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Leaf tissues proportion and chemical composition of *Axonopus jesuiticus* x *A. scoparius* as a function of pig slurry application

Proporção de tecidos foliares e composição química de *Axonopus jesuiticus* x *A. scoparius* em função da aplicação de dejetos líquido de suíno

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ABSTRACT

This study aimed to evaluate the chemical and anatomical attributes of leaves of giant missionary grass to application of 0, 62, 124, 186, 248 and 310 m³ ha⁻¹ of pig slurry. At 83 days after the last application of fertilizer, the leaf blades were collected, fixed in FAA 70%, sectioned, stained, photographed and digitalized. The transversal section of leaf blades were evaluated for proportion of epidermis, lignified vascular tissue + sclerenchyma, non-lignified vascular tissue and parenchyma with an image-processing system calibrated to 1 mm pixel⁻¹. Leaf samples were analyzed for crude protein, acid detergent fiber, neutral detergent fiber and hemicellulose content by near infrared reflectance spectroscopy. The pig slurry application up to 310 m³ ha⁻¹ significantly increased the percentage of crude protein, parenchyma, epidermis, non-lignified vascular tissue and hemicellulose, while decreasing the percentage of acid detergent fiber and lignified vascular tissue + sclerenchyma. The Pearson's correlation was positive between crude protein and non-lignified vascular tissue, and between acid detergent fiber and lignified vascular tissue + sclerenchyma. The percentage of hemicellulose was positively correlated with epidermis, parenchyma and non-lignified vascular tissue. A negative correlation between acid detergent fiber and epidermis, parenchyma and non-lignified vascular tissue was observed.

Key words: crude protein, epidermis, fiber, lignified vascular tissue + sclerenchyma, parenchyma.

RESUMO

Este trabalho teve como objetivo avaliar os atributos anatômicos e químicos da grama-missioneira-gigante à aplicação de 0, 62, 124, 186, 248 and 310 m³ ha⁻¹. Decorridos 83 dias do último corte e da aplicação do dejetos, as lâminas foliares foram coletadas, fixadas em FAA 70%, seccionadas,

coradas, fotografadas e digitalizadas. Seções transversais das lâminas foliares foram avaliadas quanto à proporção das áreas de epiderme, tecido vascular lignificado + esclerênquima, tecido vascular não lignificado e parênquima, na região entre a quilha e o segundo maior feixe vascular, com sistema de processamento de imagem calibrado para 1 mm pixel⁻¹. Amostras de folhas foram coletadas e analisadas quanto ao teor de proteína bruta, fibra insolúvel em detergente ácido e fibra insolúvel em detergente neutro, pelo método de espectrometria de reflectância no infravermelho proximal. A aplicação de até 310 m³ ha⁻¹ do dejetos líquido de suíno aumentou os percentuais de proteína bruta, hemicelulose, parênquima e tecido vascular não lignificado, reduzindo os percentuais de fibra insolúvel em detergente ácido e tecido vascular lignificado + esclerênquima. A correlação de Pearson foi positiva entre o percentual de proteína bruta e tecido vascular não lignificado, e entre o percentual de fibra insolúvel em detergente ácido e tecido vascular lignificado + esclerênquima. O percentual de hemicelulose mostrou correlação positiva com epiderme, parênquima e tecido vascular não lignificado. Houve correlação negativa entre o percentual de fibra insolúvel em detergente ácido e epiderme, parênquima e tecido vascular não lignificado. O dejetos líquido de suínos, utilizado como fonte de nitrogênio, promove melhoria em atributos químicos e anatômicos, que afetam o valor nutritivo da grama-missioneira-gigante.

Palavras-chave: epiderme, fibra, parênquima, proteína bruta, tecido vascular lignificado + esclerênquima.

INTRODUCTION

Pig slurry is applied on pastures and crop lands to supply nutrients mainly nitrogen (N) to non-legumes plants. Several studies has reported the positive effect of pig slurry application on the

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production and nutritive value of forage grasses (ADELI et al., 2005; SCHEFFER-BASSO et al., 2008; VIELMO et al., 2011). Besides of recognized effect of PS on increasing of forage production, the effects of this N source on anatomical feature are unknown, especially in native forage plants.

The accumulation of N in plants can change tissue proportions of leaves and chemical composition, affecting structural organization or anatomy of plant organs and its constituent tissues. This effect influences the intake, the nature of particles produced and their rumen passage rate, and cell wall digestibility, providing greater or smaller accessibility of its polysaccharides to rumen microorganisms (CARVALHO & PIRES, 2008). In the same way, tissue proportions and cell wall thickness are important anatomical traits influencing nutritive value of grasses. The tissues lowest digested correlate negatively with CP and *in vitro* dry matter digestibility (IVDMD) (PACIULLO, 2002). In addition, neutral detergent insoluble fiber (NDF) and ADF has been correlated with leaf anatomy (QUEIROZ et al., 2000).

The giant missionary grass is native forage originated from Vale do Itajaí, Santa Catarina. It is a hybrid resulting from natural crossing between *A. jesuiticus* Araújo (Valls) and *A. scoparius* (Flüggé) Kuhl. It is also referred as *A. catharinensis* Valls, but the publication of this name is not yet formalized. It is a perennial and warmer grass, with high yield response to pig slurry fertilization (MIRANDA et al., 2012). The objective of this study was to evaluate the effect of pig slurry fertilization on chemical and anatomical attributes that affect the nutritional value of this hybrid.

MATERIAL AND METHODS

The evaluated leaves were sampled on an experiment where the giant missionary grass (accession V14337 - BRA-002020) was fertilized with pig slurry (PS) rates for two years (MIRANDA et al., 2012). This experiment was carried out in Epagri Experimental Station, in Chapecó, Santa Catarina. The climate is subtropical classified as Cfa and the soil is classified as dystrophic oxisol. A randomized complete block design, with five replications, and plots of 30m² (5m x 6m) were used. The studied leaves were sampled in the first year of the experiment (2007/2008). Along this year the following rates of PS were applied: 0, 62, 124, 186, 248 and 310m³ ha⁻¹ (0, 100, 200, 300, 400 and 500kg N ha⁻¹, respectively). To evaluate the tissue proportion and the chemical composition of the leaves,

three samples of leaves were taken in each plot of the one block, in September 15th of 2008. The sampling method is commonly used in anatomical studies, in which the samplers constitute the replications and the experimental design is considered as completely randomized (LIMA et al., 2001). Leaves were sampled when the plants were in vegetative stage and 83 days of regrowth. This time was assumed as appropriate because the growth season (spring) of the grass was beginning and the four fractions of the PS rates had been applied. Three green leaves per treatment were taken, being the second fully expanded leaf/tiller, from the top to bottom. The leaf blades were washed, sectioned in small pieces and fixed in formaldehyde: acetic acid: ethanol 70% - 5:5:90 (FAA 70%), for 48 h, and stocked in alcohol 70%. Transversal free-hand sections were made at middle third of the blade, followed by staining with basic fuchsin diluted to 0.5% in ethanol 50% and Astra blue, to show the lignified walls and the cellulose walls in the leaf cells. The anatomical blades were photographed in light microscope Zeiss and the pictures were digitalized to measure the area of the tissues. The image-processing system Image Pro Plus 5.0 software, calibrated to 1mm pixel⁻¹, was used to quantify the tissues according the cell wall nature (ALVES DE BRITO et al., 1999): a) epidermis, except trichomes and bulliform cells; b) lignified vascular tissue + sclerenchyma (LVT+S), including xylem, fiber, sclerenchymatic sheath and other cells with lignified wall cell that had present in vascular bundles; c) non-lignified vascular tissue (NLVT), including phloem and other cells in the bundles that presented cellulosic cell wall; d) parenchyma, including bulliform cells and bundle vascular parenchymatic sheath. The tissue areas of each replication were measured along the cross section from the keel until the second major vessel of the one side of the wing. To evaluate the chemical composition, three leaf samples were taken in the same plots used to obtain the leaf samples for anatomical study. The samples were dried at 60°C for 72h, weighted, ground before passing through a 1mm screen and analyzed for percentage of CP, ADF, NDF and hemicellulose (HC) by near-infrared spectroscopy (NIRS), using the usual calibration method described by FONTANELI et al. (2004). The HC content was determined by difference between these two fractions (VAN SOEST et al., 1991). The dry matter (DM) content was determined by difference between the fresh and dried weight of the samples.

A regression analysis was established between the quantified tissues or the chemical analyses and the PS rates and a Pearson's linear correlation

was established between these two kinds of variables (chemical composition and tissue proportion), using SISVAR software (FERREIRA, 2011). The dissimilarity between PS rates was checked by clustering analysis by using Ward's method, based in Mahalanobis distance (D^2). The relative importance of chemical and anatomical attributes affecting divergence among pig slurry rates applied on giant missionary grass was calculated according Singh's method (SINGH, 1981). The multivariate analysis was performed on Genes software (CRUZ, 1997).

RESULTS AND DISCUSSION

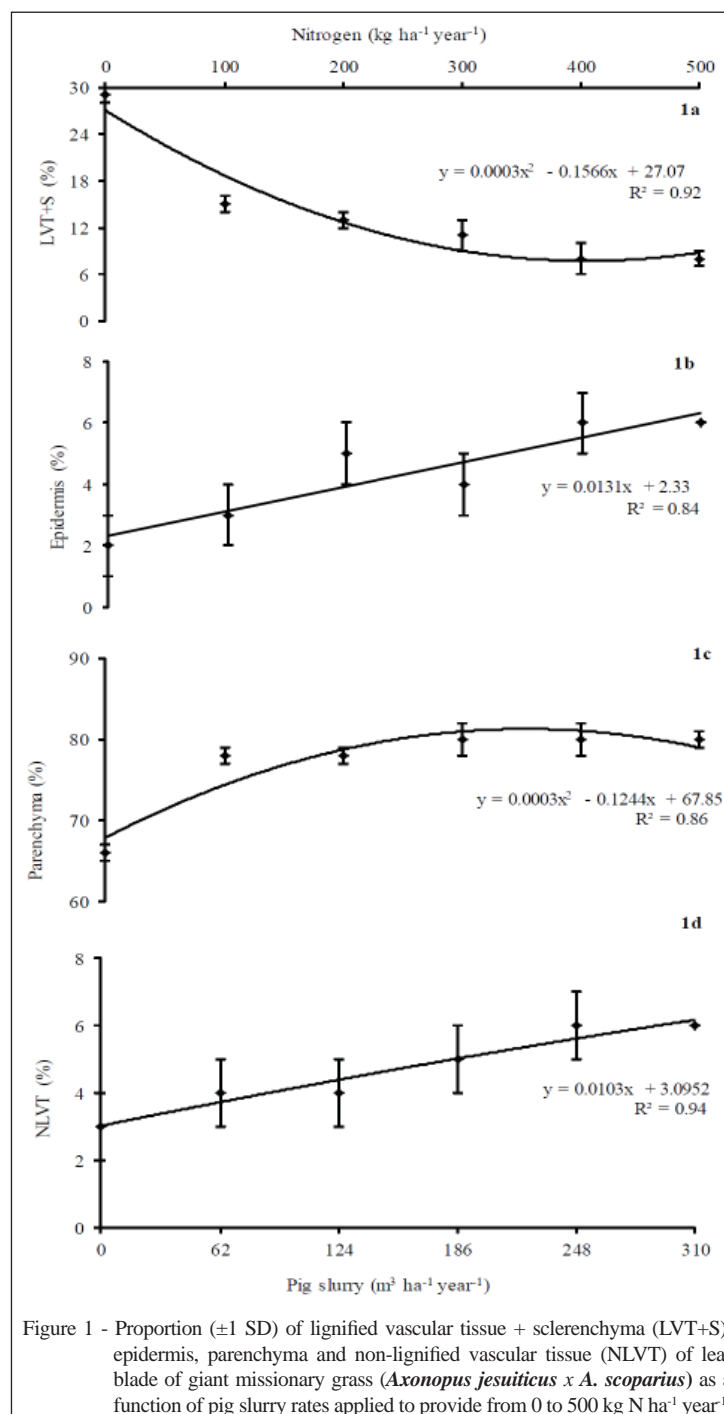
The proportion of epidermis and NVT linearly increased with increasing of PS application up to $310\text{m}^3\text{ha}^{-1}$, and a quadratic effect was observed for parenchyma tissue. A negative quadratic effect of fertilization was verified for LVT+S (Figure 1). Although N availability is known to affect leaf elongation, its effect on leaf tissue proportion remains poorly understood, especially when the N source is manure. No references were found about the effect of PS fertilization on grass leaf anatomy, but the reduction of percentage of sclerenchyma and vascular tissue was reported for *Panicum maximum* Jacq. fertilized with mineral N (BASSO, 2009). Plant growing with lower available N tend to be woody and to have thick cell walls, presumably because much of the carbohydrate is deposited in these structures, whereas if availability of this N is higher it would be used in protein synthesis (SINNOT, 1960). Under N deficiency the excess of carbon assimilated are used for lignin formation and other cell wall components, increasing the concentration of cell wall and decreasing its digestibility (BELANGER & McQUEEN, 1999). Thus, the leaf blade microanatomy and inherent characteristics of cell walls affect digestibility by rumen microorganisms (AKIN, 1989).

The effect of PS fertilization on leaf anatomy found on this study was positive since the tissue proportion improved as a result of this practice indicates higher nutritive value of the forage. The epidermis content increase is desirable because the inner wall of this tissue offers no resistance to microbial digestion, since the composition of its cell wall is equal to the mesophyll (CHESSON et al., 1986). In addition, the increase on the non-lignified vascular tissue and parenchyma proportion, which have high digestibility, and the reduction of the structural characteristics that limit forage breakdown, which are mainly the support tissue highly lignified, like sclerenchyma and xylem (AKIN, 1989), showed

an improvement of the anatomical attributes that influences the nutritive value of the grass.

In a parental of giant missionary grass, *Axonopus scoparius*, the maturity of cultivars decreased the degradability of the forage, which was related to cell wall thickening and increased lignified tissue area (LIMA et al., 2001). Dynamics of lignification in cell walls can indicate the quality of forage plants, and many authors have linked the increase of tissue lignification as a result of maturity and management effects, as cutting and fertilization of grass, with a consequent decrease of the digestibility of forages to ruminants (ALVES DE BRITO et al., 1999). Since lignin concentration and the digestibility of the giant missionary grass were not evaluated in our study, such determinations must be included in future researches about PS fertilization effect on the nutritive characterization of this forage. However, our results can indicate a good trend about this effect.

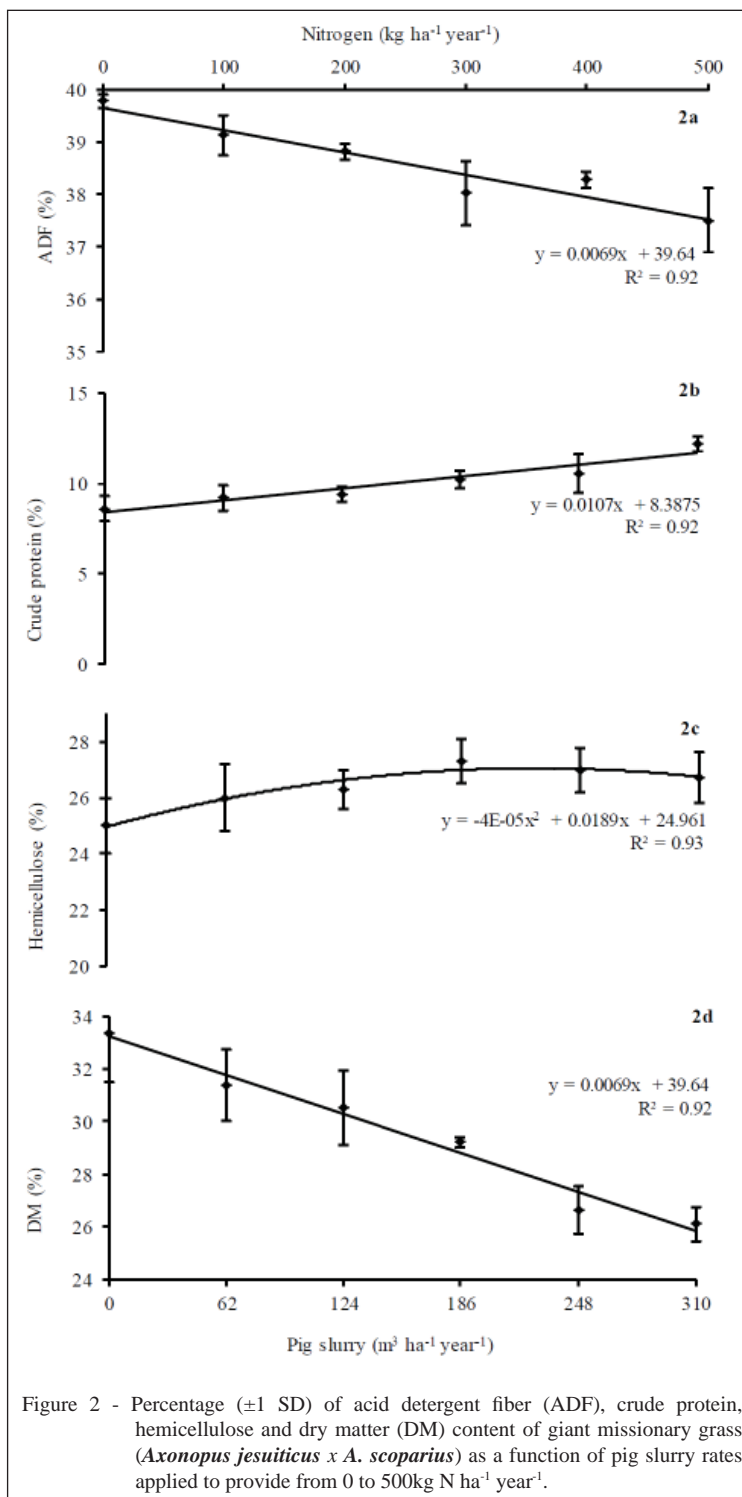
The effect of PS rates did not affect chemical composition of NDF (average= 65%). However, decreased of both ADF (-5.78%) and DM contents (-26.6%), and increased of CP (41.9%) and HC contents (between 6.8 to 9.2%) were observed (Figure 2). These results show that PS application would improve the digestibility of the giant missionary grass, since it is affected by these variables (AKIN, 1989). Determination of ADF, which consists of lignin associated with cellulose and other structural polysaccharides, has been suggested as the best chemical method for estimation of IVDMD (ROHWEDER et al., 1978). Forages with higher ADF are lower in digestible energy than forages with lower ADF, which means that ADF level increases, digestible energy levels decrease (SAHA et al., 2010). The highest CP percentage (12.2%) was found at the highest rate ($310\text{m}^3\text{ha}^{-1}$) of PS and was similar to also reported (11.0-14.7%) for the giant missionary grass (DESCHAMPS & TCACENCO, 2000). The same trend was reported for *Cynodon* spp. fertilized with pig slurry (SCHEFFER-BASSO et al., 2008; VIELMO et al., 2011). The effects found in this study are the same found with mineral N fertilization on grasses, which increases the water concentration, and at higher rates results in progressively greater concentrations of N in the plant tissue (BUXTON & FALES, 1994). A quadratic effect of PS was observed in HC (Figure 2). ADELI et al. (2005) also verified a quadratic trend of ADF for bermudagrass (*Cynodon* spp.), with the highest content of these variables, 56.3 and 37.4%, respectively, on the treatments with PS. VIELMO et al. (2011) observed decreased of



this fraction in Tifton 85 (*C. dactylon* (L.) Pers. x *C. transvaalensis* Burt Davy), and BARNABÉ et al. (2007) reported decrease in *Brachiaria brizantha* (Hochts. ex A. Rich) as a function of PS rates.

The Pearson's correlation between leaf tissue proportion and chemical composition showed positive association between HC and tissues with

higher degradability (epidermis, parenchyma and NLVT), and negative correlation with LVT+S, a poor degradability tissue (Table 1). Opposite correlation was found between these tissues and the percentage of ADF. These results were similar to found for tropical grasses, where a positive correlation between epidermis and CP, and a negative correlation between



ADF and epidermis and ADF and lignified vascular tissue were observed (QUEIROZ et al., 2000).

The nutritive value of forage plants is the result of many attributes and therefore the effect of management practices can be better analyzed when

it is considered the set of variables. The multivariate analysis showed that the attributed that exhibited greater relative contribution for divergence among the pig slurry rates were LVT+S, epidermis and parenchyma, which explained 96.9% of the variability

Table 1 - Pearson's correlation coefficients between chemical composition and tissue proportions of leaf blade of giant missionary grass (*Axonopus jesuiticus* x *A. scoparius*).

Chemical composition	Tissue proportion (%)			
	Epidermis	Parenchyma	NLVT	LVT+S
CP (%)	0.77 ns	0.62 ns	0.91*	-0.73 ns
ADF (%)	-0.82*	-0.81*	-0.91*	0.87*
Hemicellulose (%)	0.84*	0.83*	0.85*	-0.89*
DM (%)	-0.92**	-0.79 ns	-0.98**	0.90*

CP: crude protein. ADF: acid detergent fiber. DM: dry matter. LVT+S: lignified vascular tissue + sclerenchyma. NLVT: non-lignified vascular tissue. * **Significant by t test ($P < 0,05$ and $< 0,01$, respectively). ns= no significant ($P > 0,5$).

among the pig slurry rates (Table 2). Three main groups were created in the dendrogram (Figure 3). The Group II (248 and 310m³) was characterized by better indicators of nutritive value, by the lower proportion of LVT+S, and greatest proportion of parenchyma, epidermis and NLVT.

CONCLUSION

Pig slurry used as nitrogen source improves chemical and anatomical attributes that affect the nutritional value of the leaves of giant missionary grass.

Table 2 - Relative importance (R.I) of chemical and anatomical attributes affecting divergence among pig slurry rates applied on giant missionary grass (*Axonopus jesuiticus* x *A. scoparius*) and characterization of groups formed by Ward's method.

Attribute	R.I (%)	Groups		
		I (0m ³)	II (244 and 310m ³)	III (62, 124 and 186m ³)
DM	0.4359	33.3	26.4	30.4
CP	0.3440	8.9	11.3	9.6
ADF	0.2954	39.8	37.9	38.7
Hemicellulose	0.2067	25.1	27.0	26.5
Epidermis	26.1434	2.0	6.0	4.0
LVT+S	50.7069	29.0	8.0	13.0
NLVT	1.7676	3.0	6.0	4.3
Parenchyma	20.1001	66.0	80.0	78.7

DM: dry matter. CP: crude protein. ADF: acid detergent fiber. HC: hemicellulose. LVT+S: lignified vascular tissue + sclerenchyma. NLVT: non-lignified vascular tissue.

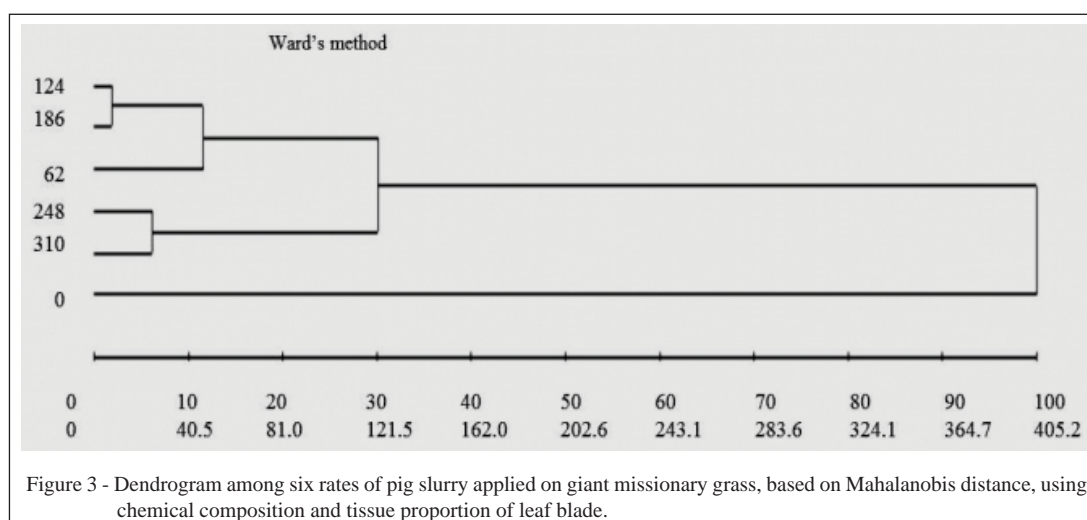


Figure 3 - Dendrogram among six rates of pig slurry applied on giant missionary grass, based on Mahalanobis distance, using chemical composition and tissue proportion of leaf blade.

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