

# Estimate of genetic diversity in germplasm of elephant grass accessions in Brazil using the Gower algorithm

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**ABSTRACT.** Management of variability in germplasm banks is essential for genetic improvement, so that the breeder can estimate the genetic similarity between cultivars, as well as maintain genetic diversity in breeding programs. Elephant grass is a forage crop plant native to Africa of great socio-economic and environmental importance; it can be used for animal feed and for bioenergy production. Understanding the genetic variability of elephant grass is essential for breeding programs. In this context, we examined the genetic divergence of elephant grass accessions using the Gower algorithm. The experiment was conducted under field conditions in the municipality of Campos dos Goytacazes-RJ, Brazil. All 85 elephant grass accessions belonging to the Active Elephant Grass Germplasm Bank of Embrapa Gado de Leite were included. These genotypes are commercial varieties from various countries. They were evaluated for morphoagronomic, morphological and phenological characteristics. The experimental design was

randomized blocks with two repetitions. The plots were composed of 5.5-meter rows, with 2.0-meter spacing between the planting rows, totaling 11.0 m<sup>2</sup>. The useful area was a sample in the center of the plot. We generated an illustrative dendrogram, obtained by the UPGMA method and the Tocher clustering, based on the Gower algorithm. Data were examined by means of the GENES statistical programs and the R program. According to the dissimilarity matrices based on the Gower algorithm, the genetic distances varied between 0.08 and 0.56, and the mean distance of the 85 evaluated accessions was 0.25, suggesting, consequently, that there is wide genetic variability between the accessions. Of the 85 genotypes, seven presented genetic distances smaller than 0.1, being indicative of duplicates in the germplasm bank, which could be eliminated without risk of loss of genetic variability.

**Key words:** *Cenchrus purpureus*; Clustering analysis; Multivariate analysis; Germplasm

## INTRODUCTION

Elephant grass (*Cenchrus purpureus*) is a forage of great socioeconomic and environmental importance, widely cultivated in various regions of the world. Its importance includes high efficiency in atmospheric CO<sub>2</sub> fixation, producing more than 60 t. ha<sup>-1</sup> year<sup>-1</sup> of dry matter (Silva et al., 2020), perenniality, rapid growth, broad adaptation, high fiber content, high C / N ratio and high calorific value (Fontoura et al., 2015; Rocha et al., 2015; Araujo et al., 2017; Vidal et al., 2019).

Africa is the center of origin and genetic diversity of elephant grass and this crop is capable of adapting to different edapho-climatic conditions due to its enormous genetic variability. In Brazil, elephant grass is found in the five regions of the country due to its wide adaptation to the tropical environment and high production potential and forage quality, being predominantly used as forage for animal nutrition purposes (Pereira et al., 2017). However, in recent years elephant grass has also gained prominence as a potential crop to be used for bioenergy production, in view of the growing search for alternative sources of renewable and less polluting energy (Rocha et al., 2018; Gravina et al., 2020).

Elephant grass is an allotetraploid (2n=4x=28), with normal diploid behavior, presenting A'A'BB genome (Hanna et al., 1984; Techio et al., 2008). Predominantly allogamous, protogenic, and with a rate of cross-pollination that depends on the flowering season of genotypes, it shows wide genetic variability for most of the morphoagronomic traits, with this variability distributed in all germplasm (Lima et al., 2011; Oliveira et al., 2017).

Variability management in germplasm banks depends on the availability of precise information on the level of genetic divergence among accessions. This information is essential for plant breeding so that the breeder can estimate the genetic similarity among cultivars to carry out the selection of parental combinations, as well as maintain genetic diversity in breeding programs (Rossi et al., 2017).

Morphological and phenological traits are used when estimating genetic diversity in plants, as this is an economic practice and requires less time when compared to quantitative and molecular traits (Marim et al., 2009). For this reason, the joint analysis of variables turns into an interesting alternative for more accurate quantification of genotype genetic variability.

Gower (1971) proposed a technique that enables the simultaneous analysis of quantitative and qualitative data. This method allows the values of the distance matrix to be between 0 and 1, requiring the standardization of quantitative and qualitative variables (Ledo et al., 2008). The joint analysis of data by means of the Gower algorithm provides a better understanding of the traits considered, along with more discerning and effective conclusions from the statistical viewpoint about the genetic relationship among the accessions under study (Torres et al., 2015).

Bearing in mind the importance of elephant grass, characterizing genotypes in germplasm banks as regards genetic dissimilarity among genotypes is of utmost significance in order to eliminate duplicates and orient new crosses in breeding programs. Along this line, we estimated the genetic divergence of elephant grass on the basis of morphoagronomic, morphological, and phenological descriptors using the Gower algorithm for the joint study of quantitative and qualitative data.

## MATERIAL AND METHODS

The experiment was implemented in an experimental area belonging to the State University of Northern Rio de Janeiro (UENF), located in the premises of the State Center for Research in Agroenergy and Waste Use (CEPEAA), of the Experimental Station of PESAGRO-RIO, in Campos dos Goytacazes - RJ, Brazil (21°44'47" S, 41°18'24" W, 11 m asl). The climate is Aw-type, tropical hot and humid, with a dry season in winter, rainy in summer, and an annual rainfall of around 1,152 mm (Alvares et al., 2013).

All Eighty-five genotypes of elephant grass from the Active Bank of Elephant Grass Germplasm (BAG-CE) of Embrapa Gado de Leite, located in the municipality of Coronel Pacheco, Minas Gerais State, Brazil (Table 1), were evaluated. These genotypes are commercial varieties from different countries.

The planting of the experiment was carried out in February 2011 after soil preparation with plowing, harrowing and row openings spaced 50 cm apart, with entire stems arranged in an inclined position, distributed in 10 cm deep furrows. After distribution of the stems, they were cut into pieces containing two or three buds. For planting fertilization, 60 g of simple superphosphate was distributed in each line, and 50 days after planting, the same quantity was used as cover fertilization using 70 g of urea and 40 g of KCl per line, corresponding to 28.6 kg of N (nitrogen) and 24 kg of K<sub>2</sub>O per hectare. After the establishment phase, on December 15, 2011, all genotypes were cut close to the soil (cut of uniformity) and replanting was done to minimize emergence failures in the planting lines.

The experiment was carried out in a completely randomized block design with two replicates. Each experimental unit consisted of a genotype planted in the row of 5.5 m spaced in 2 m, totaling 11 m<sup>2</sup>. The useful area comprised 2 m<sup>2</sup> to the center of the plot.

Ten months after planting, in December 2011, after the establishment phase, all genotypes were cut close to the soil (uniformity cut). The first cut for evaluation was made

in November 2012 and the second one, in November 2013, so that there was one cut per year over a period of two years.

**Table 1.** Identification of the 85 genotypes of the Universidade Estadual do Norte Fluminense Darcy Ribeiro Elephant Grass Germplasm Active Bank, used in this study.

No.	Genotype	Origin	Nº.	Genotype	Origin
1	Elefante da Colômbia	Colombia	44	Capim Cana D'África	Brazil
2	Mercker	Brazil	45	Gramafante	Brazil
3	Três Rios	Brazil	46	Roxo	Brazil
4	Napier Volta Grande	Brazil	47	Guaçu IZ-2	Brazil
5	Mercker Santa Rita	Brazil	48	Cuba-115	Cuba
6	Pusa Napier N° 2	India	49	Cuba-116	Cuba
7	Gigante de Pinda	Brazil	50	Cuba-169	Cuba
8	Napier N° 2	Brazil	51	King Grass	Cuba
9	Mercker S, E, A	Brazil	52	Roxo Botucatu	Brazil
10	Taiwan A-148	Brazil	53	Mineirão IPEACO	Brazil
11	Porto Rico 534-B	Brazil	54	Vruckwona Africano	Brazil
12	Taiwan A-25	Brazil	55	Cameroon	Brazil
13	Albano	Colombia	56	CPAC	Brazil
14	Hib, Gigante Colômbia	Colombia	57	Guaçu	Brazil
15	Pusa Gigante Napier	India	58	Napierzinho	Brazil
16	Elefante Híbrido 534-A	Brazil	59	IJ 7125 cv EMPASC 308	Brazil
17	Costa Rica	Costa Rica	60	IJ 7126 cv EMPASC 310	Brazil
18	Cubano Pinda	Brazil	61	IJ 7127 cv EMPASC 309	Brazil
19	Mercker Pinda	Brazil	62	IJ 7136 cv EMPASC 307	Brazil
20	Mercker Pinda México	Brazil	63	IJ 7139	Brazil
21	Mercker 86 México	Colombia	64	IJ 7141 cv EMPASC 306	Brazil
22	Taiwan A-144	Brazil	65	Goiano	Brazil
23	Napier S,E,A,	Brazil	66	CAC-262	Brazil
24	Taiwan A-143	Brazil	67	Ibitinema	Brazil
25	Pusa Napier N° 1	India	68	Australiano	Brazil
26	Elefante de Pinda	Colombia	69	13 AD	Brazil
27	Mineiro	Brazil	70	10 AD IRI	Brazil
28	Mole de Volta Grande	Brazil	71	07 AD IRI	Brazil
29	Porto Rico	Brazil	72	Pasto Panama	Panama
30	Napier	Brazil	73	BAG - 92	Brazil
31	Mercker Comum	Brazil	74	09 AD IRI	Brazil
32	Teresópolis	Brazil	75	11 AD IRI	Brazil
33	Taiwan A-46	Brazil	76	05 AD IRI	Brazil
34	Duro de Volta Grande	Brazil	77	06 AD IRI	Brazil
35	Mercker Comum Pinda	Brazil	78	01 AD IRI	Brazil
36	Turrialba	Brazil	79	04 AD IRI	Brazil
37	Taiwan A-146	Brazil	80	13 AD IRI	Brazil
38	Cameroon – Piracicaba	Brazil	81	03 AD IRI	Brazil
39	Taiwan A-121	Brazil	82	02 AD IRI	Brazil
40	Vruckwona	Brazil	83	08 AD IRI	Brazil
41	P241 Piracicaba	Brazil	84	União	Brazil
42	IAC-Campinas	Brazil	85	Pesagro	Brazil
43	Elef. Cachoei.Itapemirim	Brazil			

At the time of the cuts, the following morphoagronomic traits were analyzed: 1 - number of basal tillers per linear meter, located within the useful area of the plot; 2 - average height of the plants (m); 3 - stem diameter (mm), measurements were made approximately 10 cm from the soil; 4 - leaf blade width (cm); and 5- leaf blade length (cm), in accordance with the methodology by Daher et al. (1997).

Morphological and phenological traits were evaluated in agreement with Daher et al. (1997) (Table 2). For the morphological descriptors, only the clump shape (CS) was evaluated in plots with 12-month growth, evaluated over two years (2012/2013). All the other descriptors were evaluated in six-month growth plots, with only one evaluation. For the evaluations of phenological traits, the percentage of flowering and the percentage of flag leaf in each access for each cut had to be quantified once a week. Subsequently, it was evaluated after how many days of growth each accession had emitted 10% of flag leaf and, at the end of the flowering days, which was the total flowering of each accession. Based on these data, the analysis of variance and the Scott-Knott clustering at 5% (Scott and Knott, 1974) for each cut were carried out.

**Table 2.** Grade scales referring to each morphological and phenological descriptor evaluated in 85 accessions of elephant grass (*Cenchrus purpureus*), according to Daher et al. (1997).

Morphological Trait	Grade
1- Clump shape	1-Open; 2-semi-open; 3-upright
2-Overall color of plot plants	1- Light green; 2-dark green; 3-purple
3- Hair intensity in the sheath leaf	1- Glabrous; 2-little hairy; 3-very hairy
4- Leaf angle	1- Upright (0 to 30°); 2-semi- upright (30 to 60°); 3- prostrate (60 to 90°)
Phenological Trait	Grade
<b>(Cut 1- 2012)</b>	
1- Number of days for the emergence of 10% flag leaf	1- Super early (104 to 130 days); 2-early (131 to 146 days); 3- medium (147 to 177 days); 4-late (178 to 209 days)
2- Flowering percentage at the end of the reproductive period	1- Fully flowered (100% flowering); 2-partially flowered (60 to 45% flowering), 3-poorly flowered (9.5 to 44% flowering)
<b>(Cut 2- 2013)</b>	
1- Number of days for the emergence of 10% flag leaf	1- Super early (122 to 134 days); 2-early (134 to 153 days); 3- medium (154 to 167 days); 4-late (168 to 183 days)
2- Flowering percentage at the end of the reproductive period	1- Fully flowered (76 to 100% flowering); 2-partially flowered: 75 to 41% flowering); 3-poorly flowered (< 40% flowering)

The estimate of the genetic distance matrix was carried out by joint analysis of the variables (morphoagronomic + multicategorical + phenological), obtained from the Gower algorithm (1971), as expressed by:

$$S_{ijk} = \frac{\sum_{k=1}^p W_{ijk} S_{ijk}}{\sum_{K=1}^p W_{ijk}}, \text{ in which:}$$

K = number of variables (k = 1, 2, ..., p); i and j = two individuals representing the accession;  $W_{ijk}$  = weight given to the  $ijk$  comparison, assigning value 1 for valid comparisons and value 0 for invalid comparisons (when the value of the variable is not present in one or both individuals);  $S_{ijk}$  = contribution of variable k to the similarity between individuals i and j, with values between 0 and 1. For a qualitative variable (nominal), if the value of variable k is the same for both individuals, i and j, then  $S_{ijk} = 1$ , otherwise it is equal to 0; for a quantitative variable (continuous)  $S_{ijk} = 1 - |x_{ik} - x_{jk}| / R_k$ , in which  $x_{ik}$  and  $x_{jk}$  are the values of variable k for individuals i and j respectively, and  $R_k$  is the interval (maximum value minus minimum value) of the variable k in the sample. The

division by  $R_k$  eliminates the differences between scales of the variables, resulting in a value within the interval [0, 1] and equal weights.

For the dendrogram, cluster analysis using the UPGMA method (Unweighted Pair Group Method with Arithmetic Mean) was used based on data generated by the joint analysis of morphoagronomic, multicategorical and phenological variables. The UPGMA method seeks to verify the degree of similarity between genitors, genitors and similar clusters, or between divergent clusters (Cruz et al., 2012).

Clustering by the Tocher method was also carried out. This clustering analysis allows the researcher to form clusters in which the values of intra-cluster distances are lower than any inter-cluster distances (Cruz et al., 2012). The maximum mean value of an intra-cluster distance was defined as the maximum value of the Gower distance and obtained in the set of shorter distances involving each genotype. Data were evaluated based on the GENES statistical programs (Cruz, 2013) and the R program (R Core Team, 2014).

## RESULTS AND DISCUSSION

It was verified a great variability among the elephant grass accessions by means of the clustering generated by the joint analysis of morphoagronomic, morphological, and phenological traits on the basis of the Gower algorithm (Figure 1). In genetic dissimilarity studies, the evaluation of as many descriptors as possible is indicated so as to provide a more complete analysis of dissimilarity. Only the phenological percentage of flowering, at the end of the reproductive period, showed little variability (92.9% low flowering) in both cuts. All phenotypic classes were seen in both morphological and phenological traits.

The genetic distances varied between 0.08 and 0.56, and the mean distance of the 85 evaluated accessions was 0.25, suggesting, consequently, that there is wide genetic variability between the accessions. The values obtained were higher than those reported by Pereira et al., (2008), who, evaluating the genetic diversity in 30 elephant grass accessions, reported genetic distances ranging from 0.0 to 0.34, and the mean distance of the 30 accessions was 0.21.

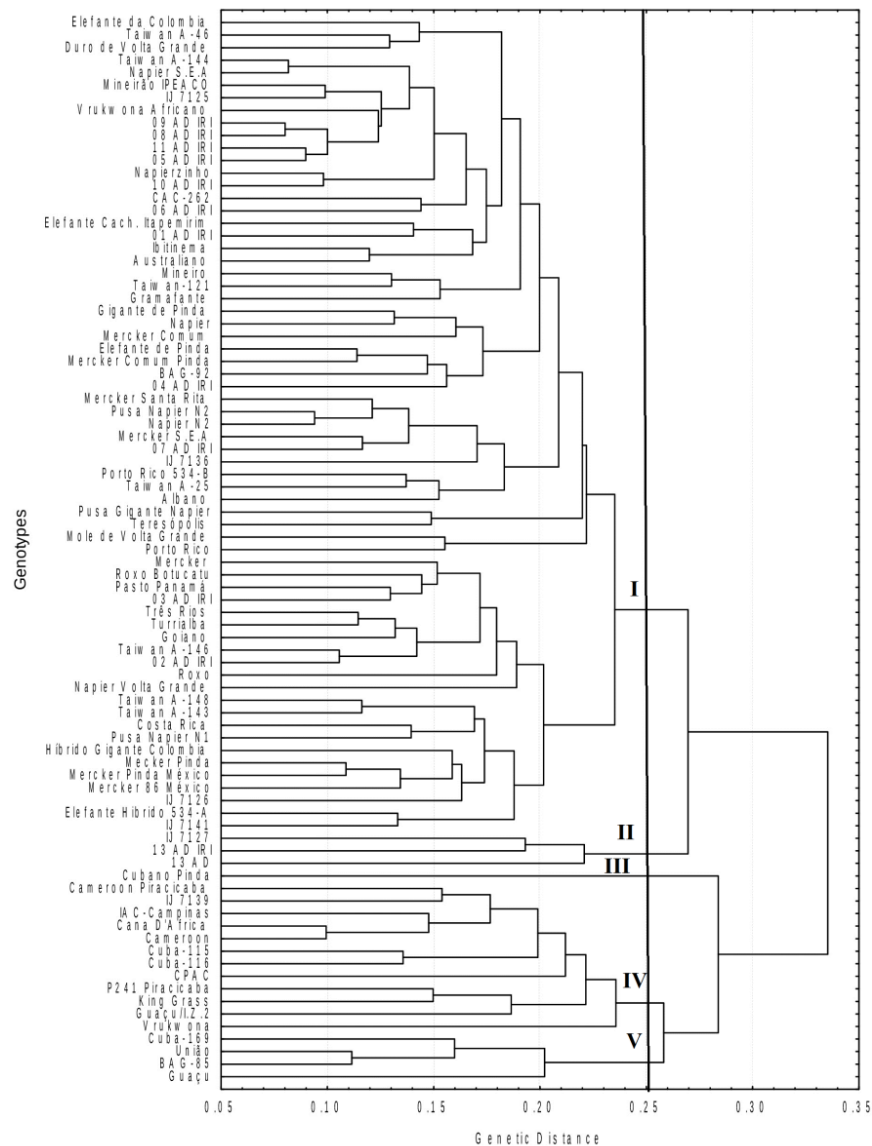
The lowest dissimilarity coefficients were observed in the combinations involving the accessions (Pusa Napier N° and Napier N°2); (Taiwan A-144 and Napier S,E,A.); (Capim Cana D'África and Cameroon); (Mineirão IPEACO and IJ 7125 cv EMPASC 308); (Vrukwna Africano and 05 AD IRI); (Napierzinho and 10 AD IRI); and (09 AD IRI and 08 AD IRI). Among these combinations, the shortest distance is between accessions 74 and 83, being they, respectively, accessions 09 AD IRI and 08 AD IRI. Interestingly, the accessions with lower dissimilarity coefficient had the same origin.

These accessions are highly similar for having genetic distances below 0.1, suggesting they may have the same genetic background. As Struwig et al. (2009) report, duplicate problems in germplasm banks are common, as a result of wrong accession names and loss of their identity. Information regarding the duplication of accessions is of great relevance to better organize the germplasm collection, in other words, some accessions can be discarded without affecting the genetic diversity of the bank (Azevedo et al., 2012).

With respect to the highest dissimilarity coefficients, we observed combinations related to the accessions (Mercker Comum and União); (Teresópolis and Cuba-169); (Vrukwna and 13 AD); (Cuba 169 and 13 AD); (IJ 7127 cv EMPASC 309 and Cuba-169); (BAG-92 and Vrukwna); (13 AD IRI and Pesagro); and (13 AD and Pesagro) had the

greatest distances, becoming promising combinations for future crosses, with a higher probability of obtaining genetic gains due to heterosis.

Figure 1 shows the dendrogram where the pairs of accessions with greater similarity among the 85 evaluated accessions were allocated. In a dendrogram, the sudden change in level points to the union of heterogeneous genotypes (Torres et al., 2015); accordingly, a cut at a distance of 0.25 was used, considering this as the point of sudden change, which resulted in the formation of five clusters.



**Figure 1.** Dendrogram obtained by the UPGMA method from dissimilarity matrices based on the Gower algorithm, by joint analysis of morphoagronomic, multicategorical and phenological traits, among the 85 genotypes of elephant grass.



In cluster I, plants with the shape of an upright and semi-open clump, light green and dark green plants, with many tillers, low and with stems with a thin diameter, were clustered. From the 85 accessions analyzed, 65 were clustered in this cluster (Figure 1), representing 76.47% of the evaluated accessions, enabling to deduce these materials are genetically similar in terms of the morphoagronomic traits evaluated. Similar results were found by Shimoya et al. (2002), who, when evaluating 99 genotypes of elephant grass, noticed the formation of 18 clusters, and the largest cluster accounted for 44.45% of the 99 genotypes. Similar results were found by Shimoya et al. (2002), who, when evaluating 99 genotypes of elephant grass, noticed the formation of 18 clusters, and the largest cluster accounted for 44.45% of the 99 genotypes.

Pereira et al. (2008) report the presence of duplicates in the Embrapa Gado de Leite germplasm bank, given that the accessions were from various collections and some materials may have had their original names changed. The Active Bank of Elephant Grass Germplasm (BAG-CE) of Embrapa Gado de Leite supplied the accessions evaluated.

The other clusters represent 23.5% of the accessions; cluster II is composed of three accessions, in which the clustered plants had an upright clump shape and light green color; they are early flowering plants, presenting many tillers and a diameter of thin stem.

In cluster III, the only accession (Cubano Pinda) had an open clump shape, light green color, late flowering, tall plants with few tillers, thin thatched diameter, short and thin leaves (Figure 1). The establishment of a cluster with only the Cuban Pinda genotype indicates that this is the most divergent accession in the active germplasm bank (Allard, 1971).

Cluster IV consisted of 12 accessions, with clump-shaped plants in the 1<sup>st</sup> year evaluation, and semi-open, in the 2<sup>nd</sup> one; dark green plants; and medium flowering (147 to 177 days to emerge 10% of the flag leaf). The plants also have a smaller stem diameter, with large and long leaves.

The last cluster (cluster V) was clustered in four accessions, where the plants have an open and semi-open clump shape, light green plants, with late flowering, small plants with thin stems, and large long leaves.

From the dissimilarity matrix among variables, in the genetic divergence study by means of the Tocher method, the accessions were clustered into 11 clusters (Table 3), which proves the great genetic variability existing among the 85 elephant grass accessions under study.

The differences among the genotypes were demonstrated in the number of clusters formed in both clusters; the Tocher clustering established more clusters ( $n = 11$ ) compared to the hierarchical clustering by the UPGMA method ( $n = 5$ ), while some clusters, such as III and X of the Tocher method, maintained the same accessions of clusters I and III of the UPGMA, respectively.

Azevedo et al. (2014) indicate that differences in the estimation of genetic variability between the Tocher method and the UPGMA are expected, considering that the methods are based on different clustering techniques. As such, according to these same authors, results should be compared from different types of multivariate analysis methods to provide a more complete interpretation of results thereby avoiding possible errors of identification.

Knowing these clusters is crucial when choosing genitors within breeding programs because new populations to be formed should be based on the magnitude of their



dissimilarities and on the potential *per se* of the genitors. In this way, the joint analysis of quantitative and qualitative data can provide a better understanding of genetic diversity contained in the germplasm bank and a more complete genotype characterization, contributing to the determination of future strategies for breeding (Gonçalves et al., 2008). In view of the importance of quantitative and qualitative traits for elephant grass, it can be affirmed that, in the study herein, this type of analysis provided greater support and understanding of the diversity among the genotypes under analysis.

**Table 3.** Tocher clustering from dissimilarity matrices estimated using the Gower algorithm, among the 85 accessions of elephant grass.

Cluster	No. of accessions	ACCESSION
I	52	Elefante da Colômbia, Mercker, Três Rios, Napier Volta Grande, Mercker Santa Rita, Pusa Napier N° 2, Gigante de Pinda, Napier N° 2, Mercker S, E, A, Taiwan A-148, Porto Rico 534-B, Taiwan A-25, Hib, Gigante Colômbia, Pusa Gigante Napier, Elefante Híbrido 534-A, Costa Rica, Mercker Pinda, Taiwan A-144, Napier S,E,A, Taiwan A-143, Elefante de Pinda, Mineiro, Napier, Taiwan A-46, Duro de Volta Grande, Mercker Comum Pinda, Turrialba, Taiwan A-146, Taiwan A-121, Elefante Cachoeira Itapemirim, Gramafante, Roxo, Mineirão IPEACO, Vrukhwona Africano, Napierzinho, IJ 7125 cv EMPASC 308, IJ 7126 cv EMPASC 310, IJ 7136 cv EMPASC 307, IJ 7141 cv EMPASC 306, Goiano, CAC-262, Ibitinema, 903-77 ou Australiano, 10 AD IRI, 07 AD IRI, 09 AD IRI, 11 AD IRI, 05 AD IRI, 06 AD IRI, 01 AD IRI, 04 AD IRI, 03 AD IRI, 08 AD IRI.
II	11	Capim Cana D'África, Cameroon, IAC-Campinas, IJ 7139, Cameroon Piracicaba, Cuba-115, 02 AD IRI, Cuba-116, King Grass, Roxo, Guaçu IZ-2
III	5	União, Pesagro, Cuba-169, CPAC, Guaçu
IV	6	Mercker Pinda México, Mercker 86 México, Albano, Roxo Botucatu, Pusa Napier N°1, Pasto Panamá
V	3	Mercker Comum, Teresopólis, BAG - 92
VI	2	Mole de Volta Grande, Porto Rico
VII	2	IJ 7127 cv EMPASC 309, 13 AD IRI
VIII	1	13 AD
IX	1	P 241 Piracicaba
X	1	Cubano Pinda
XI	1	Vrukhwona

In addition, the results of the joint analysis of data represent a motivation for the curators of germplasm collections to conduct the characterization of their collections as completely as possible, with the aim of generating safer information on the variability and genetic divergence among the accessions.

## CONCLUSIONS

There was genetic variability among the elephant grass genotypes, identified by morphoagronomic, morphological, and phenological traits, enabling selection of divergent genitors for breeding programs. Of the 85 genotypes evaluated, seven had genetic distances less than 0.1, being indicative of duplicates in the germplasm bank, which can be eliminated without risk of loss of genetic variability.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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