

Research Paper

Anatomical and nutritional characteristics of *Megathyrsus maximus* genotypes under a silvopastoral system

Características anatómicas y nutricionales de genotipos de Megathyrsus maximus en un sistema silvopastoril

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Abstract

Our objective was to measure chemical composition and anatomy of 5 *Megathyrsus maximus* (syn. *Panicum maximum*) genotypes, when grown in combination with eucalypts in a silvopastoral system. Cultivars Massai, Mombaça, BRS Tamani, Tanzânia and intraspecific hybrid accession PM44 were evaluated in full sun and a silvopastoral system at 5 different distances from eucalyptus tree rows. The experimental design was a randomized block in split plot with 2 replications. Plots corresponded with genotypes and subplots with sampling points within the system. Total forage and leaf biomass as well as nutritive value and tissue proportions were evaluated. Our results showed a decrease in biomass as radiation incidence decreased. Forage biomass was greatest in BRS Tamani and Mombaça and lowest in PM44. There was a significant interaction between sampling points and genotype for nutritive value variables, such as crude protein, in vitro digestibility of organic matter, cellulose, hemicellulose and lignin-S, while tissue proportions were not affected by the interaction between sampling points and genotypes. Genotype had more pronounced effects on chemical composition and anatomical characteristics than did sampling points. The leaves of Mombaça were the longest and had greatest total cross-sectional area, and this genotype showed greater proportions of sclerenchyma and vascular tissues than other cultivars and the lowest proportion of mesophyll. The greatest proportion of parenchyma bundle sheaths was also found in Mombaça leaves. Genotypes PM44 and Tanzânia had the lowest proportions of sclerenchyma, and PM44 and BRS Tamani had the lowest proportions of vascular tissues. On the other hand, PM44 and Tanzânia had the greatest proportions of mesophyll. BRS Tamani was comparable with the most used cultivars, Mombaça and Tanzânia, and had forage quality slightly superior to that of Mombaça. Tropical grasses growing under shade can potentially produce less forage but with better nutritive value, in terms of chemical composition and tissue proportions, than grasses grown under full sun. However, as the degree of shading in silvopastoral systems does not occur uniformly across the whole area, the improved nutritive value would not be uniform and may not be very prominent overall.

Keywords: Agroforestry, anatomical tissues, chemical composition, *Panicum maximum*, shading, tropical grasses.

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Resumen

Nuestro objetivo fue medir la composición química y la anatomía de 5 genotipos de *Megathyrsus maximus* (syn. *Panicum maximum*), cuando se cultivan en combinación con eucaliptos en un sistema silvopastoril. Los cultivares Massai, Mombaça, BRS Tamani, Tanzânia y la accesión híbrida intraespecífica PM44 se evaluaron a pleno sol y en un sistema silvopastoril a 5 distancias diferentes de las hileras de eucaliptos. El diseño experimental fue un bloque al azar en parcela dividida con 2 repeticiones. Las parcelas se correspondieron con genotipos y subparcelas con puntos de muestreo dentro del sistema. Se evaluó la biomasa total de forrajes y hojas, así como el valor nutritivo y las proporciones de tejido. Nuestros resultados mostraron una disminución en la biomasa a medida que disminuyó la incidencia de radiación. La biomasa del forraje fue mayor en BRS Tamani y Mombaça y menor en PM44. Hubo una interacción significativa entre los puntos de muestreo y el genotipo para las variables de valor nutritivo, como proteína cruda, digestibilidad in vitro de materia orgánica, celulosa, hemicelulosa y lignina-S, mientras que las proporciones de los tejidos no se vieron afectadas por la interacción entre los puntos de muestreo y los genotipos. El genotipo tuvo efectos más pronunciados sobre la composición química y las características anatómicas que los puntos de muestreo. Las hojas de Mombaça fueron las más largas y tuvieron mayor área de corte transversal total, y este genotipo mostró mayores proporciones de esclerenquima y tejidos vasculares que otros cultivares y la menor proporción de mesófilo. La mayor proporción de vainas de haces de parénquima también se encontró en las hojas de Mombaça. Los genotipos PM44 y Tanzânia tenían las proporciones más bajas de esclerenquima, y PM44 y BRS Tamani tenían las proporciones más bajas de tejidos vasculares. Por otro lado, PM44 y Tanzânia tuvieron las mayores proporciones de mesófilo. BRS Tamani fue comparable con los cultivares más utilizados, Mombaça y Tanzânia, y tuvo una calidad de forraje ligeramente superior a la de Mombaça. Los pastos tropicales que crecen bajo sombra pueden producir potencialmente menos forraje pero con mejor valor nutritivo, en términos de composición química y proporciones de tejido, que los pastos cultivados a pleno sol. Sin embargo, como el grado de sombreado en los sistemas silvopastoriles no ocurre de manera uniforme en toda el área, el valor nutritivo mejorado no sería uniforme y podría no ser muy prominente en general.

Palabras clave: Agroforestería, composición química, *Panicum maximum*, pastos tropicales, sombreado, tejidos anatómicos.

Introduction

In the tropics, Guinea grass [*Megathyrsus maximus* (syn. *Panicum maximum*)] is one of the major grasses used as forage for cattle feeding, characterized by both high forage biomass and high nutritive quality, which favors weight gain of bovines in grazing systems (Euclides et al. 2015). Although 24 cultivars are registered in the official site of the Brazilian Ministry of Agriculture, Livestock and Food Supply (http://sistemas.agricultura.gov.br/snpc/cultivarweb/cultivares_registradas.php), the most used *M. maximus* cultivars in Brazil are Tanzânia and Mombaça, comprising about 90% of the seed of this species being sold (Fernandes et al. 2014). This lack of diversity carries the risk that traditional cultivars might succumb to a disease or pest, such as *Bipolaris maydis* fungus, which has caused significant losses in cv. Tanzânia (Braz et al. 2015). Therefore, there is an ongoing need for research to develop and evaluate new cultivars, in a range of different environmental conditions, in order to promote pasture diversification in livestock production systems.

Aiming at achieving resource-efficient and sustainable animal production in the tropics, integrated crop-livestock

and integrated livestock-forestry and crop-livestock-forestry systems have been studied by Brazilian Agricultural Research Corporation (Embrapa). The standard silvopastoral systems combine livestock and forestry in the same location, providing benefits in terms of soil quality (Sousa Neto et al. 2014), forage quality (Gamarra et al. 2017), greenhouse gas balance (Alves et al. 2015) and animal welfare (Karvatt Junior et al. 2016).

The presence of trees in the systems modifies the environment for forage grass development and, consequently, its growth patterns. When exposed to shade, some forage grasses can modify their leaf anatomy, in order to increase light absorption efficiency and continue growing under tree canopies (Gobbi et al. 2011). Further factors like gaseous exchanges and, consequently, the forage primary production process and nutritive value are also affected (Batistoti et al. 2012).

An increase in the specific leaf area of plants growing under shade has been reported widely in the literature (Gomes et al. 2019; Pezzopane et al. 2019). This greater specific leaf area under shade than in full sun may affect the leaf blade proportion of tissues, such as lower mesophyll proportion, decrease in sclerenchyma and vascular tissues plus thinner cell walls and bulliform cells

([Lambers et al. 1998](#)). Quantifying the proportion of various tissues in leaf blades should indicate the potential digestibility of forage, although some leaves are highly digestible, while others are poorly digestible or even indigestible ([Batistoti et al. 2012](#)). In addition, leaf anatomy consists of morphologically and functionally distinct types of photosynthetic cells ([Berry and Patel 2008](#)). For instance, C₄ leaf anatomy was identified with lower proportions of mesophyll and greater less-digestible epidermis, bundle sheath, sclerenchyma and vascular tissues than C₃ leaf anatomy, which negatively affects pasture digestibility of C₄ species ([Wilson and Hattersley 1989](#)).

Several studies have been conducted with tropical grasses under different shading levels, focusing on responses in biomass yield, nutritive value, physiology and morphology of forage ([Gobbi et al. 2011](#); [Baldissera et al. 2016](#); [Geremia et al. 2018](#)). For these parameters, *M. maximus* cultivars have shown medium tolerance of shaded environments ([Santiago-Hernández et al. 2016](#)), which indicates that they have potential for use in silvopastoral systems ([Paciullo et al. 2016](#)). However, those studies are more focused on cv. Tanzânia and Mombaça, although cv. BRS Tamani, launched in 2014, has shown high performance under shading ([Pereira et al. 2015](#)).

The increased interest in silvopastoral systems has boosted research into forage grasses suitable for use in shaded environments. We aimed at investigating the leaf anatomy of different *M. maximus* genotypes under shaded conditions in a silvopastoral system, and the relationship between anatomical and nutritional characteristics of leaf blades of these genotypes and different degrees of shade.

Materials and Methods

Experimental design

The field experiment was conducted at Embrapa Beef Cattle, located in the municipality of Campo Grande - MS (20°24'54.9 S, 54°42'25.8 W; 530 masl). The climate corresponds to the humid tropical type, sub-type Aw (Köppen classification), with hot and rainy summers. Soil is classified as clayey Oxisol. The evaluations were carried out in full sun and in a silvopastoral system, both established in 2008, and briefly described as follows:

- Full sun, representing the Control (CON), with soybean (*Glycine max*) planted every 4 years in a no-tillage system in rotation with tropical grass pasture for 3 consecutive years.
- A silvopastoral system with *Eucalyptus urograndis* H13 clone trees (*Eucalyptus grandis* × *E. urophylla*) planted in rows in an east-west direction. Distance

between rows was 22 m and between trees within rows 2 m, totalling 227 eucalyptus trees/ha. During the experimental period, the average tree height was 22 m measured by a Haglöf compass clinometer (Haglöf Sweden AB, Långsele, Sweden). As for the CON treatment, soybean was sown every 4 years by sod-seeding, in rotation with tropical grass pasture for 3 consecutive years.

A detailed description of the establishment and design for both the silvopastoral system and CON is given by Oliveira et al. ([2014](#)) and Gamarra et al. ([2017](#)). The management of the systems was similar throughout. The soybean was harvested in April 2013, and in October of the same year, 5 *Megathyrsus maximus* genotypes, i.e. cvv. Massai, Mombaça, BRS Tamani, Tanzânia and hybrid accession PM44 were sown in plots of 20 × 1.5 m, at a row spacing of 0.25 m, as an understory for eucalyptus trees. There was an application of 109 kg urea/ha and 300 kg N:P:K/ha (0:20:20) at seeding. Grass seeding rates were adjusted to apply 200 pure viable seeds/m². Grasses were first harvested at 70 days after sowing and 30 cm above ground level, and then 35 days after the first cut, in order to evaluate regrowth potential. The average height of the cultivars at harvest was 60 cm. Evaluation of the anatomical and chemical characteristics of the genotypes was conducted during the rainy season at the beginning of February 2014.

The experimental design was a randomized block in split plot with 2 replications/ paddock. Plots corresponded with *M. maximus* genotypes and the subplots with sampling points within each plot. Five sampling points were defined: 1 m from the northern tree rows (P1N); 1 m from the southern tree rows (P1S); 6 m from the northern tree rows (P6N); 6 m from the southern tree rows (P6S); and midway between the tree rows, i.e. 11 m from each (P11). For the CON system 2 sampling points were randomly collected in the system. The photosynthetically active radiation (PAR) was recorded at grass canopy height at each sampling point by a portable ceptometer (model AccuPAR-LP 80, Decagon Devices Inc., Pullman, WA, USA), in the morning and afternoon on sunny days (Figure 1), and at random points under full-sun conditions. The leaf greenness index was measured with a Soil Plant Analysis Development (SPAD) optical chlorophyll meter (SPAD-502 Plus, Minolta, Japan) on 3 leaves of randomly selected plants at each sampling point.

Field plots were mechanically defoliated 70 days after establishment and 35 days later at a cutting height of 30 cm above ground to determine forage biomass (total dry matter, TDM) within a metallic frame of 1 × 1 m. All forage samples collected were individually weighed after harvesting. A subsample from each sampling point was

taken, placed in a paper bag and dried in a forced-air oven at 65 °C until constant mass for determination of DM. Another subsample from each sampling point was selected and separated into its morphological components – leaf blade, stem with sheath and senescent material, which were weighed and subsequently dried in a forced-air oven at 65 °C for DM determination. Leaf biomass (LDM) was then estimated for the purpose of this study.

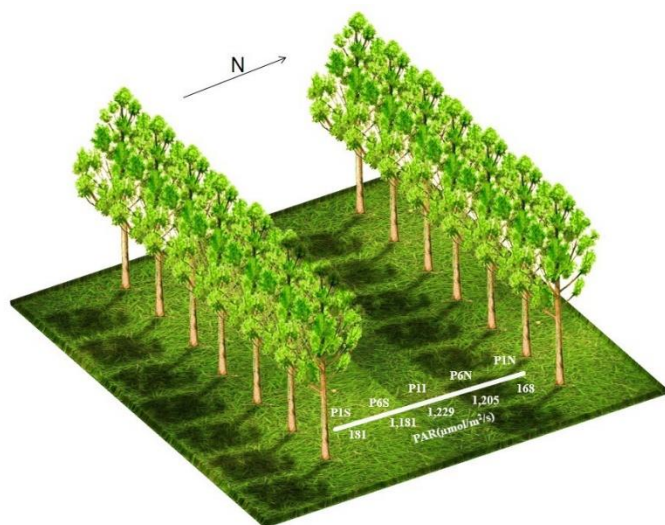


Figure 1. Schematic representation of forage sampling points (P1N: 1 m from the northern tree rows; P1S: 1 m from the southern tree rows; P6N: 6 m from the northern tree rows; P6S: 6 m from the southern tree rows; P11: midway between the tree rows, i.e. 11 m from each) sited in a silvopastoral system (grass + 227 trees/ha), with values for respective photosynthetically active radiation reaching pasture surface. (Adapted from [Oliveira et al. 2019](#)).

Anatomical parameters

The penultimate expanded leaf blade, with exposed ligules, cut from the basal region of the main vegetative shoot of 5 tillers randomly selected from each cultivar, was taken at each sampling point. Leaf blades were identified and width at the central point and length (from the apex of the blade to the base of the ligule insertion) were measured. Sample fragments of 1 cm were taken from the central area of 5 blades and stored in bottles filled with formalin aceto-alcohol solution. The 1 cm leaf blade fragments were stored in a tertiary butyl ethanol series ([Daykin and Hussey 1985](#)).

After dehydration, fragments were processed with paraplast. Fragments were sectioned with a thickness of 10 μm, using a manual rotary microtome, followed by triarch quadruple staining of tissues before permanent blade mounting, following the methodology proposed by Hagquist ([1974](#)). The image analyzing system (AxioVision version

3.1) was coupled to the binocular optical microscope to estimate proportions of each tissue in the leaf blades. Total cross-sectional area projected in the video was measured first (TA), followed by determination of the area occupied by abaxial (ABAep) and adaxial (ADAep) epidermal tissues from the parenchyma bundle sheath (PBS), the sclerenchyma (SCL) and the vascular (VT) system. The mesophyll (MES) area was calculated as the difference between total anatomical area and the area of other tissues.

Nutritional parameters

Leaf samples from the respective sampling points (P1N, P1S, P6N, P6S, P11 and CON) as for anatomical parameters were weighed and dried in a forced-air oven at 65 °C until constant weight was reached. After grinding to pass a 1-mm sieve, samples were analyzed for crude protein (CP), in vitro organic matter digestibility (IVOMD) and cellulose, while hemicellulose was calculated by subtracting acid detergent fiber values from neutral detergent fiber values, and lignin-S values determined for the predictive equation by the method of solubilization of cellulose with sulfuric acid, using near infrared reflectance spectroscopy (FOSS NIR System 5000), according to Marten et al. ([1985](#)), at the Embrapa Beef Cattle facilities.

Statistical analysis

Shapiro-Wilk test was used to assess the normality of the residual data. No transformation was required. Pearson correlation was used to evaluate the relationship between anatomical and chemical characteristics of data, and was also applied for the PAR, SPAD index, forage biomass, and leaf biomass of the genotypes. These data were also subjected to an analysis of variance using the General Linear Model (GLM) procedure of SAS with genotype, sampling point and their interaction as main effects. Statistical significances were defined for $P < 0.05$, and mean differences were analyzed by the Tukey test.

Results

The anatomy of the penultimate expanded leaf blade of the 5 *Urochloa maximus* genotypes evaluated is depicted in Table 1 and Figure 2. A distinguishing feature of C_4 pathways of photosynthesis, which occurs in tropical grasses, is the Kranz anatomy, i.e. 2 morphologically and functionally different types of photosynthetic cells, PBS and MES, with numerous well-developed chloroplasts. The bundle sheath of specialized cells, with normally thick walls, surrounds the VT and MES cells, surrounding the bundle sheath beneath the epidermis (Epi). The carbon

Table 1. Means of anatomical traits and length of leaf blades of *Megathyrsus maximus* genotypes, in a silvopastoral system.

Variable ¹	Massai	Mombaça	PM44	BRS Tamani	Tanzânia	s.e.m.	P-value
TA (μm^2)	98,477ab	131,631a	120,054ab	89,044b	120,035ab	4.56	<0.01
Relative tissue proportion (%)							
Epi	28.6	25.9	26.1	28.4	24.9	7.24	<0.01
ADAep	18.8a	16.9a	16.4a	17.9a	16.0a	5.37	<0.01
ABAep	9.8ab	9.0b	9.8ab	10.5a	8.9b	0.38	<0.01
PBS	36.3b	40.0a	36.7b	36.0b	38.0ab	2.25	0.01
VT	7.6ab	9.3a	6.8b	6.9b	7.3ab	1.62	0.01
SCL	4.8a	4.5a	3.4b	4.4a	3.5b	0.22	<0.01
MES	22.7ab	19.7b	27.0a	24.3ab	26.3a	7.20	<0.01
SPAD	40.7a	40.1a	43.9a	35.4a	43.6a	49.7	<0.01
Length (cm)	53.4ab	66.3a	51.0b	53.7ab	56.0ab	49.5	0.01

¹Variables: TA, total anatomical area of the leaves; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; SPAD, Soil Plant Analysis Development. Within rows, genotypes without a common letter are significantly different (P<0.05).

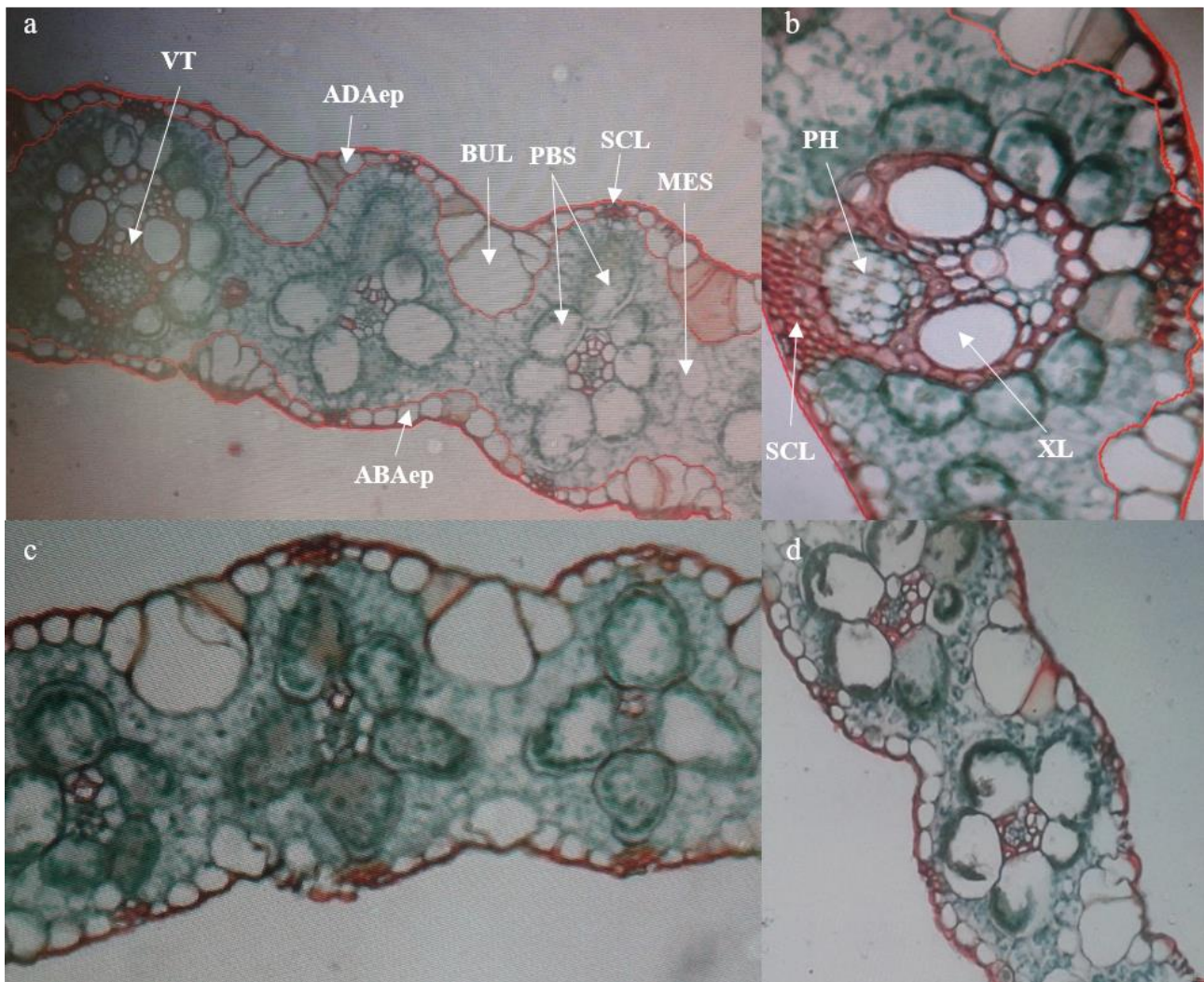


Figure 2. Cross-sectional area of leaves of *Megathyrsus maximus* genotypes in a silvopastoral system (grass + 227 trees/ha) and full sun conditions. a. ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; BUL, bulliform cells. b. An example of a leaf of cv. Massai with the Girder structure. PH, phloem; XL, xylem. c and d. Examples of leaves with PBS and MES with chloroplasts.

dioxide-assimilation C_4 pathway utilizes 2 different sets of biochemical reactions, and the compartmentalization and functional separation of those structural frameworks are provided by this wreath-like arrangement ([Berry and Patel 2008](#)). *M. maximus* (biochemical characterization of phosphoenolpyruvate carboxykinase, PEP-CK subtype of C_4) has an undifferentiated MES with few intercellular spaces and a single layer of concentric bundle sheath cells around VT. The chloroplasts in the bundle sheath cell are centrifugal and the leaves contain stomata in both surfaces (amphistomatic). The epidermis contains a single layer of juxtaposed cells with bulliform cells placed between the vascular bundles in the ADAep ([Habermann et al. 2019](#)). While their leaves are relatively resistant to degradation by rumen microorganisms, they afford great photosynthetic carbon conversion efficiency and hence above-ground biomass production ([Wilson et al. 1983](#)).

In our study the level of PAR that reached the forage canopy varied greatly according to the distance from the trees, with pasture close to the tree lines receiving only 10% as much radiation as open pasture received. While points P6N, P11 and P6S in the silvopastoral system received levels of PAR above $1,100 \mu\text{mol}/\text{m}^2/\text{s}$, or the amount required for tropical forage grass development, [for instance, $800 \mu\text{mol}/\text{m}^2/\text{s}$ for the genus *Brachiaria* ([Gifford 1974](#); [Sousa et al. 2010](#))], points P1N and P1S received only 22% of this requirement (Figure 1). The PAR of full sun conditions was on average $1,697 \mu\text{mol}/\text{m}^2/\text{s}$. As expected, forage growth decreased as level of PAR reaching the grass canopy also decreased, so leaf biomass was lower in the silvopastoral than the full-sun environment.

Total forage biomass (TDM) and leaf biomass (LDM) were greater ($P < 0.05$) in CON (means of 6,249 kg DM/ha and 2,408 kg DM/ha, respectively) than at the silvopastoral sampling points, where TDM and LDM decreased as distance from tree lines decreased ([Pereira et al. 2021](#)). Means for TDM were 4,915, 4,211, 5,699, 4,656 and 5,613 kg DM/ha and for LDM were 1,511, 1,339, 1,694, 1,192 and 1,814 kg DM/ha, at points P1N, P1S, P6N, P6S and P11, respectively (data not shown). By comparing the genotypes, TDM of BRS Tamani (6,490 kg DM/ha) and Mombaça (6,033 kg DM/ha) was greater ($P < 0.05$) than that of PM44 (3,596 kg DM/ha), while those of Massai (5,356 kg DM/ha) and Tanzania (4,644 kg DM/ha) were intermediate ($P > 0.05$). Massai (2,385 kg DM/ha) showed the greatest LDM and Tanzania (1,142 kg DM/ha) the lowest. Mombaça (1,873 kg DM/ha) was greater ($P < 0.05$) than PM44 (1,189 kg DM/ha), while BRS Tamani (1,708 kg DM/ha) was intermediate ($P > 0.05$) (data not shown).

There were significant interactions ($P < 0.05$) between sampling point and genotype for all chemical parameters measured, so data are presented for all combinations in

Table 2. The CP concentration for BRS Tamani for P1N exceeded that for P6S ($P < 0.05$). Similarly, IVOMD for PM44 at P1N exceeded that at P6S ($P < 0.05$) and hemicellulose concentration of BRS Tamani at P1N exceeded those for P1S, P6S and CON ($P < 0.05$).

Effects of genotype were much more pronounced, although genotype had no significant effect on CP concentration or IVOMD ($P > 0.05$). At P6S, cellulose concentration of BRS Tamani exceeded those for PM44 and Tanzania and that of Mombaça exceeded that of PM44 ($P < 0.05$). At P1N hemicellulose concentration of BRS Tamani exceeded that of PM44 and at P6N concentration of Tanzania exceeded that of PM44 ($P < 0.05$). Lignin concentration of PM44 at P1N and P6S exceeded that of Tanzania and lignin concentration of BRS Tamani at P1S and P6N exceeded that of Tanzania ($P < 0.05$).

Regarding anatomical characteristics, effects of genotype were much more pronounced than effects of sampling points. Mombaça showed the highest total cross-sectional area (TA), which was significantly greater ($P < 0.01$) than that of BRS Tamani, and leaves of Mombaca were longer than those of PM44 (Table 1). In general, Mombaca showed higher proportions of poorly digestible or even indigestible tissues, e.g. SCL and VT, than other cultivars and lower proportion of mesophyll. Genotypes PM44 and Tanzânia had the lowest proportions of sclerenchyma, while PM44 and BRS Tamani had the lowest proportions of vascular tissues. On the other hand, PM44 and Tanzânia had higher proportions of MES, a rapidly and completely digested tissue, than Mombaça ($P < 0.01$). In relation to partially degradable tissues, BRS Tamani showed higher ABAep than Mombaça and Tanzânia, while Mombaça had higher PBS than Massai, PM44 and BRS Tamani. Total cross-sectional area of leaf blades in the full-sun system (CON) was greater than for the sampling points near trees (P1N, P1S and P6S) (Table 3). Effects of sampling points on the remaining anatomical parameters were not significant ($P > 0.05$).

Overall, weak correlations were found between anatomical and nutritional parameters ($P \leq 0.49$; Table 4). CP was positively related to IVOMD, hemicellulose and MES, but negatively related to cellulose, Epi, ADAep and SCL. These final 3 tissues were positively related to cellulose, whereas lignin-S was positively correlated with Epi and ADAep. As expected, IVOMD showed positive correlation with hemicellulose, and negative correlation with cellulose and lignin-S. SPAD index was positively related to CP, IVOMD, TA and MES, and negatively related to cellulose and Epi (Table 4).

PAR was positively related to LDM and TA, and negatively related to CP, IVOMD, hemicellulose and ABAep. In addition, LDM showed positive correlation with SCL, and negative with MES.

Table 3. Means of anatomical traits and length of leaf blades of *Megathyrus maximus* genotypes at various sampling points¹, in a silvopastoral system.

Variable ²	P1N	P1S	P6N	P6S	P11	CON	s.e.m.	P-value
TA (µm ²)	97,259b	105,178b	114,790ab	108,796b	117,163ab	130,304a	1.17	0.01
Relative tissue proportion (%)								
Epi	27.5	27.4	26.3	27.1	26.3	26.1	1.75	0.25
ADAep	17.2	17.2	17.0	17.8	17.0	17.0	1.46	0.63
ABAep	10.3a	10.2a	9.4a	9.3a	9.3a	9.1a	0.45	0.03
PBS	37.6	37.3	37.7	36.8	35.8	38.8	4.80	0.19
VT	7.7	7.1	7.0	7.8	7.4	8.5	2.81	0.46
SCL	4.2	4.0	4.5	3.9	4.1	4.1	0.33	0.56
MES	22.9	24.2	24.6	24.3	25.3	22.6	10.5	0.56
SPAD	40.2	40.6	44.4	38.4	41.9	38.9	15.1	0.19
Length (cm)	58.8	58.8	61.1	51.8	49.9	53.2	43.2	0.07

¹Sampling points: P1N, 1 m from the northern tree rows; P1S, 1 m from the southern tree rows; P6N, 6 m from the northern tree rows; P6S, 6 m from the southern tree rows; P11, midway between the tree rows, i.e. 11 m from each. ²Variables: TA, total anatomical area of the leaves; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; SPAD, Soil Plant Analysis Development. Within rows, means without a common letter are significantly different (P<0.05).

Table 4. Correlation coefficients among anatomical and chemical characteristics of leaf blades of *Megathyrus maximus* genotypes in a silvopastoral system.

	PAR	TDM	SPAD	IVOMD	Cel	Hemi	LigS	Epi	ADAep	ABAep	PBS	VT	SCL	MES
LDM	0.33	0.69	-	-	-	-	-	-	-	-	-	-	0.37	-0.27
CP	-0.40	-	0.30	0.49	-0.76	0.43	-	-0.26	-0.34	-	-	-	-0.35	0.29
IVOMD	-0.44	-	0.30	-	-0.37	0.44	-0.34	-	-	-	-	-	-	-
Cel	-	-	-0.28	-	-	-	0.37	-	-	-	-	-	-	-
Hemi	-0.42	-	-	-	-	-	0.40	-	-	-	-	-	-	-
TA	0.38	-	0.34	-	-0.28	-	-	-0.73	-0.49	-0.78	0.44	0.26	-0.26	-
Epi	-	-	-0.27	-	0.37	-	0.28	-	-	-	-0.47	-0.30	-	-
ADAep	-	-	-	-	0.40	-	0.26	0.90	-	-	-0.38	-	-	-
ABAep	-0.44	-	-	-	-	-	-	0.71	0.33	-	-0.40	-0.35	-	-
VT	-	-	-	-	-	-	-	-	-	-	0.76	-	-	-
SCL	-	0.31	-	-	0.36	-	-	-	-	-	-	0.37	-	-
MES	-	-	0.26	-	-	-	-	-	-	-	-0.70	-0.82	-0.60	-
Length	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-

PAR, photosynthetically active radiation; TDM, total dry matter; SPAD, Soil Plant Analysis Development; IVOMD, in vitro organic matter digestibility; Cel, cellulose; Hemi, hemicellulose; LigS, lignin determined by solubilization of cellulose with sulfuric acid; Epi, epidermis; ADAep, adaxial epidermis; ABAep, abaxial epidermis; PBS, parenchyma bundle sheath; VT, vascular tissue; SCL, sclerenchyma; MES, mesophyll; LDM, leaf dry matter; CP, crude protein; TA, total anatomical area of the leaves. Values are shown when P<0.05 and are highlighted when correlations are negative.

Discussion

The complex adjustments in plant physiology and the structure of leaf tissues provide the mechanism for plants to survive in a limited or impaired environment. Despite neglecting leaf anatomy in the studies evaluating plant responses to different conditions (Xu et al. 2012), plant physiology, productivity and animal performance are closely related to leaf anatomy (Habermann et al. 2019). Mesophyll characteristics influence carbon assimilation rates and leaf function, while leaf tissue thickness plays a vital role in thermal regulation, light interception and CO₂ and water vapor diffusion (Terashima et al. 2011; Habermann et al. 2019) and different proportions of leaf tissues are important in the digestibility and nutritional value of forage for animals (Batistoti et al. 2012).

There was a positive correlation between forage biomass and leaf biomass plus indigestible tissues, such as SCL and VT and a negative correlation between leaf biomass and MES. In agreement with our findings, Britto de Assis Prado et al. (2016) reported that large biomass for expanded leaves tended to result in thinner MES. Even though those authors assessed the effects of temperature and CO₂ concentrations on foliage development, our findings of leaf biomass being negatively correlated with MES suggest further studies are warranted to measure differences in ambient air temperature between full-sun and silvopastoral systems, which are likely to favor full sun (Karvate Junior et al. 2016). Although some genotypes had a greater quantity of highly digestible tissues, their forage biomass, leaf:stem ratio and tiller density, as the case of PM44 in contrast to Mombaça, should be considered for use in systems with a forest component, to support grazing.

The negative correlation between level of radiation and CP was expected due to the increase of nitrogen concentration of forage in shaded environments, already widely reported in the literature (Lin et al. 2001; Soares et al. 2009; Gobbi et al. 2010; Paciullo et al. 2016), but still undefined for IVOMD (Lima et al. 2018). In addition, the negative correlation between radiation and hemicellulose is indicative of increase of primary wall in forage developing with light restriction due to changes in the partitioning of photosynthate. Light restriction decreases the availability of photosynthate, resulting in the partitioning of photosynthate to priority areas to improve the efficiency of the photosynthesis, instead of developing secondary walls. In addition, leaves grown in full sun tend to be thicker than shaded leaves (Deinum et al. 1996).

The positive correlations between SPAD index, CP and MES result from high amounts of photosynthesis-related enzymes in MES tissues, because most of the

nitrogen in MES is present in the chloroplasts and involved in photosynthesis, which can be measured by SPAD (Yamamoto et al. 2002; Batistoti et al. 2012). Moreover, the positive relationships between cellulose and lignin-S and Epi, ADAep, SCL and VT were also expected, because these tissues are cell types with thickened secondary walls, and are highly lignified, contributing to poor quality of forage (Wilson 1994). Positive correlation between PBS and VT is the reason for thickened secondary walls and the lignification of parenchyma bundle sheath tissues (Batistoti et al. 2012).

Although shaded plants displayed changes in anatomical development relative to plants grown in full sun, such as lower mesophyll proportion, decrease of sclerenchyma and vascular tissues, thinner cell walls and bulliform cells (Lambers et al. 1998), in silvopastoral systems the light environment in the understory of trees is dynamic, varying according to the incline of the earth's axis in relation to the sun, as well as the growth of the trees. Consequently, the amount of light reaching plants under trees could have varied with time (Geremia et al. 2018). However, most of the chemical and anatomical differences between locations and cultivars were likely due to genetic variability. Some interaction was found between genotype and sampling points, meaning an interaction between the genotype and the PAR reaching the canopy or degree of competition for nutrients and water. Presumably, effects of soil moisture competition between trees and forage grasses would be minimal since the measurements occurred in the rainy season. Nevertheless, we propose that research should be undertaken in the areas of soil moisture and nutrient uptake in silvopastoral systems, especially since eucalypts have a very large root system.

Depending on the plant's response, shading will not necessarily result in improvements in the forage nutritive value. While we failed to record any significant effects of degree of shading on CP% (except for cv. BRS Tamani), according to Paciullo et al. (2016), there was an increase in both CP and acid detergent insoluble protein in leaves of cv. Massai under intense shading. The nitrogen fraction accounting for the unavailable protein contained in the cell walls is undegraded in the rumen and is non-digestible in ruminant intestines, Sniffen et al. (1992) suggested that shading may compromise forage nutritive value. Our study failed to clarify this hypothesis.

The high proportion of tissues with thickened cell walls and increased lignification for Mombaça is related to the greater length of leaves. Lengthy leaf blades require strong structural support to maintain their erect configuration, and this support is provided by xylem and SCL (Paciullo et al. 2002). Mombaça presents visually

erect leaf configuration and cespitose growth habit. Gomes et al. (2011) found greater structural tissues, such as SCL and VT, in genotypes with lengthy leaf blades and lower MES, in agreement with our finding as MES of Mombaça leaf blade was the lowest reported in this study.

Regarding cv. Massai, the high SCL is associated with the Girder structure present in its leaves. This structure has an arrangement of SCL cells among Epi and PBS (Brâncio et al. 2002), reducing the nutritive value of forage produced (Lempp 2007). BRS Tamani showed results similar to those of Massai, being slightly superior to Mombaça, but slightly inferior to Tanzânia and PM44. According to Martuscello et al. (2015), BRS Tamani was clustered together with Massai based on graphic dispersion of canonical variables, indicating also a similarity among them for low plant height, but high leaf:stem ratio and leaf proportion. However, our study did not evaluate the presence of Girder structure in the genotypes, as those authors evaluated genotypes of *M. maximus* for productive characteristics.

Conclusions

This study has shown that the tropical forages evaluated had similar nutritive value in both full sun and shade in contrast to other published findings of better nutritive value in terms of chemical composition and tissue proportions in grasses grown in shade. Since the degree of sunlight reaching the grass canopy under trees can vary dramatically depending on time of day and distance from the tree lines, differences in nutritive value may not be very prominent. This issue seems to warrant more studies.

Acknowledgments

We thank Embrapa Beef Cattle, Fundect, Unipasto (Associação para o Fomento à Pesquisa de Melhoramento de Forrageiras) and the Research Training Group 'Water-People-Agriculture' at the University of Hohenheim funded by Anton & Petra Ehrmann Foundation for financial support, and Roberta Alves Gomes for contributions with laboratory analyses.

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(Note of the editors: All hyperlinks were verified 15 April 2021.)

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(Received for publication 28 January 2020; accepted 3 January 2021; published 31 May 2021)

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