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Morphophysiological characterization of giant missionary grass accessions[1]

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ABSTRACT - In this study, the phenotypic diversity of five accessions of giant missionary grass (*Axonopus jesuiticus* × *A. scoparius*) was evaluated by using morphophysiological traits. Accessions V 14337, V 14403, V 14404, V 14405 and V 14406 are hybrids derived from spontaneous crossing that occurred in Vale do Itajaí, Santa Catarina, Brazil. Plants were cultivated in greenhouse and evaluated at 60, 90, 120, 150, 180, 210 and 240 days of growth. Variation was observed for dry matter production, phenology and morphological traits, showing the possibility of selection. Flowering started at 210 days of growth and only in accessions V 14337 and V 14404. The Mahalanobis distance among accessions ranged from 35.64 (V 14403 and V 14405) to 183.38 (V 14337 and V 14405), and three groups were formed, based on 17 vegetative morphophysiological traits evaluated in plants with 180 days of growth: G1 (V 14403, V 14405), G2 (V 14406) and G3 (V 14337, V 14404). Group I presented the greatest dry matter production of stolon and aboveground, which were the traits with the largest relative contribution to genetic divergence, 38.67% and 38.31%, respectively. Accessions V 14403 and V 14405 are the most promising for agronomic evaluations that address their records as forage cultivars.

Key Words: *A. catharinensis*, dry matter production, growth analysis, phenology

Introduction

The acquisition, characterization and evaluation of wild germplasm adapted to climate and soil of each region are fundamental for the continuing animal production on pasture, which is the predominant system in Brazil.

In a germplasm collection of forage plants maintained by the Empresa de Pesquisa Agropecuária e Extensão (Epagri), in Vale do Rio Itajaí, Santa Catarina, the introduction of Tcacenco/Ramos 037 (*Axonopus* spp.) prevailed for its height, productivity and acid soil adaptation (Tcacenco & Soprano, l997). The grass is popularly known as *missioneira-gigante*, in this study called giant missionary grass, because of its bigger size in relation to jesuit grass (*Axonopus jesuiticus* (Araújo) Valls).

The giant missionary grass is a triploid hybrid (2n = 3x = 30) resulting from natural crossing between *A. jesuiticus* and *A. scoparius* (Flüggé) Kuhlm., and without viable seeds because the cell meiosis does not produce perfect gametes (Valls et al., 2000). It is a stoloniferous plant, a trait inherited from *A. jesuiticus*, with branched inflorescence, typical of *A. scoparius*, and it is vegetatively propagated. It is also referred to as *A. catharinensis* Valls (Perez, 2008; Probst et al., 2009); however, the publication of this new name is not formalized yet.

Researches have proven the forage potential of this grass related to dry matter production in warm season (11,700 kg DM/ha), and its nutritive value, with levels of up to 11.9% of crude protein and 70.3% of *in vitro* digestibility of organic matter (Tcacenco, 1994; Tcacenco & Soprano, 1997). Dufloth (2002) stressed its high persistence, animal acceptability and the possibility of ensuring an average daily gain of 0.814 kg/animal.

The spontaneous hybridization between *A. jesuiticus* × *A. scoparius* occurred in several opportunities, favoring the perpetuation of distinct genotypes (Valls et al., 2000). Epagri added other problable hybrids to its germplasm bank; hybrids whose accessions, analyzed in this study, are available at Embrapa Recursos Genéticos e Biotecnologia, in Brasília, Distrito Federal, Brazil. Limited information regarding the variation that exists in this germplasm is available.

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This study evaluated the divergence between accessions of giant missionary grass, guided by the assumption that there is variability in the germplasm, and that the morphophysiological traits enable its efficient characterization. The information can allow the development of a breeding program by indicating the most promising materials.

Material and Methods

The research was developed at the research station of Epagri, in Chapecó, Santa Catarina (27° 07' South and 52° 37' West), between August 2008 and April 2009. The climate of the region is Cfa type (Mota et al., l971) (Figure 1).

Five accessions of giant missionary grass (Table 1) were evaluated at 60, 90, 120, 150, 180, 210 and 240 days of continuous growth, using randomized complete block design, with five replications. Plants were grown in plastic pots filled with 0.5 kg (60, 90 and 120 days), 2.0 kg (150 and 180 days) and 4.0 kg (210 and 240 days) of dried substrate, according to the harvest age. The substrate (Ferticel®) presented the following attributes: pH = 7.1; dry matter content = 0.64%; N = 5.72 g/kg; P = 1.97 g/kg; K = 2.02 g/kg; Ca = 11.01 g/kg; Mg = 13.12 g/kg; Cu = 51.46 mg/kg; Zn = 45.80 mg/kg; Fe = 9863.70 mg/kg and Mn = 198.35 mg/kg.

Stolon cuttings, presenting one tiller with about 15 cm of height, two visible nodes, two fully expanded leaves and one expanding leaf, were planted in pots and placed on benches exposed to air under a polyethylene net to avoid rain or hail damages. Plants were watered twice a day, and pots, in each block, were rotated weekly to provide equal light condition to all plants, and minimize variation due to border effects. There was no need for fertilizers or chemical plant protection.

Plants were evaluated according to the following routine: plant height was measured at the highest point, between the ground and the curvature of the upper leaves (vegetative

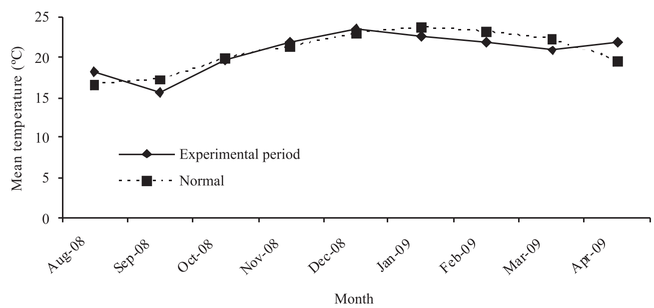

Figure 1 - Mean monthly temperatures during the experimental and normal regional periods(thirty-year mean). Chapecó, Santa Catarina, Brazil.

Table 1 - Identity, denomination and probable origin of giant missionary grass accessions

Identity	Accession code	First denomination	Origin
V 14337	*Axonopus* BRA-002020	Tcacenco & Ramos 037 (*grama missioneira gigante*)	Originally collected in rural property in Rio do Oeste, SC; kept in cultivation at Epagri, Itajaí, SC, under EEI 85269 code; collected for the herbarium, as documental exemplar, in screenhouse at Embrapa Cenargen, Brasília, DF, under V 14337 code
V 14403	*Axonopus* BRA-002429	Tcacenco & Vetterle 048 (*missioneira gigante 2*)	Originally collected in rural properties in Dona Emma, SC; kept in cultivation at Epagri, Itajaí, SC, under EEI 89028 code; collected for the herbarium, as documental exemplar, in bed cultivation at Epagri, Lages, under VTsRcRMJr 14403 code
V 14404	*Axonopus* BRA-002437	(*missioneira gigante*)	Kept in cultivation in the Experimental Station at Epagri, in Lages, SC, coming from Ituporanga, SC, where it was considered as a probable the EEI 85269 accession; collected for the herbarium, as documental exemplar, in bed cultivation at Epagri, Lages, under VTsRcRmJr 14404 code
V 14405	*Axonopus* BRA-002445	Tcacenco & Ramos 046 (*missioneira-pitã-açu*)	Originally collected in rural properties in Presidente Getúlio, SC; kept in cultivation at Epagri, Itajaí, SC, under EEI 89009 code; collected for the herbarium, as documental exemplar, in bed cultivation at Epagri, Lages, under VTsRcRmJr 14405 code
V 14406	*Axonopus* BRA-002453	(*missioneira-pitã-mirim, missioneira roxa*)	Kept in cultivation in the Experimental Station at Epagri, in Itajaí, SC, under a double denomination, EEI 89004 and *missioneira roxa Ituporanga* at Epagri; collected for the herbarium, as documental exemplar, in bed cultivation at Epagri, Lages, under VTsRcRmJr 14406 code

height) and tip of inflorescences (reproductive height). The angle of insertion of two peripheral opposite tillers to the ground was determined with a protractor, and after that, plants were removed from the pots. The length of the longest root was measured and the root system volume was determined by the water-level difference, after immersion in a graduated cylinder. The number of tillers was recorded, and in the longest tiller, the diameter of the second basal internode and the number of leaves were estimated. Stolons were counted and their length measured; the longest stolon was evaluated as for diameter of the second internode, counted from the plant insertion, number of nodes and internode average length. Leaf color (green, green-purple) was registered, and the second fully expanded leaf, counted from the tip to the base of the highest tiller, was evaluated as for blade width and its angle of insertion in the tiller. Inflorescences were characterized for length, branch number, length of the longest branch and spikelet number per branch. Roots, green and senesced leaves, stolons, tillers and inflorescences were dried in a forced ventilation oven at 60ºC during 72 h and weighed.

Data were submitted to split-plot analysis of variance (main plot: accessions; subplot: days of growth) and regression analysis, using the software package SISVAR (Ferreira, 2000). Means were compared using Tukey's test at the 5% probability. Genetic divergence was evaluated by multivariate procedures, using 17 vegetative morphophysiological traits recorded in plants with 180 days of growth: Mahalanobis distance, relative importance of characters to genetic divergence, by Singh method (1981), and hierarchical clustering (UPGMA method). Multivariate analysis was performed using the software package Genes (Cruz, 2001).

Results and Discussion

Aboveground and root dry matter accumulation increased quadratically as function of growing days, with variation between accessions (Figure 2). In plants with 240 days of growth, the dry matter production of these components was 2.0 times greater for V 14403 and V 14405 accessions compared with the other ones. These accessions were also superior as for stolon dry matter accumulation (Figure 3).

In all accessions, the stolon dry matter production was late, only from 120 days, stabilized between 150 and 210 days of growth, followed by resumption of growth rate. The cubical trend observed for this attribute illustrated its rhythmic growth, which is common in stem structures (Barthélémy & Caraglio, 2007), and suggests a slow establishment of the giant missionary grass. On the other hand, there is reference about its easy establishment and dominance over other species in pastures (Nascimento et al., 1990). This discrepancy is an important aspect to be considered in future field essays because, in this study, stolons went over the edge of the pots and their rooting was restricted. Lateral expansion is one of the most important traits for stoloniferous species, as it implies in soil recovering, competition with other species, efficiency in intercepting radiant energy, and, consequently, faster utilization by animals.

Plants showed significant allocation of dry matter in roots and stolons (Figure 4). Accession V 14337 presented the largest root allocation (43%), and the opposite was

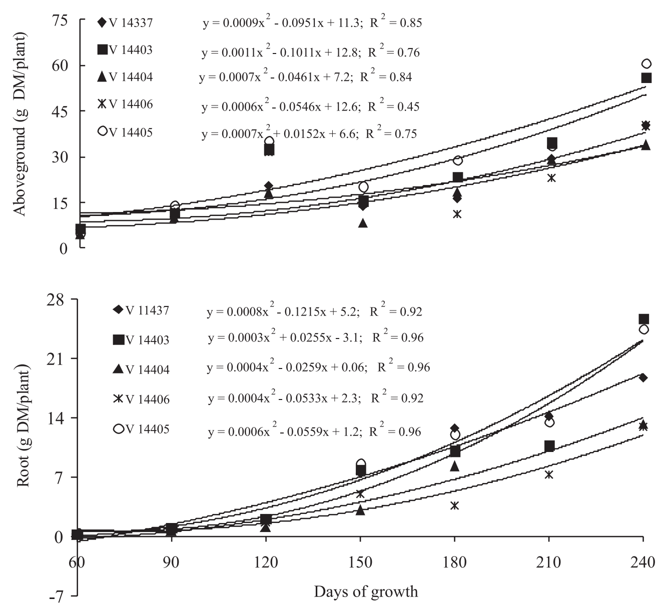

Figure 2 - Aboveground and root dry matter accumulation of giant missionary grass accessions as for days of growth.

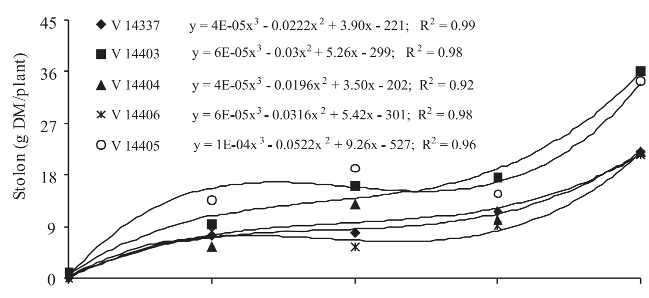

Figure 3 - Stolon dry matter accumulation of giant missionary grass accessions as for days of growth.

verified in V 14406 (24%). Nascimento et al. (1990) also reported the high root vigor of V 14337, which, among the hybrids of giant missionary grass, is the most cultivated in Santa Catarina. This feature must be a contributing factor to its high persistence under grazing (Dufloth, 2002) and acid soil tolerance (Tcacenco & Soprano, 1997).

Tropical grasses, in general, have expressive partitioning in subterranean structures, as it was pointed out by Grise et al. (2006) in cv. Pensacola (*Paspalum notatum* Flüggé), which allocated between 4 and 12 times more dry matter in roots + rhizomes in relation to aboveground. In the present study, the dry matter partitioning in leaves and tillers was 17% and 10% of total dry matter at 240 days of growth, respectively (Figure 4). In aboveground, these components represented 60% and 40% of dry matter, respectively, which it is excellent, considering the advanced plant age. This aspect suggests the aptitude of this grass, also, for hay production and deferment.

The relationship between leaf dry matter accumulation and days of growth was linear to accessions V 14337 and V 14403 and quadratic to the other ones (Figure 5). Maximum leaf production was recorded in V 14405 (10 g DM/plant) and the minimum occurred in V 14404 (5.9 g DM/plant), which also presented the smallest leaf senescence (26%) (Figure 6). In accessions V 14337 and V 14405, senescence reached the highest values, of 39% and 42%, respectively, suggesting variability in the morphogenesis. Santos (2005) registered high leaf durability in this grass (accession not mentioned), which was above 1,000 degrees/day (basal temperature = 0ºC).

Accessions varied in relation to phenology, since flowering was only observed in accessions V 14337 and V 14404, from 210 days of growth. These materials exhibited differences as for reproductive canopy height and inflorescence morphological traits (Table 2), except for inflorescence dry matter production, which was negligible. However, through cluster analysis, which considered only the vegetative characters, these materials remained in the same group (Figure 7). In the dendrogram generated by

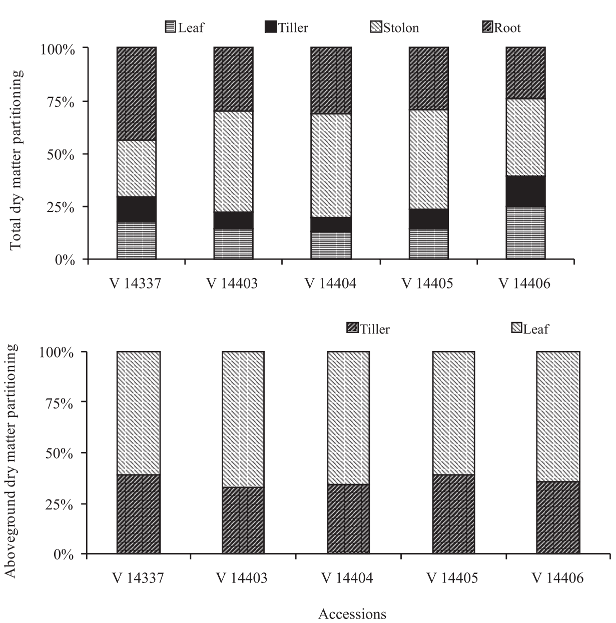

Figure 4 - Partitioning of total (aboveground + roots) and aboveground (leaves + tillers) dry matter of giant missionary grass accessions at 240 days of growth.

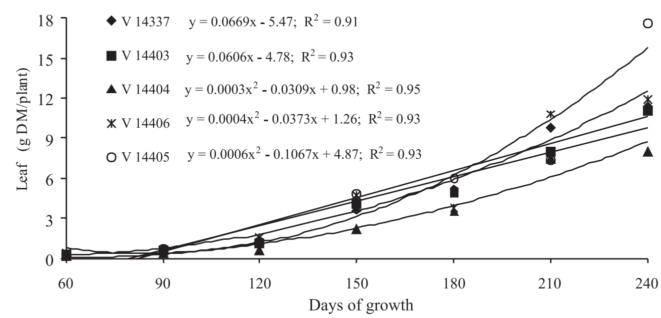

Figure 5 - Leaf dry matter accumulation of giant missionary grass accessions as for days of growth.

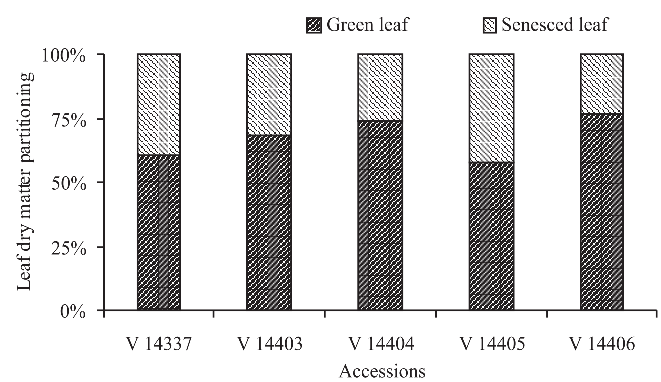

Figure 6 - Leaf dry matter partitioning of giant missionary grass accessions at 240 days of growth.

Table 2 - Reproductive morphophysiological traits of two giant missionary grass accessions

Traits	V 14337	V 14404
Reproductive canopy height (cm)	76.28a	42.85b
Reproductive tiller length (cm)	74.28a	17.14b
Branch number/inflorescence	13.42a	2.85b
Longest branch length (cm)	9.71a	3.14b
Spikelet number/longest branch	24.57a	7.71b
Inflorescence dry matter production (g DM/plant)	0.15a	0.05a

Means followed by same letter in the row do not differ (P>0.05) by Tukey test.

UPGMA method, assuming 50% of relative distance among accessions, three groups were formed: group I: V 14403 and V 14405 (D^2 = 35.64), group II: V 14406 and group III: V 14337 and V 14404 (D^2 = 56.36). The greatest divergence was verified between V 14337 and V 14405 (D^2 = 183.38).

Considering the traits with the highest relative contribution for genetic divergence (Table 3), stolon and aboveground dry matter production, the clustering was very consistent and expressed the accession yield performance. Group I was the most productive, and the opposite characterized the group II (Figure 8). The only accession that formed group II, V 14406, stood out from the others by its upright growth habit, demonstrated by greater angle of tiller insertion with the ground (Table 4), as well as by color leaf, which is green-purple. These attributes are characteristic of *A. scoparius* (Lima et al., 2001; Giraldo-Cañas, 2008), one progenitor of this hybrid.

Table 3 - Relative contribution for the genetic divergence (R.C.G.D), by Singh method (1981), of vegetative morphophysiological traits evaluated in giant missionary grass accessions at 180 days of growth

Traits	R.C.G.D (%)
Green leaf dry matter production (g/plant)	5.76
Senesced leaf dry matter production (g/plant)	1.10
Tiller dry matter production (g/plant)	1.78
Stolon dry matter production (g/plant)	38.67
Aboveground dry matter production (g/plant)	38.31
Root dry matter production (g/plant)	0.044
Total dry matter production (aboveground + root; g/plant)	6.08
Stolon (nº/plant)	0.16
Diameter of the longest stolon (mm)	1.37
Length of the longest stolon (cm)	0.66
Node (nº/longest stolon)	0.53
Internode of the longest stolon (cm)	0.22
Vegetative canopy height (cm)	0.35
Tiller (nº/plant)	1.10
Leaf (nº/plant)	2.26
Leaf blade width (cm)	0.25
Leaf blade length (cm)	0.17

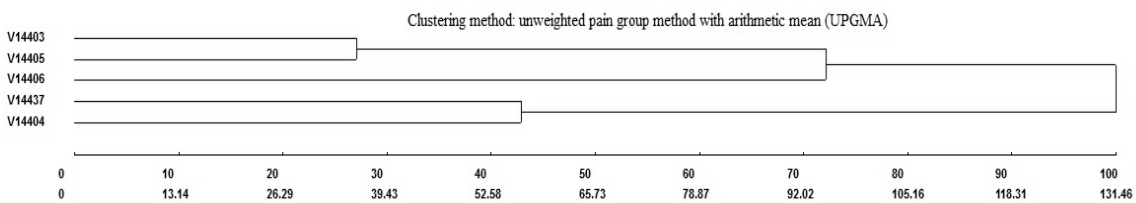

Figure 7 - Dissimilarity dendrogram of five giant missionary grass accessions obtained by UPGMA method, based on Mahalanobis distance, using 17 vegetative morphophysiological traits evaluated in plants with 180 days of growth. Cutting point = 50% of relative distance.

Table 4 - Vegetative morphophysiological traits of giant missionary grass accessions, at 180 days of growth

Traits	Accessions				
	V 14337	V 14403	V 14404	V 14405	V 14406
Green leaf dry matter production (g/plant)	4.08ab	3.88ab	2.48b	5.35a	3.12b
Senesced leaf dry matter production (g/plant)	1.16ns	1.12	0.98	0.60	0.63
Total leaf dry matter production (g/plant)	5.24ab	5.00ab	3.46b	5.95a	3.76b
Tiller dry matter production (g/plant)	3.38ns	2.45	1.81	3.85	2.08
Stolon dry matter production (g/plant)	7.88ab	16.06a	12.98ab	19.12a	5.47b
Aboveground dry matter production (g/plant)	16.45bc	23.45ab	18.27bc	28.93a	11.32b
Root dry matter production (g/plant)	12.74a	10.12a	8.33b	12.07a	3.61b
Total dry matter production (aboveground + root; g/plant)	29.19b	33.57ab	17.72c	41.00a	14.94c
Stolon (nº/plant)	2.20a	1.40ab	1.40ab	2.00ab	0.80b
Diameter of the longest stolon (mm)	5.28b	6.90b	6.80b	9.00a	6.93b
Length of the longest stolon (cm)	1.63ns	1.63	1.60	2.25	1.70
Node (nº/stolon)	16.40b	21.20ab	21.00ab	32.80a	13.20b
Internode length of the longest stolon (cm)	6.01ns	5.54	6.36	6.31	5.04
Tiller (nº/plant)	10.80a	10.00a	8.00ab	10.80a	5.40b
Tiller diameter (mm)	5.28b	6.90b	6.88b	9.00a	6.93ab
Tiller length (cm)	16.30ns	17.60	16.00	27.00ns	18.00
Leaf (nº/plant)	15.60b	20.20b	18.60b	14.17ab	12.45b
Vegetative canopy height (cm)	40.60ns	53.20	36.00	57.00	36.20
Length of the longest root (cm)	67.80ns	63.80	56.00	70.40	58.40
Root volume (cm^3/plant)	32.00ab	29.80ab	31.80ab	44.60a	12.00b
Leaf blade width (cm)	1.20ab	1.36b	1.20b	1.16b	1.64a
Leaf blade length (cm)	12.60b	8.02b	14.30a	8.20b	13.10ab
Leaf angle close to tiller (º)	66.00ab	64.00ab	60.00ab	42.00b	80.00a
Tiller angle of insertion with the ground (º)	56.00ab	54.00ab	50.00ab	32.00b	70.00a

Means followed by the same letter in the row do not differ (P>0.05) by Tukey test; ns = not significant.

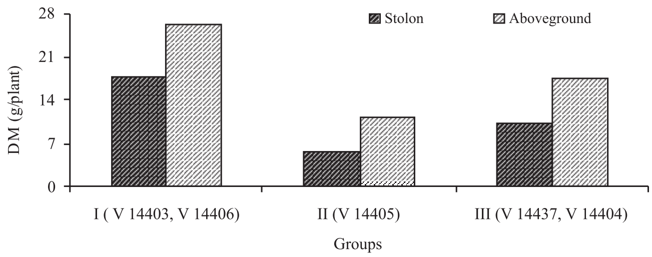

Figure 8 - Average dry matter production of aboveground and stolon of the groups formed by UPGMA method, based on Mahalanobis distance among accessions of giant missionary grass.

The morphophysiological traits used to characterize the giant missionary grass accessions showed that there is variability in the germplasm (Table 4), which credits its evaluation in field experiments, to describe the variation for attributes of agronomic importance (Jaramillo & Baena, 2000). The new information about the accessions can contribute to their conservation, use and management, offering subsides to the species breeding program.

Conclusions

Morphophysiological traits are efficient to evaluate the variability in the giant missionary grass accessions, which vary as for growth habit, morphology, dry matter accumulation and phenology. Accessions V 14337 and V 14404 are earlier for flowering, and V 14403 and V 14405 are the most promising to further agronomic evaluation aiming at cultivar registration.

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