

Tropical forages: morphoanatomy of plants grown in areas with the death of pasture syndrome

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Abstract

Roots and leaves of *Panicum maximum* Tanzânia, Mombaça and Massai; *Urochloa brizantha* Piatã, Marandu and Xaraés; *Urochloa humidicola* Llanero; *Urochloa ruziziensis* Ruzizienses; *Urochloa hybrida* Mulato II and *Cynodon nlemfuensis* Estrela-roxa were analyzed, seeking to identify characters for better adaptation to the environment that may interfere with digestibility of tissue from the point of view of the rumen in cattle. Were planted ten cultivars in a completely randomized blocks with three repetitions. Was collected vegetative material, which histological slides were prepared from middle third of the sections of roots and leaves. Were observed differences ($p>0.05$) in the roots: higher volume of epidermal cells (28.62 μm) and overall diameter (1926.41 μm) of Llanero; thicker vascular cylinder (975.09 μm) and more protoxylem (42.25) in Estrela-roxa and occurrence of aerenchyma in cultivars Piatã, Mulato II, Xaraés, Massai, Llanero and Estrela-roxa; Were found higher proportions of bulliform cells in the leaves (121.07 μm) and thicker leaf mesophyll in *U. humidicola* Llanero (263.63 μm); higher proportion of sclerenchyma fibers in Xaraés and Marandu; lower results for amount of fibers in *P. maximum* Massai. We conclude that the cultivars Estrela-roxa, Llanero and Massai have greater adaptability to the environment and better nutritional quality.

Keywords: *Urochloa*, *Cynodon*, digestibility, *Panicum*.

Forrageiras tropicais: anatomia e morfometria de plantas cultivadas em áreas com síndrome da morte de pastagens

Resumo

Foram analisadas raízes e folhas de *Panicum maximum* Tanzânia, Mombaça e Massai; *Urochloa brizantha* Piatã, Marandu e Xaraés; *Urochloa humidicola* Llanero; *Urochloa ruziziensis* Ruzizienses; *Urochloa hybrida* Mulato II e *Cynodon nlemfuensis* Estrela-roxa, procurando identificar caracteres relacionados à melhor adaptação ao ambiente e à qualidade nutritiva das forrageiras. As dez cultivares foram semeadas em blocos inteiramente casualizados com três repetições. Após estabelecidas foi coletado material vegetativo, do qual lâminas histológicas foram confeccionadas a partir de seções do terço médio de raízes e folhas. Foram observadas diferenças significativas ($p>0,05$) nas raízes: maior volume de células epidérmicas (28,62 μm) e diâmetro total (1926,41 μm) de Llanero; em Estrela-roxa maior espessura do cilindro vascular (975,09 μm) e número maior de protoxilemas (42,25) e formação de aerênquimas nas cultivares Piatã, Mulato II, Xaraés, Massai, Llanero, e Estrela-roxa; Nas folhas, foram constatadas maiores proporções de células buliformes (121,07 μm) e mesófilo foliar mais espesso (263,63 μm) em Llanero; em Xaraés e Marandu maiores proporções de fibras esclerenquimáticas; em Massai menores resultados para quantidade de fibras. Conclui-se que as cultivares Estrela-roxa, Llanero e Massai apresentam maior adaptabilidade ao ambiente e melhor qualidade nutritiva.

Palavras-chave: *Urochloa*, *Cynodon*, digestibilidade, *Panicum*.

1. Introduction

The grasses are constituted by an intricate arrangement of roots, stem, leaves and inflorescences, and each structure has its genesis of tissues properly organized for particular functions (Valente et al., 2011). The tissues differ chemically and physically, depending on their role of assimilation, transportation, support or reserve accumulation (Queiroz et al., 2000).

The leaf anatomy influences not only the production of forage but also its nutritional value and the animal performance (Paciullo et al., 2002). The digestion of thin-walled cells like the foliar mesophyll and the phloem is faster than the cells of the xylem and the bundles of sclerenchyma fibers.

The anatomy of the organs of the plant and its constituent tissues have an influence on the consumption because of the effect they produce on the ease of fragmentation of the particles of the forages, the nature of the particles produced and their rate of passage through the rumen (Carvalho and Pires, 2008). The anatomical knowledge of the forage grasses may contribute to their best use in the ruminant feed (Brito and Deschamps, 2001).

The several studies reported in the literature and methodologies for the development of new cultivars evaluate the production of green and dry mass in the flowerbeds, digestibility of the aerial parts of the forage, resistance to soil waterlogging and pests, which requires time and a multidisciplinary team. By normally cover a large number of genotypes, the improvement programs mainly use the agronomic characteristics of the forage, to the detriment of the anatomical and morphophysiological, in the selection of genotypes (Gomes et al., 2011). This generates little knowledge about the internal structural characteristics of the cultivars.

The low nutritional value of tropical forages is reported in the literature (Gerdes et al., 2000), based mainly by the higher proportion of fibers in relation to the low content of crude protein and minerals, when the comparison is made to natural grass from temperate areas. Knowing the anatomy along with the chemical composition and the digestibility may improve the comprehension about the factors that limit the utilization of the forage by ruminants (Queiroz et al., 2000).

In this paper we attempted to describe anatomically and morphometrically the roots and the leaf blades of ten cultivars of forage grasses sown in area of sudden death pasture (DPS), noting characters that may be related to a better adaptation of the plant to the environment and / or that may interfere in the digestibility of the tissues, from the point of view of the ruminal degradation in cattle.

2. Materials and Methods

2.1. Study area

The forages *Panicum maximum* Jacq, cultivars: Tanzânia, Mombaça e Massai; *Urochloa brizantha* Hochst Stapf, cultivars: BRS Piatã, Marandu and Xaraés;

Urochloa humidicola (Rendle) Schweick cv. Llanero; *Urochloa ruziziensis* Germain et Evrard cv. Ruzizienses; *Urochloa hybrida* cv. Mulato II and *Cynodon nlemfuensis* Vanderyst (Estrela-roxa or Estrela-roxa-africana) were cultivated in the Experimental area of the University of the State of Mato Grosso in partnership with EMBRAPA Beef Cattle - Sinop, in 2014.

The site is located in the extreme north of Mato Grosso, southern Amazon, in latitude of 9°51'03"S, longitude of 56°12'35"E and altitude of 255 m. The region is part of the South American Amazon depression, with a humid continental equatorial climate and presents two well-defined seasons: dry winter, from May to July, and rainy summer. The annual rainfall can exceed 2,300 mm m⁻² and the average temperature is 24.8 °C (SEPLAN, 2006).

2.2. Experimental delineation

The experimental delineation used for planting was the randomized blocks, with three repetitions for each cultivar. The plants were planted in blocks an area previously used with an extensive system of pasture with *U. brizantha* cv. Marandu for 12 years. The grass in this area had focus of death by the death of pastures syndrome (DPS). The area of the experiment, as well as 5 meters of its surroundings, was desiccated with the use of the glyphosate herbicide, followed by the disking for the soil decompression and subsequent liming for the correction of the soil chemical properties. Thirty fractions were assembled, measuring 5.0 m long and 6.0 m wide each, with the distance of 1.0 m between them. Once the area was prepared, the portions were numbered and randomly selected one at a time for seeding of the 10 forages. From the established plants, about 40 days after seeding, the pruning cycles at 10 cm above the soil were initiated, and they were repeated every 28 days. After four cutting cycles (pruning), in April 2014, the collection of plant material for an anatomic analysis was performed. With a hoe, a clump was withdrawn in each fractions, respecting the distance of 1 meter from the edge. Four root samples were removed from these portions, and the organs with the largest diameter of each cultivar were selected. Eight leaves of each cultivar per portion were also cultivated, always standardizing by the second leaf of the tiller. This material passed through the fixing process in FAA 50 (formaldehyde, glacial acetic acid and ethanol 50% 5:5:90, v/v), it was maintained for 48 hours in a vacuum pump, and later was stored in ethanol at 70% (Johansen, 1940) in the Laboratory of Vegetal Biology of the University of the State of Mato Grosso, campus of Alta Floresta.

2.3. Laboratory procedure

Transverse sections of the middle third region of the roots and leaves were obtained freehand with a razor blade, discolored with the use of sodium hypochlorite at 2%, stained with double staining, Astra blue and basic fuchsin (Roeser, 1962) and mounted in permanent and semi-permanent histological slides.

For the analysis of the foliar epidermis, paradermic cuts in the adaxial and abaxial surface were made freehand with a razor blade. The dissociation method Kraus and Arduin (1997) modified was used, in which the leaf portions were placed in test tubes with hydrogen peroxide (30 volumes) and glacial acetic acid in the proportion of 1: 1 and they were kept in a greenhouse at 60 °C for about 48 hours. After this period, the samples were washed in distilled water and ethanol 50%. With a paint brush, the two epidermal surfaces were separated, and then they were stained with basic fuchsin (Roeser, 1962) and mounted in glycerinated gelatine.

For a histochemical analysis it was used the same material selected for anatomical study, as well as fresh samples. Tests using lugol were carried out on leaves and roots for the detection of starch (Johansen, 1940), phloroglucinol for the detection of lignin and suberin (Jensen, 1962) and toluidine blue for mucilage (O'Brien et al., 1965).

The slides were analyzed in a trinocular photonic microscope Leica® ICC50, coupled to a computer, and photomicrographs were recorded with the assistance of the software LAZ EZ 1.7.0.

In the images qualitative and quantitative anatomical characters were analyzed. They quantitatively assessed: (1) diameter of the root; (2) thickness of the root epidermis; (3) count of cores of protoxylem; (4) diameter of the vascular cylinder of the root; (5) proportion of the stomata and (6) subsidiaries cells; (7) size of the bulliform cells; (8) thickness of the foliar mesophyll; (9) thickness of the group of fibers of the abaxial and adaxial foliar rib; (10) diameter of the central vascular bundle of the foliar blade. Were qualitatively evaluated: (1) characteristics of epidermic cells; (2) cuticle; (3) thickness of the endoderm; (4) thickness of the exodermis; (5) hypodermis; (6) trichomes; (7) mesophyll; (8) vascular and extravascular fibers and (9) vascular bundles.

From the photomicrographs, the tissues were measured and the stomatal count was done through the Anati Quanti

program 2® UFV (Aguilar et al., 2007). For the calculation of the stomatal density, it was used the following formula: density = number of stomata mm² per leaf area. For the statistical analysis there were selected four images of transversal sections of root, four images of paradermic sections and four images of transversal sections of leaf per portion of collect, which totaled 12 repetitions per cultivar and 120 independent variables for each character analyzed quantitatively.

The data were subjected to analysis of variance by the F test and the averages were compared by the Tukey test at 5% of probability (Ferreira, 2011). Posteriorly, images of each cultivar were selected and boards were set up to evidence anatomical patterns and differences among cultivars.

3. Results and Discussion

During the collection of the vegetal material, it was observed a large volume of roots with reduced diameter in the first 10 cm of soil, in the cultivars Tanzania and Mombaça. It was also noted the presence of a large amount of absorbent hairs in the roots of larger caliber of Estrela-roxa. Such characteristics may arise as a response to the anoxia in depth. These replacement roots usually emerge from the aereal base and/or in superficial layers of the soil (Vartapetian and Jackson, 1997).

From transversal sections, the roots of Llanero stood out with a larger diameter and a larger volume of epidermic cells compared to the other cultivars (Table 1).

It was registered unistratified epidermis, with cells with irregular size and shape, with walls slightly thickened in the ten cultivars analyzed (Figure 1a, 1b, 1c), which are common characteristics to the Poaceae (Alquini et al., 2003).

The cortex, bulky in the studied species, was composed of parenchyma of filling arranged in layers. Below the epidermis it was observed a layer of cells with thin walls, larger in Tanzania (Figure 1b). Exodermis, isodiametric cells with thickened walls with two well-defined layers was

Table 1. Morphometry of the middle third of the root of the ten cultivars of tropical forage. Epidermis (Epi), diameter of the root (Root diam), diameter of the vascular cylinder (VC diam), protoxylem (n), coefficient of variation (CV) and simple medium deviation (SMD).

Cultivar	Epi (µm)	Root diam (µm)	VC Diam (µm)	Protoxylem (n)
Tanzânia	20.57bc	1663.36ab	912.38ab	36.83b
Mombaça	18.47bc	1327.02c	765.24bc	34.33b
Llanero	28.62a	1926.41a	670.54cd	28.16cd
Piatã	23.27b	1373.10bc	632.70cd	31.91bc
Ruzzienses	19.36bc	879.22e	416.81e	22.00e
Mulato II	22.12bc	1237.46cd	553.04de	28.91cd
Massai	18.83bc	945.61de	523.73de	25.16de
Marandu	19.89bc	1661.73ab	892.65ab	35.50b
Xaraés	21.73bc	1407.13bc	679.22cd	34.75b
Estrela-roxa	17.76c	1679.38ab	975.09a	42.25a
CV (%)	19.08	17.01	21.52	12.21
SDM	5.29	316.34	199.26	5.14

Same letters in the column do not differ significantly ($p > 0.05$), according to the Tukey's test.



Figure 1. Details of the transversal sections of the middle third of the roots of tropical forages. Epidermis (Ep), hypodermis (Hi), exodermis (Ex), cortex (Co), endoderm (En), aerenchyma (Ae), metaxylem (MX), protoxylem (PX) phloem (Ph), pericycle (Pe) and parenchymatic marrow (Ma). (a) and (f) Llanero; (b) Tanzânia; (c) and (g) Xaraés; (d) Marandu; (e) Piatã; (h) Estrela-roxa. Bars = 50 μ m.

seen in most cultivars. Llanero presented the largest cells in the exodermis (Figure 1a), while the cultivar Xaraés presented greater wall thickening (Figure 1c).

Baruch and Merida (1995), analyzing the *Urochloa mutica* (Forssk.) Stapf, classified the first layers of the cortex as hypodermis, further stating that probably the wall thickness of some layers occurs by accumulation of suberin and lignin. The abovementioned characteristics are similar to those observed in this study, and it was confirmed the presence of the substances through the test with phloroglucinol.

Tanzania, Mombaça, Marandu and Ruzizienses presented, in the central region of the cortex, isodiametric parenchymatic cells, with thin cell walls and symmetrically arranged layers (Figure 1d). The other cultivars showed the formation of aerenchyma (Figures 1e, 1f).

Aerenchyma were also observed in maize (*Zea mays* L.) under continuous flooding condition (Pereira et al., 2008), in 91 species tolerant to soil drenching (Justin and Armstrong, 1987), and in *U. mutica*, both in dry environment and swampy environment (Baruch and Mérida, 1995). The development of thicker roots, with a higher proportion of aerenchyma and a reduction of lateral

roots may act together to a more efficient system for O₂ transport (Cardoso et al., 2013).

Llanero presented the highest formations of aerenchyma (Figure 1f), which corroborates with the adaptive characteristic of the cultivar to soils with high level of humidity (Vilela, 2011).

The endoderm showed thickening of the periclinal and anticlines walls in the ten cultivars. Pereira et al. (2008) affirm that this suberised layer imposes resistance to the water flux to the vascular cylinder, its reduction can be related with the increase of aerenchyma and the environment in which the plant is inserted.

Mulato II, Llanero and Estrela-roxa presented a higher thickening of the walls of the endoderm (Figure 1g), with an increase of intercellular spaces in the cortex of Mulato II. The list of changes in the structures over time was not analyzed in this study.

The vascular cylinder (VC) was polyarc in the ten cultivars (Figure 1g), with significant differences in the diameter, which is larger in Tanzânia, Estrela-roxa and Xaraés (Table 1). Prominent pericycle formed by three or four layers of sclerified cells in Xaraés and Estrela-roxa (Figure 1h).

Pericycle with sclerified layers as well as thick endoderm, are common in plants more resistant to flooding. Plants well adapted to situations of radicular hypoxia have lower loss of dry mass of root in relation to poorly adapted plants (Cardoso et al., 2013). The maintenance of the sclerified layers of the pericycle is directly related to the root dry mass due to the low accumulation of fluid in the fibers.

Ruzizienses presented the lowest frequency of poles of protoxylem, while Estrela-roxa presented the greatest quantities (Table 1). A greater arrangement of protoxylem is related to the anatomy of the vascular cylinder (VC), the bundles of metaxylem and phloem, smaller, did not

occupy as much space as other cultivars with VC of similar caliber (Figure 1h).

At the center of the VC it was registered a parenchymatic marrow with cells with thin walls in all cultivars. Estrela-roxa showed higher this tissue compared to other cultivars (Figure 1h), and a higher concentration of starch. This characteristic was proven by the test with lugol. The starch accumulation in parenchymatic cells can provide greater resistance to the plant and facilitate the process of regrowth, for providing energy quickly to the meristematic tissues (Machado et al., 2008).

In the leaves, the epidermic cell walls presented anticlinal walls with sinuous juxtaposition (Figure 2a, 2c, 2d, 2e), which can increase the contact surface among adjacent cells and hinder the tissue disruption (Carvalho and Pires, 2008). Cells very attached to each other, as occurred in the forages analyzed in this study make it difficult for the microorganisms to access the mesophyll, very easily digested region (Valente et al., 2011).

It was also recorded the presence of short cells, lined up, which received little color on treatment with basic fuchsin. They were described by Alquini et al. (2003) as siliceous cells (Figure 2b, 2c, 2d, 2e), very common in Poaceae (Ellis, 1979; Vieira et al., 2002). The amount of silicon in forage leaves is not decisive for the digestibility, due to the fact that plants with different silica contents also present different levels of other important components that greatly affect the digestibility, such as lignin contents, differentiated arrangements of cellulose fibrils (Silva et al., 2005).

There was a significant difference in the stomatal density among the cultivars (Table 2). The size of the stomata among the cultivars was visually discrepant. The cultivar Llanero presented polar measurement of the stomata about three times larger than the cultivar Estrela-roxa (Figure 2a, 2b).

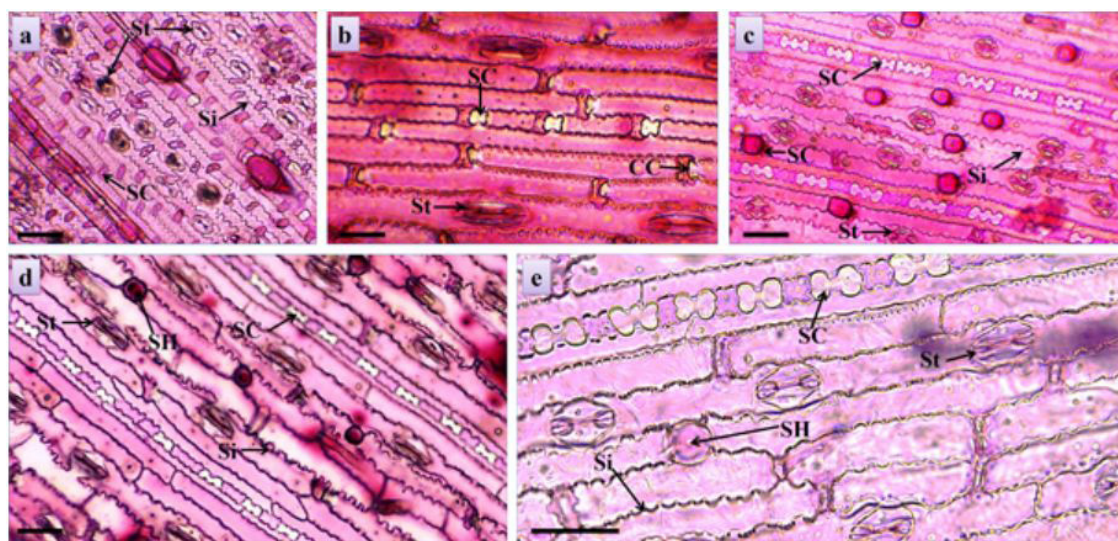


Figure 2. Paradermic sections of the leaves of tropical forages. Stomata (St), siliceous cells (SC), short cells (SH) and sinuosities (Si). (a) Estrela-roxa; (b) Llanero; (c) Massai; (d) Piatã; (e) Mombaça. Bars = 50 μm.

Estrela-roxa presented greater stomatal density by adding the two faces of the leaf, which can provide higher digestibility, since microorganisms are dependent on the epidermis breakage by chewing, or on open stomata, initiating the digestion of the tissues, generally by the substomatal chamber (Brito and Deschamps, 2001).

It was registered a thin cuticle on both sides of the leaves of the cultivars (Figure 3a, 3b), unistratified epidermis composed by notably larger abaxial cells in all

analyzed cultivars (Figure 3c), most notably the volume of this layer in Piatã (Figure 3d). Bulliform cells were observed on both sides of the leaves, and in the adaxial face they are bulkier. This characteristic is common in representatives of Poaceae (Metcalfe and Chalk, 1960). The samples of Llanero stood out with bulkier groups of cells than the other cultivars (Table 2).

The analyzed cultivars presented these structures in higher quantities. This characteristic is common in tropical

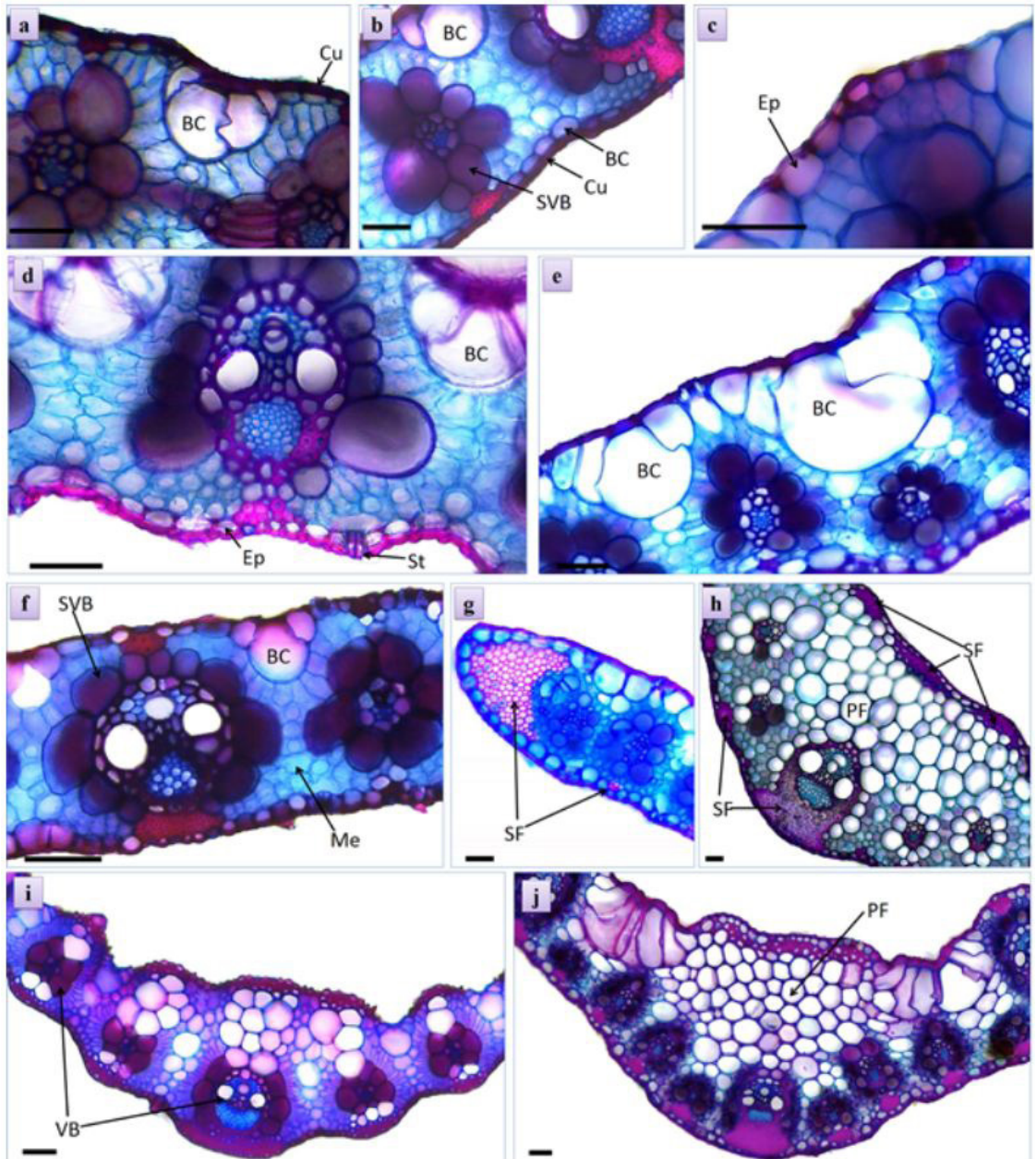


Figure 3. Transversal sections of the leaves of tropical forages. Cuticle (Cu), epidermis (Ep), stomata (St), bulliform cell (BC), mesophyll (Me), sheath of the vascular bundle (SVB), sclerenchymatic fibers (SF), parenchyma of filling (PF), and vascular bundle (VB). (a) Marandu; (b) and (h) Xaraés; (c) Mombaça; (d) Piatã; (e), (g) and (j) Llanero; (f) Tanzânia; (i) Estrela-roxa. Bars = 50 µm.

Table 2. Foliar morphometry of ten cultivars of tropical forage. Stomatal index (Stom index), stomatal density (Stom dens), midrib, bulliform cells (BC), sclerenchyma fibers (Fibers), mesophyll of the leaf blade (Mes), coefficient of variation (CV) and simple medium deviation (SMD).

Cultivar	Stom dens	Stom dens	BC (µm)	Mibrid (µm)	Mes (µm)	Fibers	Fibers
	(mm ²)	(mm ²)				(µm)	(µm)
	Abaxial	Adaxial	Adaxial			Abaxial	Adaxial
Tanzânia	155.14ab	119.45b	79.11b	834.13a	206.38bc	78.28bcd	23.86cd
Mombaça	127.46bc	65.54cd	85.26b	532.86cde	198.80bcd	79.80bcd	34.09abc
Llanero	33.44e	23.37e	121.07a	636.70abc	263.63a	62.00d	29.04bcd
Piatã	53.60e	47.36de	79.57b	542.93bcde	241.59ab	90.89b	35.54abc
Ruzzienses	91.34cd	122.97b	70.90b	573.85bcd	196.81bcd	63.56cd	33.54abc
Mulato II	100.79c	28.69e	74.69b	381.58de	210.64b	85.80bc	29.76abcd
Massai	173.05a	96.48bc	63.02b	567.17bcd	152.81d	57.42d	17.57d
Marandu	39.81e	51.30de	70.59b	616.57bc	225.02ab	115.06a	37.53ab
Xaraés	62.02de	46.92de	73.27b	739.48ab	247.93ab	118.28a	42.27a
Estrela roxa	158.39ab	238.25a	65.29b	361.00e	156.34cd	60.33d	34.35abc
CV (%)	12.99	13.85	24.81	26.34	18.57	21.57	31.08
SDM	37.38	33.64	25.61	200.98	51.41	23.08	13.01

Same letters in the column do not differ significantly ($p > 0.05$), according to the Tukey's test.

grasses and may increase as a result of the adaptation to the environment, for being responsible for the winding and unwinding of the leaf in warm periods, avoiding the loss of water (Paciullo et al., 2002).

The mesophyll was homogeneous, as described by Brito and Rodella (2002) to C4 grasses. It was verified the occurrence of a similar anatomical organization among the representatives of the three genera analyzed in this study. The thickness of the limbo of the Llanero (Table 2) is highlighted. Carvalho and Pires (2008) conclude that a higher proportion of these tissues in the leaf blade of a forage can mean a better utilization of nutrients by animals and thus a higher nutritional quality.

Cardoso et al. (2013) state that plants resistant to marshy soil can develop spaces among the tissues of the root, stem and leaves to better aeration. In this study it was not observed formation of aerenchyma in the leaves of the cultivars, maybe because they were not exposed to flooding or the waterlogging period to which these plants were exposed have been short.

Collateral vascular bundles of second order (Figure 3i) occurred periodically in the mesophyll of the leaf blade, interspersed with smaller frequency by first-order bundles (Figure 3g). The distance between the bundles differs among the cultivars, Massai presented the lowest average distances (124,8 µm), and *U. brizantha* presented the highest averages (220 µm) in the Marandu and Xaraés cultivars. More spaced bundles on the foliar limb may facilitate the rupture of limbo at the moment of the grazing, especially for bovine animals (Brito and Deschamps, 2001).

The cultivars Xaraés and Marandu presented higher proportion of sclerenchyma fibers on both sides of the leaf, with greater emphasis on the abaxial face (Figure 3h), while the Massai cultivar presented the lowest results for the quantity of fibers (Table 2), increasing its digestibility, since the condition of higher amount of subepidermic fibers

may negatively interfere on the digestibility of the forage by the cattle, due to the lignin in the walls of sclerenchyma cells (Paciullo et al., 2002).

The cultivars of the Panicum gender analyzed here presented cells of the sheath of the vascular bundle (SVB) with thinner cell walls than the cultivars of the genus *Urochloa*, which may contribute to a higher productivity/yield of ruminant that eat these forages (Vilela, 2011).

The cells of SVB are rich in photosynthetic enzymes and non-structural carbohydrates, which can positively interfere on the productivity and chemical composition of the leaf blades (Gomes et al., 2011). However, Carvalho and Pires (2008) highlighted that their digestion is slow or incomplete due to their thickened cell wall, which hinders the access of the ruminal microorganisms to the nutrients inside these cells.

4. Conclusions

Massai, Piatã, Xaraés, Llanero, Mulato II, Estrela-roxa and Llanero presented the highest formations of aerenchyma, which may be related to the adaptation to the environment subject to flooding. The higher stomatal density of Estrela-roxa, adding the two sides of the leaf, the size of cells bulliform and chlorophilian parenchyma of Llanero, plus the low proportion of the fibers from both, provide highlights on the digestibility in relation to the other cultivars, as well as Massai that presented the smallest results for amount of fibers.

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