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Anatomical characteristics and leaf blade digestibility of five *Panicum* genotypes under integrated crop-livestock-forest system

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Introduction

Integrated crop-livestock-forest systems (ICLF) are intended to increase land use efficiency and to harvest benefits from interactions among the components involved. Thus, cattle husbandry success on such systems depends on the suitability and adaptability of the forages used. Shadow causes stress to plants growing in the understory of ICLF systems due to limitation of photo synthetically active radiation, whose intensity varies with location, time of the year and the tree component.

Reduction of light incidence on forage leads to limited growth rates as a function of energy restriction necessary to the photosynthetic processes, requiring a number of morphological, physiological, structural and anatomical adaptations from the plant, called acclimatization (Gobbi *et al*., 2011). In this context, this work aimed to evaluate anatomical characteristics of tropical *Panicum* grasses under an ICLF system in the Brazilian Cerrado.

Materials and Methods

The experiment is located at Embrapa Beef Cattle research station in Campo Grande-MS (20º27'S and 54º37'W; mean altitude of 530 m). The climate corresponds to the humid tropical type, sub-type Aw (Köppen classification), with hot and rainy summers. Soil is classified as clay Oxisoil. The ICLF system is based on 227 Eucalyptus trees⁄ha planted in 2009 (*Eucalyptus urophylla* x *Eucalyptus grandis* - clone H13). Pastures are kept for three years, followed by one season soybeans. A similar system with no trees was also seeded as reference. Forages evaluated were seeded in October 2013, in plots of 20 m by 1.5 m, in 0.25 m spaced rows, receiving 50 kg⁄ha NPK 0-20-20 at seeding and 90 kg⁄ha in April 2014, after second soybean harvest. Grass seeding rates were adjusted for 200 pure viable seeds⁄ha. Grasses were cut at 70 days after sowing and after 35 days from the first, in order to evaluate regrowth potential.

Experimental design was a randomized block in split plot with two replications. Plots corresponded to forages (*Panicum maximum* cvs. Massai, Mombaça, Tanzania, *Panicum spp*. cv. Tamani and access PM44) and the subplots corresponded to sampling points (A, B, C, D, E and F). Samples were taken at five equidistant points (A, B, C, D and E) between Eucalyptus rows. Sampling points A and E were the points closest to trees. Point F was located in the reference system, considered having 100% photosynthetically active radiation. Light incidence was measured using a portable ceptometer. Plants were cut close to the ground, weighted and dried in forced-air oven at 65ºC until constant weight was reached. After grinding, samples were analyzed for in vitro organic matter digestibility (IVOMD) using NIRS.

For the anatomical study, samples were collected from leaf blades in six sampling points. Within the sample area, five tillers were randomly selected, collecting one leaf blade of each. Lab samples were the penultimate expanded leaf blade, with exposed ligules, cut in the basal region of the main vegetative shooting. Leaf blades were identified and measured regarding width at (central area) and length (from the apex of the blade to the base of the ligule insertion). Sample fragments of 1 cmwere taken from the central area of five blades and stored in bottles filled with FAA solution (formalin aceto-alcohol).

After fragment sampling leaf area area was measure using Licor 3100. Afterwards, dry matter content of leaf blades was obtained in forced air circulation oven at 65°C. The 1 cm leaf blade fragments underwent progressive alcoholic series with butyric tertiary alcohol (Dankin and Hussey, 1985). After dehydration fragments were processed with paraplast. Fragments were sectioned with a thickness of 10 micrometres, using a manual rotary microtome, followed by a 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. First, the cross sectional area projected in the video was measured followed by determination of the area occupied by abaxial and adaxial epidermal tissues from the parenchyma bundle sheath (GMP) from the sclerenchyma from vascular system. The mesophyll area was calculated through the difference between total area and the other tissues area.

Results were presented as a ratio of each tissue's area in relation to the total area. Tukey test was used adopting a probability level of 5% through statistics software SISVAR application version 5.3.

Results and Discussion

There was no interaction between variables for forage dry matter production and IVOMD as well as for anatomical characteristics of leaf blades. For forage dry matter production, there was no difference among cultivars of *Panicum* spp. nor between sampling points, being the average 6139 kg DM/ha.

Regarding the anatomical characteristics analyzed, the Mombaça grass had a higher proportion of vascular tissue when compared to grasses PM44 and Tamani, higher proportion of sclerenchyma tissue and smaller proportion of mesophyll compared to grasses PM44 and Tanzania. These are attributes that can compromise the digestibility of Mombaça grass, since mesophyll tissues are highly digestible while sclerenchyma and vascular tissues, in this case the xylem, are lignified tissues and therefore, not digestible. Indeed, Mombaça and Tamani grasses had lower IVOMD, 57% and 58%, respectively, compared to Tanzania grass (62%). Grasses Massai (60%) and PM44 (60%) had IVOMD intermediate, not differing from the others.

In the case of tropical grasses, that during photosynthesis produce their first stable organic compound with 4 carbons, cells containing chloroplasts are mesophyll cells and parenchymal cells of sheath bundles, so an increase in proportion of these tissues contributes to photosynthesis efficiency of these forages under shade.

Regarding the relationship with sampling points, there was less area occupied by cell tissues of leaves from points A and E, with higher shading degree in relation to point F, under plain sun. Indeed shaded plants have fewer cells and lower leave thickness. Point A had the highest IVOMD, reaching 64% compared to points C (59%), D (58%) and F (56%).

Table 1: Area covered by cell tissues of leaves (AT) ratio (%) occupied by the vascular tissue (TV), Sclerenchyma, the adaxial epidermis, abaxial epidermis, parenchyma bundle sheath (GMP) and the mesophyll in cross sections of leaf blades as well as specific leaf area (SLA) of *Panicum* grasses.

Forage	AT (μm^2)	TV	Sclerenchyma	Adaxial epidermis	Abaxial epidermis	GMP	Mesophyll	SLA (dm^3/g)
PM44	120054^{ab}	6.8^{b}	3.4^{c}	16.4^{a}	9.8^{ab}	36.7^{ab}	27.0^{a}	0.8^{a}
Massai	89044^{b}	7.6^{ab}	4.8^{a}	18.8^{a}	9.8^{ab}	36.3^{b}	22.7^{ab}	0.6^{a}
Mombaça	98477^{ab}	9.3^{a}	4.5^{a}	17.0^{a}	9.0^{b}	40.6^{a}	19.7^{b}	1.1^{a}
Tamani	131631^{a}	6.9^{b}	4.4^{ab}	18.0^{a}	10.5^{a}	36.0^{b}	24.3^{ab}	0.6^{a}
Tanzania	122035^{ab}	7.3^{ab}	3.5^{bc}	16.0^{a}	8.9^{b}	38.0^{ab}	26.3^{a}	1.3^{a}
CV (%)	18.7	17.1	11.6	13.3	7.3	5.9	11.1	83.8

Means followed by the same letter in the columns do not differ by Tukey test at 5% probability.

Table 2: Area covered by the cell tissues of leaves (AT) ratio (%) occupied by the vascular tissue (TV), Sclerenchyma, the adaxial epidermis, abaxial epidermis, parenchyma bundle sheath (GMP) and the mesophyll, in cross sections of leaf blades as well as specific leaf area (SLA) of *Panicum* grasses in different sampling points.

Sampling point	Degree of shading	AT (μm^2)	Sclerenchyma	Epidermis + GMP	Mesophyll	SLA (dm^3/g)
A	87	97259[b]	12.0[a]	65.1[a]	23.0[a]	1.1[a]
B	8	114789[ab]	11.4[a]	64.0[a]	24.6[a]	1.0[a]
C	5	117163[ab]	11.6[a]	63.2[a]	25.3[a]	0.6[a]
D	15	108796[ab]	11.7[a]	64.0[a]	24.3[a]	1.0[a]
E	90	105178[b]	11.1[a]	64.7[a]	24.2[a]	0.9[a]
F	0	130304[a]	12.6[a]	64.8[a]	22.6[a]	0.7[a]
CV (%)	-	14.1	17.9	3.0	14.5	63.1

Means followed by the same letter in the columns do not differ by Tukey test at 5% probability.

Conclusion

The Tanzania and PM44 cultivars showed leaf blades with lower proportion of sclerenchyma and higher proportion of mesophyll than Mombaça grass while Tanzania grass showed higher in vitro organic matter digestibility than Mombaça and Tamani grasses.

Points closer to tree rows, with a higher shading degree, resulted in thinner leaf blades. Point A showed higher in vitro organic matter digestibility than the points C, D and F.

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