]	5.69[a]
Medium	4.88[a]	4.96[a]	1.23[a]	2.20[a]	9.90[a]	16.63[a]	88.92[a]	81.16[a]	11.91[a]	5.95[a]
Basal	2.85[b]	4.93[a]	1.58[a]	1.57[a]	16.35[a]	10.26[a]	82.05[a]	88.15[a]	13.43[a]	6.71[a]
Taiwan-146-2.6										
Apical	3.81[a]	4.00[a]	2.18[a]	0.95[a]	15.29[a]	4.26[a]	82.52[a]	61.44[b]	5.93[a]	2.96[a]
Medium	1.58[b]	5.00[a]	1.46[a]	1.49[a]	15.74[a]	8.48[a]	82.78[a]	56.70[b]	6.96[a]	3.48[a]
Basal	1.25[b]	4.87[a]	1.22[a]	1.28[a]	13.04[a]	7.74[a]	85.73[a]	91.08[a]	7.46[a]	3.73[a]
Itambé I-1.5										
Apical	4.55[a]	5.00[a]	1.51[a]	1.98[a]	7.83[a]	5.72[a]	57.31[b]	92.29[a]	5.39[a]	2.69[a]
Medium	2.27[b]	4.66[a]	0.99[a]	0.78[a]	11.09[a]	5.05[a]	87.91[a]	94.16[a]	6.37[a]	3.18[a]
Basal	1.08[b]	4.66[a]	1.81[a]	1.01[a]	12.98[a]	7.06[a]	85.19[a]	91.92[a]	7.17[a]	3.58[a]
Pusa Napier or 419-76 X Buaçu/122-11.2										
Apical	4.77[a]	4.94[a]	1.58[a]	1.52[a]	6.19[a]	8.11[a]	92.22[a]	90.36[a]	6.58[a]	3.29[a]
Medium	3.55[ab]	4.33[a]	1.57[a]	1.45[a]	12.52[a]	8.08[a]	85.90[a]	90.46[a]	7.86[a]	3.93[a]
Basal	2.83[b]	4.72[a]	1.40[a]	1.23[a]	11.12[a]	9.34[a]	87.46[a]	89.42[a]	8.82[a]	4.41[a]
Taiwan-146-2.03										
Apical	3.88[a]	4.83[a]	1.90[a]	1.58[a]	15.86[a]	7.77[a]	82.22[a]	90.64[a]	4.39[a]	2.19[a]
Medium	3.05[ab]	3.38[b]	1.54[a]	0.97[a]	10.47[a]	8.97[a]	87.98[a]	90.03[a]	6.03[a]	3.01[a]
Basal	1.22[b]	4.27[ab]	1.69[a]	0.95[a]	15.55[a]	5.51[a]	81.97[a]	93.53[a]	6.97[a]	3.48[a]
Taiwan-146-2.85										
Apical	5.00[a]	5.00[a]	2.28[a]	1.12[a]	20.77[a]	4.13[a]	76.45[a]	93.08[a]	6.70[a]	3.35[a]
Medium	3.88[ab]	4.83[a]	1.30[a]	0.90[a]	8.80[a]	7.00[a]	89.89[a]	92.08[a]	7.96[a]	3.98[a]
Basal	2.99[a]	4.94[a]	1.86[a]	0.79[a]	14.41[a]	5.14[a]	83.72[a]	94.05[a]	8.72[a]	4.36[a]
Itambé II-2.46										
Apical	4.83[a]	4.94[a]	3.62[a]	1.81[a]	16.09[a]	9.40[a]	80.28[a]	88.78[a]	5.82[a]	2.91[a]
Medium	1.22[b]	3.44[b]	1.29[b]	1.45[a]	13.88[a]	10.25[a]	84.82[a]	88.29[a]	7.13[a]	3.56[a]
Basal	1.00[b]	4.00[ab]	2.06[b]	1.51[a]	16.24[a]	9.28[a]	81.68[a]	89.19[a]	7.48[a]	3.74[a]
Pusa Napier or 419-76 X Cuba-116-12.3										
Apical	5.00[a]	4.90[a]	2.29[a]	1.14[a]	20.77[a]	4.13[a]	76.45[a]	93.08[a]	6.70[a]	3.35[a]
Medium	3.88[ab]	4.83[a]	1.31[a]	0.90[a]	8.80[a]	7.00[a]	89.89[a]	92.08[a]	7.96[a]	3.98[a]
Basal	2.99[a]	4.94[a]	1.86[a]	0.80[a]	14.41[a]	5.14[a]	83.72[a]	94.05[a]	8.72[a]	4.36[a]
Pusa Napier or 412-76 X Buaçu/122-8.22										
Apical	4.83[a]	4.94[a]	3.62[a]	1.81[a]	16.09[a]	9.40[a]	80.28[a]	88.78[a]	5.82[a]	2.91[a]
Medium	1.22[b]	3.44[b]	1.29[b]	1.45[a]	13.88[a]	10.25[a]	84.82[a]	88.29[a]	7.13[a]	3.56[a]
Basal	1.00[b]	4.00[ab]	2.06[b]	1.51[a]	16.24[a]	9.28[a]	81.68[a]	89.19[a]	7.48[a]	3.74[a]

[1]CRC= Cortex red cells; [2]RCMR= red cells in the medullary region; [3]PHLOC= % of phloem in the cortex region; [4]PHLOM= % of phloem in the medullary region; [5]XSFC= % of xylem plus sclerenchyma fibers in the cortex region; [6]XSFM= % of xylem plus sclerenchyma fibers in the medullary region; [7]PARCR + SCL= % of parenchyma plus sclerenchyma in the cortex region; [8]PARMR + SCL= % of parenchyma plus sclerenchyma in the medullary region; [9]STEM DIAM= stem diameter (mm). Same superscripts in a row indicate no difference in Tukey test (P>0.05).

Table 4. Histological characterization of elephant-grass stems in three tillers of the plant.

Tillers	Variables									
	CRC[1]	RCMR[2]	PHLOC[3]	PHLOM[4]	XSFC[5]	XSFM[6]	PARCR[7]	PARMR[8]	STEM DIAM[9]	RADIUS (mm)
Itambé IV-46										
Primary	3.50[a]	4.87[a]	2.45[a]	1.82[a]	13.87[a]	10.41[a]	83.66[a]	87.75[a]	13.61[a]	6.80[a]
Secondary	3.48[a]	4.80[a]	2.15[a]	1.70[a]	18.20[a]	9.61[a]	81.03[a]	88.68[a]	12.98[a]	6.49[a]
Tertiary	3.20[a]	4.45[a]	1.62[a]	1.64[a]	16.49[a]	9.29[a]	81.88[a]	89.05[a]	10.92[a]	5.46[a]
Itambé I-1.20										
Primary	3.33[a]	4.00[a]	2.77[a]	2.73[a]	16.16[a]	14.28[a]	81.06[a]	82.97[ab]	16.31[a]	8.15[a]
Secondary	3.33[a]	4.97[a]	2.00[a]	1.64[ab]	17.42[a]	9.00[ab]	80.56[a]	89.34[a]	13.53[ab]	6.76[ab]
Tertiary	3.66[a]	5.00[a]	2.10[a]	1.17[b]	12.18[a]	6.92[b]	85.70[a]	58.56[b]	11.38[b]	5.69[b]
Itambé I-1.4										
Primary	3.66[a]	5.00[a]	1.97[a]	1.28[a]	15.16[a]	7.83[a]	82.85[a]	89.43[a]	12.37[ab]	6.18[ab]
Secondary	3.00[a]	4.33[a]	1.71[a]	0.99[a]	12.53[a]	6.74[a]	85.99[a]	92.34[a]	9.28[b]	4.64[b]
Tertiary	3.66[a]	5.00[a]	2.08[a]	1.61[a]	17.69[a]	9.33[a]	82.88[a]	89.05[a]	12.82a	6.41[a]
Hybrid Milheto X Buaçu/112-23.4										
Primary	3.00[a]	3.66[a]	1.76[a]	1.58[a]	21.03[b]	9.63[a]	77.19[a]	88.77[a]	10.81[ab]	5.40[ab]
Secondary	4.33[a]	4.66[a]	1.56[a]	1.58[a]	19.45[b]	11.11[a]	78.97[a]	87.29[a]	8.20[b]	4.10[b]
Tertiary	3.33[a]	4.66[a]	2.45[a]	2.25[a]	47.22[a]	14.03[a]	78.69[a]	83.71[a]	11.69[a]	5.84[a]
Cuba-116-29.3										
Primary	4.97[a]	5.00[a]	2.10[a]	1.78[a]	22.15[a]	12.03[a]	75.77[a]	86.18[a]	13.85[a]	6.92[a]
Secondary	3.66[ab]	4.95[a]	1.65[a]	1.79[a]	19.32[a]	8.25[a]	79.02[a]	89.95[a]	11.91[a]	5.95[a]
Tertiary	2.76[b]	4.87[a]	1.54[a]	1.62[a]	20.76[a]	10.76[a]	79.02[a]	87.60[a]	14.85[a]	7.42[a]
CAC-262-12.102										
Primary	3.00[a]	4.82[a]	2.09[a]	1.34[a]	21.69[a]	7.79[a]	76.01[a]	90.85[a]	10.65[b]	5.32[b]
Secondary	2.64[a]	4.66[a]	1.71[a]	1.26[a]	20.52[a]	7.48[a]	77.75[a]	91.23[a]	14.26[a]	7.13[a]
Tertiary	2.68[a]	4.95[a]	1.64[a]	1.41[a]	16.79[a]	8.92[a]	81.57[a]	89.66[a]	13.86[ab]	6.93[ab]
Roxo de Botucatu X CAC-282-18.29										
Primary	4.44[a]	4.91[a]	1.67[a]	2.53[a]	10.35[a]	14.95[ab]	87.97[a]	82.50[a]	10.69[b]	5.34[b]
Secondary	3.97[a]	4.92[a]	1.66[a]	2.17[a]	13.00[a]	16.64[a]	85.39[a]	81.17[a]	11.59[ab]	5.79[ab]
Tertiary	4.24[a]	4.97[a]	1.72[a]	1.63[a]	13.74[a]	9.19[b]	84.52[a]	89.16[a]	14.46[a]	7.23[a]
Taiwan-146-2.6										
Primary	2.12[a]	4.66[a]	1.64[a]	2.32[a]	15.49[a]	11.66[a]	82.85[a]	86.01[a]	7.92[a]	3.96[a]
Secondary	2.35[a]	4.33[a]	1.78[a]	1.07[ab]	18.85[a]	6.48[ab]	79.36[a]	92.46[a]	6.76[a]	3.38[a]
Tertiary	2.16[a]	4.87[a]	1.44[a]	0.33[b]	9.73[a]	2.34[b]	88.81[a]	30.76[b]	5.67[a]	2.83[a]
Itambé I-1.5										
Primary	2.38[a]	4.71[a]	1.80[ab]	0.91[a]	12.55[a]	5.13[a]	85.64[a]	93.94[a]	6.22[a]	3.11[a]
Secondary	2.53[a]	4.93[a]	0.61[b]	1.87[a]	7.18[a]	6.27[a]	58.86[b]	91.84[a]	6.00[a]	3.00[a]
Tertiary	2.99[a]	4.68[a]	1.91[a]	0.99[a]	12.17[a]	6.42[a]	85.91[a]	92.58[a]	6.71[a]	3.35[a]
Pusa Napier or 419-76 X Buaçu/122-11.2										
Primary	4.16[a]	4.33[a]	1.23[a]	1.30[a]	10.83[a]	10.13[a]	87.92[a]	88.55[a]	7.68[a]	3.84[a]
Secondary	3.66[a]	4.94[a]	1.65[a]	1.66[a]	7.24[a]	8.96[a]	91.09[a]	89.37[a]	8.61[a]	4.30[a]
Tertiary	3.33[a]	4.72[a]	1.67[a]	1.23[a]	11.76[a]	6.44[a]	86.56[a]	92.31[a]	6.97[a]	3.48[a]
Taiwan-146-2.03										
Primary	2.66[a]	3.72[a]	1.57[a]	0.80[a]	13.41[a]	5.76[a]	85.01[a]	93.43[a]	4.57[a]	2.28[a]
Secondary	2.55[a]	3.77[a]	1.32[a]	1.12[a]	14.05[a]	7.50[a]	84.62[a]	91.34[a]	6.71[a]	3.35[a]
Tertiary	2.94[a]	5.00[a]	2.24[a]	1.58[a]	14.42[a]	8.99[a]	82.53[a]	89.42[a]	6.12[a]	3.06[a]
Taiwan-146-2.85										
Primary	2.66[b]	4.77[a]	1.77[a]	0.87[a]	20.01[a]	3.77[a]	77.72[a]	93.69[a]	8.82[a]	4.41[a]
Secondary	5.00[a]	5.00[a]	1.82[a]	0.81[a]	11.51[a]	5.81[a]	86.66[a]	93.36[a]	7.27[a]	3.63[a]
Tertiary	4.22[ab]	5.00[a]	1.85[a]	1.13[a]	12.46[a]	6.70[a]	85.68[a]	92.16[a]	7.29[a]	3.64[a]
Itambé II-2.46										
Primary	2.33[a]	4.50[a]	2.30[a]	1.27[a]	16.80[a]	8.96[a]	80.89[a]	89.76[a]	7.45[a]	3.72[a]
Secondary	2.33[a]	4.11[a]	2.03[a]	1.61[a]	14.25[a]	10.15[a]	83.70[a]	88.23[a]	6.11[a]	3.05[a]
Tertiary	2.38[a]	3.77[a]	2.64[a]	1.90[a]	15.15[a]	9.82[a]	82.19[a]	88.27[a]	6.88[a]	3.44[a]
Pusa Napier or 419-76 X Cuba-116-12.3										
Primary	2.77[a]	3.61[a]	1.52[a]	0.87[a]	5.72[a]	3.65[a]	88.05[a]	95.47[a]	9.01[a]	4.50[a]
Secondary	3.55[a]	5.00[a]	1.56[a]	1.27[a]	8.11[a]	8.45[a]	90.31[a]	90.27[a]	6.53[a]	3.26[a]
Tertiary	3.05[a]	3.83[a]	1.11[a]	1.21[a]	9.14[a]	8.60[a]	89.73[a]	90.17[a]	8.13[a]	4.06[a]
Pusa Napier or 412-76 X Buaçu/122-8.22										
Primary	4.05[a]	5.00[a]	1.49[a]	1.32[a]	15.33[a]	10.19[a]	84.74[a]	88.39[a]	9.23[a]	4.61[a]
Secondary	3.44[a]	5.00[a]	1.33[a]	1.09[a]	19.44[a]	10.10[a]	79.22[a]	88.79[a]	9.49[a]	4.74[a]
Tertiary	2.77[a]	4.16[a]	1.55[a]	1.55[a]	14.40[a]	12.32[a]	84.03[a]	86.11[a]	7.73[a]	3.86[a]

[1]CRC= Cortex red cells; [2]RCMR= red cells in the medullary region; [3]PHLOC= % of phloem in the cortex region; [4]PHLOM= % of phloem in the medullary region; [5]XSFC= % of xylem plus sclerenchyma fibers in the cortex region; [6]XSFM= % of xylem plus sclerenchyma fibers in the medullary region; [7]PARCR + SCL= % of parenchyma plus sclerenchyma in the cortex region; [8]PARMR + SCL= % of parenchyma plus sclerenchyma in the medullary region; [9]STEM DIAM= stem diameter (mm). Same superscripts in a row indicate no difference in Tukey test (P>0.05).

Discussion

It is noteworthy that the time between cuts is a management factor that contributes to determining production and forage quality, as concluded by Gonçalves *et al.* (2002), who found that shorter time between cuts was associated with a higher nutritional value. Santos *et al.* (2008), working with five elephant-grass clones at 120 days old, observed values ranging from 2.73 to 3.85 for clones Taiwan-146-2.26 and Buaçu/112 x Cubra 116-15.2, respectively.

The cortex with higher lignin values was composed of non-digestible lignified sclerenchyma cells and parenchyma filler, characterized as living cells, showing lignified primary and secondary cell walls. Therefore, the degree of lignification of the cell wall is also a limiting factor in the digestibility of forage. However, according to Silva *et al.* (2006), histological studies have shown that tissues containing lignin are little or practically undegraded by rumen microorganisms. Although, in addition, some non-lignified tissues also have low ruminal digestion due to the binding of these components with low molecular weight molecules.

Phloem is a vascular tissue composed of sieve elements (living cells without nuclei and vacuoles, generally elongated, containing a primary wall with riddled areas), parenchymal cells (intermediate role of reservation) and fibers (sclerenchyma cells). The phloem allows for the fast transportation (around 1 m /h) of phloem sap, which contains approximately 250 mg of sugar/liter, other nutritious substances and hormones (Grenet, 1997).

The xylem is composed of lignified elements and may contain fibers, vessels and parenchyma cells (Larrosa and Duarte, 2005). The tracheids and xylem vessels are lignified water-conducting elements. Some tracheids are also supporting tissues. The xylem has the function of conducting crude sap and water. According to Esau (1965), the fibers and xylem sclerenchyma serve to support and store starch. Tissues formed by cells with a thick secondary wall, such as sclerenchyma and xylem, are the main contributors to the low quality of forage. Paciullo *et al.* (2002), studying three-stem forage from the C4 group, found that the xylem and sclerenchyma fibers did not disappear after 46 hours of *in vitro* incubation.

According to Álves de Brito and Rodella (2002), the largest diameter of the internode causes stem to be more resistant to seizure, suggesting a greater number of vascular bundles and, consequently, a higher percentage of lignified tissues. This is in agreement with Mello *et al.* (2002), who found in a study of 71 elephant-grass clones that the clones that showed larger diameters were more resistant to drought.

The stem diameter ranged from 5.80 to 13.74 mm for the Taiwan-146-2.03 and Itambé I-1.20 clones, respectively. However, the values were lower than those reported by Mello *et al.* (2002) for clones of smaller and larger diameter. The filler parenchyma cells have been characterized as living cells, generally polyhedral in shape, with lignified primary and secondary walls (Krans and Pisaneschi, 1998).

It was observed that all clones had a higher cellulose concentration in apical and medium strata (Table 3). This higher proportion of cellulose in the medium and apical strata is positive because, although animals show little selectivity in grazing due to how they perceive the food (through the oral apparatus, tongue and lower incisors), they tend to consume the higher parts of the plants formed by leaves and immature stems (Santos *et al.,* 2008).

Some clones had low percentages of combined xylem and sclerenchyma fibers in the three extracts tested. Conversely, the same clones showed high percentages of parenchyma plus sclerenchyma, both in the region of the cortex and in the medullar region. As cells increase with age, the deposition of phenolic compounds in the secondary wall cell in the formation of cell walls begins. However, there are certain cells that deposit only primary wall leading to a consequent lack of lamellae and lignin. These cells, located mainly in certain areas of parenchymal forage, present few problems regarding rumen degradation (Wilson and Mertes, 1995).

As observed in table 4, there were few significant differences ($p<0.05$) between tillers in the studied variables (e.g., in the proportion of lignified cells in the cortex and medullar region). These differences may have been derived from tillers in the plants of different ages and, therefore, with different degrees of lignification. As the tiller ages, there is greater deposition of lignin in cell walls. Paciullo *et al.* (2002) registered a significant increase in sclerenchyma areas with the development of the stems, and a greater proportion of xylem. In mature stems, the epidermis, sclerenchyma, xylem and the parenchyma cells near the sclerenchyma remain undigested. According to Alves de Brito *et al.* (2004), the degradation of tissue obeys the following order: parenchyma tissue > phloem > epidermis > parenchyma sheath cells > xylem and sclerenchyma. The decrease in parenchyma digestion, associated with the age of the stem, can be attributed to the progressive deposition of phenolic compounds in the cell walls.

The high values of cellulose in the middle and apical regions of the studied stems lead to positive characteristics for grazing animals if the basal region is not consumed by the animals. Among tillers studied, there was variation which yielded a consistent effect upon anatomical features. Depending upon the presence or absence of lignin and cellulose, clones evaluated differed with more pronounced proportions in the cortex region, highlighting a positive reaction to safranin for the presence of phenolic compounds in wall cells.

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