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Lista, Fábio Nunes; Neto, João Virgínio Emerenciano; Almeida, João Carlos de Carvalho;
Deminicis, Bruno Borges; Rocha, David Ramos da; Difante, Gelson dos Santos

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Nutritive value and anatomical characterization from *Pennisetum purpureum* genotypes

Fábio Nunes Lista^{1*}, João Virgínio Emerenciano Neto¹, João Carlos de Carvalho Almeida², Bruno Borges Deminici³, David Ramos da Rocha¹ and Gelson dos Santos Difante⁴

¹Universidade Federal do Vale do São Francisco, *Campus* Ciências Agrárias, Rodovia BR-407, Lote 543, Projeto de Irrigação Nilo Coelho, s/n, 56300-000, Petrolina, Pernambuco, Brasil. ²Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brasil. ³Universidade Federal do Sul da Bahia, Ilhéus, Bahia, Brasil. ⁴Universidade Federal do Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brasil. *Author for correspondence. E-mail: fabio.lista@univasf.edu.br

ABSTRACT. The research submitted samples from stems and leaf blades from tree genotypes of *Pennisetum purpureum* called 93-32-02, 92-70-02, and 91-06-02 (EMBRAPA - Dairy cattle) and elephant grass cv. Napier (reference cultivar) to the chemical, anatomical evaluations, and *in vitro* dry matter digestibility (IVDMD) measurement. The anatomical characteristics of the stems and leaf blades, the chemical composition, and the IVDMD of these genotypes at 70 days of re-growth were correlated. Concerning IVDMD, the data highlighted differences, and the cultivar Napier presented the smallest value. Digital images obtained by light microscopy from cross-section reveal that all the stem and leaf blade have similar structural organization. Quantitative differences were verified mainly in the stem. The leaves displayed differences only in the mesophyll thickness. The genotypes showed higher potential in the rainy season since they had the largest IVDMD when compared to the cultivar Napier.

Keywords: cell wall; elephant grass; fiber; grasses; leaf blade; stem.

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Introduction

The elephant grass has considerable potential as feed for dairy animals (Negawo, Teshome, Kumar, Hanson, & Jones, 2017). This grass adapted very well to the tropical cattle production systems, but still, it is crucial to study its growth, because its nutritional quality decreases in function of the accelerated increase in the production during the rainy season (Van Soest, 1994).

According to Oliveira, Santana Neto, and Valença (2013), the percentage of cell wall and resistance of the anatomical structures of the feed to ruminal digestion affect the intake and digestibility of the forage. This interference is due to the high percentage of the organic matter represented by the cell walls (35 to 80%).

The internal organization and proportion of the system tissues and cell types may influence the intake and the proportion of polysaccharides suitable for the microbial attack into the rumen. Microbial digestion occurs according to the nature of the produced particles, and the ruminal passage rate (Guimarães, 2010).

Macedo Júnior, Zanine, Borges and Pérez (2007) analyzed the relation between the elements of the cell wall and its relationship with the voluntary intake. The authors observed decreases in the intake associated with the increase of the cell wall fraction of the forage. The relationship among the anatomy of the above-ground vegetative organs (stems plus leaves), considering its organization, the relative proportion of tissues and the nutritional entities of the forage, highlighted highly significant correlations with the digestibility of the forage (Velásquez et al., 2010; Batistoti et al., 2012). However, the volume of information and the number of species studied are still scarce.

Therefore, this work evaluated the anatomical characteristics of the tree genotypic access of elephant grass and Napier grass as reference genotype, and correlate them with the *in vitro* dry matter digestibility and forage components.

Material and methods

The experiment was carried out in the district of Campos of Goytacazes, RJ – Brazil, in the unit of Animal Science research support, of the State University of the North Fluminense. The study analyzed the genotypes 93-32-02, 92-70-02, and 91-06-02 (EMBRAPA – Dairy Cattle) and the cultivar Napier. The plants were assessed during the rainy season, at 70 days of age after the standardization cut, performed at 20 cm above the ground.

The levels of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and lignin (LIG) were analyzed according to Association of Official Analytical Chemists (AOAC, 1990). The *in vitro* dry matter digestibility (IVDMD) followed the methodology described by Tilley and Terry (1963) with one incubation stage.

Samples of the stem (between the third and fourth nodes) and leaves blades (the last leaf completely expanded) of the three genotypes and Napier cultivar were selected for light microscopic analyses.

The stems were cut in segments of 0.5 to 1.0 cm length. The leaf blades were cut in segments of 0.5 cm width, and 1.0 cm length. The samples were immersed in a phosphate buffer solution 50 mM at pH 6.8 and later processed for observation by light microscopy. Cross-section of the leaf blades segments was obtained using a hand microtome and soon afterward colored with phloroglucinol acidified during five minutes (Johansen, 1940), followed by temporally mount in microscopic slides under distilled water and coverslip. The stem samples were colored with phloroglucinol acidified during 24 hours, in the containers where the stem slices were soaked. Later, from the colored piece of stems, using a steel cylindrical perforator, three sub-samples with 5.0 cm of diameter were obtained from the central area of the stem. These samples were used to obtain the number of vascular bunches per transverse section by using a magnifying glass. It was also obtained traverse section ($n=3$), manually, in the samples soaked in the solution, which were mounted in temporary microscopic slides with distilled water for the observation by optic microscopy. Therefore, three segments of the stems were measured with three repetitions for each genotype, totaling nine observations per genotype.

The digital photomicrographs were performed by an optic microscope Olympus coupled with a system of video-microscopy, through the software Image Pro-Plus® (Media Cybernetics, Rockville, USA). The analyzed variables were measured through the program Image J (Schneider, Rasband, & Eliceiri, 2012). For the leaf blades were measured, length of both epidermis layer, length of the mesophyll, and the total area of the vascular bunches (including the area of the sclerenchymatous tissue) around the xylem and phloem vessels). The analyzed variables of the stem were: the area occupied by parenchymatic tissue, number of vascular bunches, and the total area of the vascular bunches (including sclerenchymatous ring, xylem, and phloem tissue).

Tukey's test compared the averages of the segments of the stems, leaf blades, and chemical composition, as well as IVDMD at 5% significance. Linear correlations were estimated among the anatomical components, chemical composition, and IVDMD.

Results

The dry matter average accumulation was 25314.9 kg ha⁻¹. However, the DM production did not display significant differences ($p > 0.05$) among the genotypes and the Napier cultivar at 70 days of re-growth (Table 1).

The average lignin levels represented 7.47% of the dry matter, at 70 days of re-growth (Table 1). The results were not significantly different among the treatments ($p > 0.05$).

As to the lignin levels, the results obtained with NDF did not display significant differences among the treatments ($p > 0.05$). At 70 days of re-growth, the average accumulation of NDF in the dry matter was 71.43%.

The crude protein (CP) level displayed significative differences ($p < 0.05$) among the genotypes at 70 days of re-growth. The genotype 93-32-02 displayed the highest CP content compared to the other genotypes.

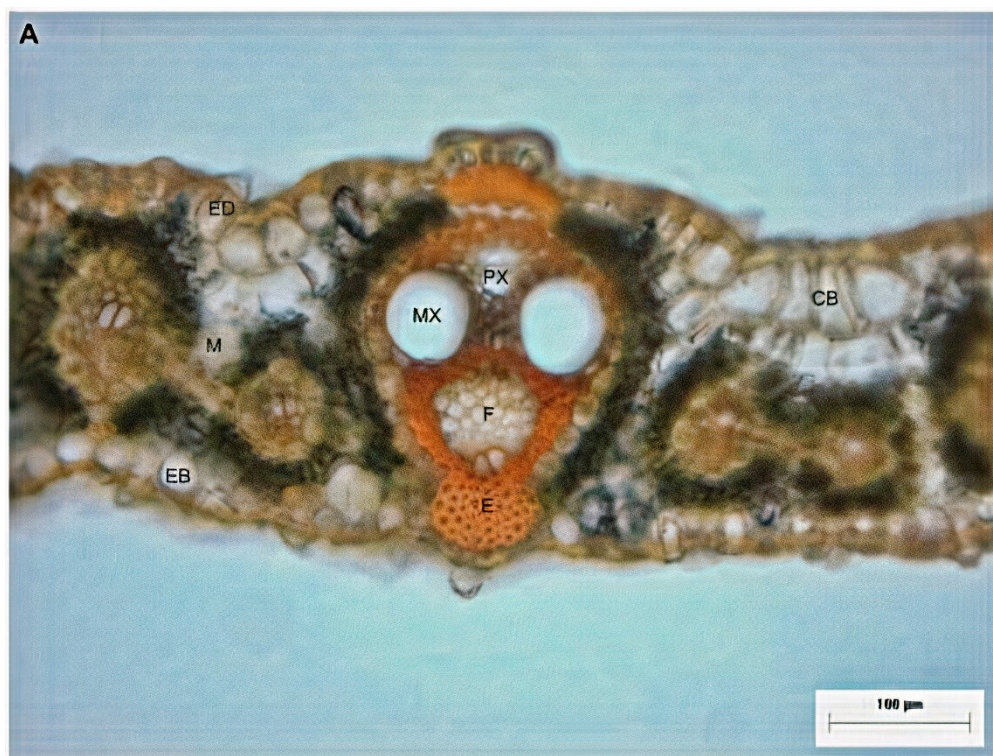
The average *in vitro* dry matter digestibility (Table 1) was 58.20%, displaying significative differences ($p < 0.05$) between the genotype 93-32-02 and the Napier cultivar. The digestibility of the genotype 93-32-02 was 11.26% higher than the cultivar Napier.

Table 1. Production and quality of forage of the three genotypes tested and cultivar of elephant grass with 70 days of re-growth.

Variables	Genotype				CV (%)
	91-06-02	92-70-02	93-32-02	Napier	
Forage accumulation (kg ha ⁻¹)	30034.6 a	24518.3 a	23937.3 a	22769.3 a	22.5
Neutral detergent fiber (%DM)	73.11 a	70.16 a	71.01 a	71.43 a	1.76
Lignin (%DM)	7.35 a	7.48 a	7.15 a	7.89 a	7.18
Crude protein (%DM)	9.92 b	9.94ab	10.73 a	9.44 b	2.84
In vitro dry matter digestibility (%)	56.77 ab	59.88 ab	63.71 a	52.45 b	5.43

Means in the line followed by different letters are different ($p < 0.05$) by Tukey's test.

In the present study, as expected, all of the four genotypes of the elephant grass presented the same anatomical organization of the leaf blade (Figure 1). Significant differences were obtained only for quantitative anatomical data related to the thickness of the mesophyll tissue (Table 2).

**Figure 1.** Light Microscopy. Anatomy of the leaf blade of elephant grass stained with acidified phloroglucinol. Central vascular tissue showing the lignin deposition at the sclerenchymatous cell wall and xylem vessels (red stain). Abbreviations: E, sclerenchyma; ED, epidermis adaxial layer; EB, abaxial epidermis layer; M, mesophyll; PX, protoxylem; MX, metaxylem; F, phloem; CB, buliform cells.**Table 2.** Anatomical measurements of specific tissues from the leaf blade of three genotypes and of cultivar Napier at 70 days of re-growth.

Variables	Genotype				CV (%)
	91-06-02	92-70-02	93-32-02	Napier	
Adaxial epidermis (μm)	25.00a	27.33a	25.33a	22.33a	13.47
Abaxial epidermis (μm)	28.00a	28.66a	25.00a	26.66a	8.55
Mesophyll (μm)	179.00a	146.66b	136.33b	190.66a	11.80
Total area (μm ²)	29835.0a	27642.3a	28699.6a	31462.6a	6.59
Sclerenchyma (μm ²)	15199.0a	13391.6a	15615.6a	16566.6a	8.12
Xylem (μm ²)	9190.3a	6565.6a	9162.6a	10556.3a	33.20
Phloem (μm ²)	2854.6a	3402.0a	2830.0a	3198.3a	13.14

Averages followed by different letters, in the line, differ statistically by the Tukey' test at 5%.

Regarding stem anatomy, all the genotypes of elephant grass presented the same structural organization (Figure 2). However, there were quantitative differences in the proportion of the different constituent tissues (Table 3).

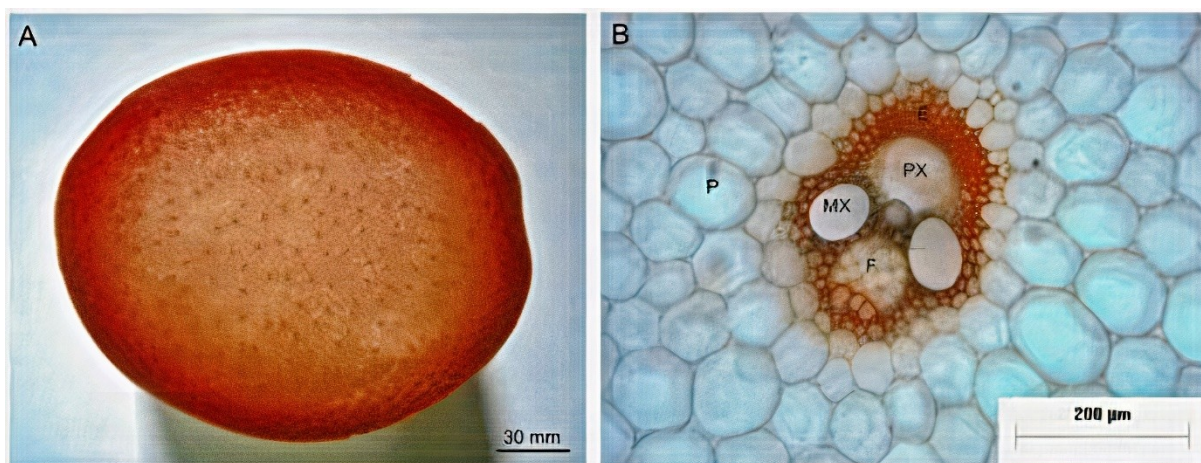


Figure 2. Light Microscopy. Anatomy of the stem of elephant grass stained with acidified phloroglucinol; (A) Traverse section of the stem, the periphery of the section showing numerous vascular bundles and; (B) Detail of a vascular bundle, evidencing the lignin (red coloration) deposition into the sclerenchymatous and xylem cell walls. Abbreviations: P, Ground parenchyma; E, sclerenchyma; MX, metaxylem; PX, protoxylem lacunae; F, phloem.

Table 3. Anatomical characteristics of the stem of different genotypes of elephant grass and the cultivar Napier at 70 days of re-growth.

Variables	Genotype				CV (%)
	91-06-02	92-70-02	93-32-02	Napier	
Parenchyma (μm^2)	18295349b	18200663b	18716757c	17848287a	0.71
Number of vascular bundle	36.6b	30.6b	21.0c	40.0a	9.7
Total area (μm^2)	1339604b	1434290b	918197c	1786666a	9.5
Sclerenchyma (μm^2)	604682b	604491b	465101c	803573a	9.8
Xylem (μm^2)	473684c	555628b	320446d	695213a	7.7
Phloem (μm^2)	129408a	149673a	120449a	182613a	17.8

Averages followed by different letters, in the line, differ statistically by the Tukey's test at 5%.

Tables 2 and 3 describe the anatomical features of the leaf blades and stems of the different genotypes and the Napier cultivar.

The proportion of mesophyll tissue of the elephant grass leaves, at 70 days of re-growth, was correlated negatively ($r = -0.73$) with IVDMD (Table 4). The parenchymatic ground of the stem displayed positive correlation coefficients ($r = 0.84$) and highly significant with IVDMD.

Table 4. Correlations among the anatomical components of the leaf blade and the stem of elephant grass (70 days of re-growth) with the chemical components and with the *in vitro* dry matter digestibility.

	NDF	LIG	IVDMD	NB	P	E	X	F	M
DM	0.97**	-0.46	0.07	0.21	0.17	-0.23	-0.26	-0.52*	0.16
NDF	-	-0.64*	0.25	0.07	0.27	-0.37	-0.36	-0.62*	0.02
LIG	-	-	-0.71*	0.37	-0.42	0.62*	0.42	0.61*	0.44
IVDMD	-	-	-	-0.87**	0.84**	-0.95**	-0.83**	-0.83**	0.73*

DM, dry matter; NDF, neutral detergent fiber; LIG, lignin; IVDMD, *in vitro* dry matter digestibility; NB, number of bunches; P, parenchyma; E, sclerenchyma; X, xylem; F, phloem; M, mesophyll. * ($p < 0.05$), ** ($p < 0.01$).

Discussion

The association between the average NDF and LIG levels suggests the correlation between the increase of the age and lignification. Digestibility correlates with the complexity of the insoluble fibrous matrix, and the lignification degree (Van Soest, 1994). Tropical forages convert the cellular content in structural components more quickly than the corresponding temperate climate species, reducing their digestibility (Van Soest, 1994).

Krutzmann et al. (2014) suggested the forage associations between IVDMD and NDF as indicators of the quality because the NDF increments are correlated negatively with the digestibility. However, according to Van Soest (1994) and Queiroz, Gomide and Maria (2000) when the association among lignification degree of the different plant cell wall types, contents of NDF and acid detergent fiber (ADF) was low, the content of fiber into the forage tissue was not a useful quality indicator.

Possibly, the best IVDMD, presented by the genotype 93-32-02, can be explained by the content of lignin and CP. Although the results did not display significant differences among the genotypes, Table 1 displayed that the cultivar Napier presented the most significant levels of lignin, mainly in comparison with the genotype 93-32-02, which presented the best in vitro digestibility.

Lignin is the main chemical component limiting the digestibility of the forage (Cavalcanti et al., 2016). The present work presented significantly negative correlation ($r = -0.71$) LIG and IVDMD, corroborating with the results of the literature, even if analyzed by different methodologies (Queiroz et al., 2000). In such direction, anatomical traits have been considered, such as the arrangement and thickness of the cell wall. The correlation between NDF and LIG (Table 4) was significant ($r = -0.64$) corroborating with Van Soest (1994) and Gallo, Moschini, Cerioli, and Masoero (2013), who demonstrated that there is an increase on the lignin content of fibrous portion.

Sanchês et al. (2018) analyzed the association of the degradability and their correlations with the anatomical components. The authors verified that the younger the forage, the higher the digestibility. They also noted that for leaf blades and stems of young forage, the values were higher; besides, smaller digestibility values were found for the stem and for stem associated with leaves, correlating smaller digestibility with the lignin presence, mainly in the oldest stems.

High significant correlations among the proportion of individual tissues (epidermis, ground and vascular tissues), or its combination, and the nutritional values suggested that, in general, plant tissues which digestion is rapid, such as the mesophyll tissue, display a positive correlation with the digestibility coefficients and the levels of CP, and a negative correlation with the levels of the cell wall (Sanchês et al., 2018). On the other hand, tissues resistant to digestion such as xylem vessels and sclerenchymatous tissue, or which digestion is partial and slow, as the parenchymatic bunches of the stems display a positive correlation with the lignin contents and a negative correlation with the digestibility (Table 4). Batistoti et al. (2012) corroborated this observation. Mauri et al. (2019) pointed out that, as there is a significant presence of xylem and sclerenchymatous tissue, these overcome the tissue with higher digestion potential and reduce the forage potential. Forages with higher proportions of xylem and sclerenchymatous tissue have a higher lignin deposition on the cell walls.

A monolayer epidermis with thickened and lignified external periclinal cell wall delimited the leaf blade of the four elephant grass genotypes that have been studied, with stomatic complex in both faces and cells. At the abaxial face, the epidermis presented buliform cells. The mesophyll tissue organization reflects the typical Kranz-type leaf anatomy, displaying cells that are associated with the vascular bundles (Liu, Maimaitijiang, Zhang, Ma, & Lan, 2020). The vascular bundles present collateral disposition surrounded by sclerenchymatous tissue and xylem differentiated into protoxylem and metaxylem. According to Ribeiro Júnior et al. (2017), a more extensive arrangement of the protoxylem may associate with the anatomy of the vascular cylinder, as the bundles of the metaxylem and phloem are smaller and do not occupy so much space.

The transverse section of the elephant grass stem highlighted numerous vascular bundles in the outlying area. In the central area, the bunches are dispersed in the fulfillment of parenchymatic tissue, characterizing the vascular system of the atactostele type (Figure 2, A). A sclerenchymatous vascular bundle entraps the collateral vascular one (Figure 2, B).

The proportion of sclerenchymatous tissue, number of vascular bundles, and the area occupied by parenchymatic ground tissue at the stems had displayed a negative correlation with IVDMD. The parenchyma tissue displayed high rates of degradation and occupied a large part of the area in the different organs and fractions, which is therefore crucial for the forage quality (Brito & Deschamps, 2001).

Parenchymatic ground correlation with IVDMD of the stem suggested that a decrease of the digestibility of the material may occur in those genotypes, where vascular bundles occupy a large proportion of the transversal area of the stems. It is fascinating to quote that studies of digestion of the different plant tissues, observed differences in the proportions of the tissues degraded as a function of the time of incubation (Paciullo, Gomide, Silva, Queiroz, & Gomide, 2002a).

The digestibility differences among the forages probably linked, among others, to tissue proportion and the arrangement of the cells that compose the organs of the plants. Paciullo, Gomide, Silva, Queiroz, and Gomide (2002b) found that in the residues of the digestion of the traverse sections of forage, the structure "girder" remained intact. Lempp (2007) corroborated the results when they analyzed different grasses. The same author verified that the structure "girder" was resistant to the digestion, even in leaf blades recently-expanded and that, those species, with a smaller incidence of the "girder" structure in the leaf blades,

displayed more significant rates of the tissues fragmentation when compared with those with high incidence.

Basso and Barbero (2015) pointed out the association between the digestibility and the anatomical components. The authors pointed out the relevance of the use of these characteristics in the programs of plants forages breeding aimed at the selection of species with higher digestibility.

Conclusion

This study pointed out no anatomical differences among the leaf blades of the four genotypes under investigation, except for the relative mesophyll proportion. As the anatomical characteristics of the stem, the genotypes that presented a larger area fulfilled by the vascular bundles resulted in smaller *in vitro* dry matter digestibility. The genotype 93-32-02 showed higher utilization potential when compared to cultivar Napier.

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