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Morphological characterization of 19 Brachiaria brizantha accessions on an acid soil

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ABSTRACT: The objective of this study was to characterize a collection of 19 Brachiaria brizantha accessions, obtained from the International Center of Tropical Agriculture (CIAT), Colombia, and preserved in the germplasm bank of the Pasture and Forage Research Station of Cascajal, Cuba. A total of 17 (13 quantitative and 4 qualitative) international descriptors for forage grasses and others of interest were used. The measurements and observations were performed in the vegetative and reproductive stages. The principal components analysis allowed to prove the existence of a relatively high variability, because the accumulated reached 81,22 % in the first five components. In component one, the indicators with higher influence were: number of spikelets, number of stalks, and the inflorescence width. With the use of the cluster analysis six groups were differentiated; the accessions CIAT-16317 and CIAT-26290, which formed group V, showed the best values in several indicators. That phenotypical variability is important, because it allows to assert that there is a differentiated *B. brizantha* germplasm, with an acceptable morphological richness, which must be known in order to prevent duplications. The characterization of this collection allowed to prove its relative variability, where the highest differentiation degree was found in the quantitative traits. It is suggested to perform this activity as initial stage of the evaluation process, and to conduct essays in which their potential for agroproductive indicators of interest, with selective purposes, is evaluated.

Key words: evaluation, germplasm, grasses

INTRODUCTION

To maintain and improve the forage production in any country that is dedicated to livestock production, it is necessary to collect, introduce and preserve the forage resources that enhance its development, due to their importance in livestock feeding (Castillo et al., 2010). For such purpose, this material should be known, which is achieved by making a characterization process that comprises the morphological, cytogenetic, biochemical, ecological, geographic, physiological and agronomic aspects (Franco and Hidalgo, 2003). In addition, such process allows to complement biodiversity studies (Veteläinen et al., 2005), to know or differentiate the germplasm collections (Negi et al., 2004) and to determine the genetic stability of the regenerated plants (Sharma et al., 2007).

The morphological characterization is the description of the phenotypic expression of each

studied individual, from a set of quantitative and qualitative traits. This activity has three components: representative population, list of descriptors and instrument of measurement/ record (Jaramillo and Baena, 2000). Such studies have been conducted in diverse collections of grasses as well as legumes, with different purposes (Machado and Olivera, 2008; Olivera *et al.*, 2009), and have allowed to know the morphological characteristics of the species in a certain environment.

This research deals with individuals from a collection of species of the genus *Brachiaria*, which were selected in initial studies (Olivera, 2004), and at present are being evaluated in their higher stages, in order to identify the most outstanding types for the ecosystems of acid soils, of low fertility and without irrigation. This collection is kept preserved *ex situ* in that environment, for which this type

of study would allow its correct identification to maintain its varietal purity.

For all the above-explained reasons, the objective of this study was to learn the morphological characteristics of 19 *Brachiaria brizantha* accessions, established on an acid soil.

MATERIALS AND METHODS

The accessions were established in areas of the Pasture and Forage Research Station of Cascajal, located in the Santo Domingo municipality (Villa Clara province, Cuba), at 60 m.a.s.l. The soil of these areas is Alitic, of low clayey activity, loam-sandy texture, high acidity (pH ~ 4,9), low OM (2,5 %) and total nitrogen content (0,4 %), as well as low cation exchange capacity (< 20,0 cmol kg⁻¹). In addition, it shows a thick layer of ironstone in its profile (Hernández *et al.*, 2003), characteristics that define it as a low-fertility acid soil.

The treatments were 19 *B. brizantha* accessions selected in a previous trial conducted in this locality, in which a collection of 69 accessions was evaluated (Olivera, 2004). They were: *B. brizantha*: CIAT-16300, CIAT-16317, CIAT-16809, CIAT-16154, CIAT-16322, CIAT-16132, CIAT-16128, CIAT-1633, 1539, CIAT-26290, CIAT-16332, CIAT-16819, CIAT-16303, CIAT-16334, CIAT-16448, CIAT-26646, CIAT-16485, CIAT-16197 and CIAT-26032.

The accessions were planted in 5,0 x 4,0 m plots, separated by 1,50 m, and a randomized block design with three replications was used.

Experimental procedure. For the characterization, the qualitative and quantitative indicators shown in table 1 were taken into consideration.

The observations and measurements were made during two phenological stages: vegetative and reproductive. In the case of the vegetative one, it was determined from the emergence of the flag leaf. The quantitative indicators measured in each stage were determined on one occasion and 15 repetitions were used, with the objective of comprising, as much as possible, the whole plot area.

Most quantitative descriptors (SL, LL, WL, LS, LIN, LI, WI, LS and RH) were measured with a graduated ruler, and the SD, with a caliper; while the NI, NSt and NS were numerically counted.

The leaves were eliminated to facilitate measuring the SL, which included from the basal crown to the apical bud. On the other hand, the LIN was measured from node to node; while in the case of the NI, the quantity of internodes in the stems of the selected plants was counted. In the laboratory of germplasm, the following measurements were also performed: a) in the limbo: LL, from the ligule to the limbo apex, and WL, in its central part; b) in the sheath: SL, from the ligule to its insertion in the node; c) in the inflorescence: IL, for which the central axis was measured from its insertion in the plant to the higher end; IW, from end to end of the stalks; d) in the stalk: the NSt, by counting the number of stalks inserted in the central axis; LS, from their insertion in the central axis to their apex; NS, by counting the total number of spikelets, with or without formed seeds. The RH was determined from the soil surface to the apex of the inflorescence, and the SD, at a height of 2 cm in the stem basis.

The qualitative indicators were visually observed, through a stereoscope, to determine the presence or absence of hairs or trichomes. In the color indicators it was not possible to use the international chart recommended by Pantone (2004) for these purposes. Nevertheless, the observations were made by the same person so that the observation criterion did not differ.

Statistical analysis. To determine the variability of the quantitative indicators, and reduce the dimensionality, a principal components analysis (PCA) was applied, in which the selection of those components that showed a cumulative variance higher than 80 %, and sum or preponderance factors over 0,70, was taken as criterion. Afterwards, the cluster analysis was performed to determine the prevailing characteristics in each of the groups formed, by means of the statistical pack SPSS version 15.1 for Windows.

RESULTS AND DISCUSSION

The results of the PCA are shown in table 2. In the first five components 81,22 % of the total variance was extracted. In CP1 (24,01 %), the most influential indicators were: number of spikelets and width of the inflorescence, which were positively related between themselves. In addition, they were related to the seed production, for which this axis is called reproduction axis of the plant. Such indicators are very important, because they are part of the species stability. Although it can be vegetatively propagated (Olivera et al., 2007), there are studies that define it as a good seed producer, and its propagation this way has brought about very good results (Argel et al., 2007). Pérez et al. (2010) also reported that it is one of the species of the genus Brachiaria with the highest production.

Quantitative	Qualitative					
SD: stem diameter (mm)	CL: color of the limbo					
SL: stem length (cm)	CS: color of the sheath					
LL: length of the limbo of the third leaf (cm)	HF: hairs on the front					
WL: width of the limbo of the third leaf (cm)	HB: hairs on the back					
LS: length of the sheath of the third leaf (cm)						
LIN: length of the internodes in the aerial stems (cm)						
IL: inflorescence length (cm)						
IW: inflorescence width (cm)						
NI: number of internodes						
NSt: number of stalks						
LSt: stalk length (cm)						
RH: reproductive height (cm) NS: number of spikelets						

Table 1. Indicators used in the morphobotanical characterization.

Table 2. Relation between variables and indicators that explain the variance.

Indicator	Principal component						
Indicator	CP1	CP2	CP3	CP4	CP5		
SD	0,211	0,921	-0,120	0,115	0,014		
SL	0,455	-0,348	0,731	-0,034	-0,081		
LL	0,316	0,024	-0,056	0,904	0,054		
WL	-0,019	0,722	0,174	-0,408	0,071		
LS	0,698	-0,304	-0,027	0,151	-0,159		
LIN	0,037	-0,127	0,798	-0,234	-0,277		
NI	0,355	-0,737	0,245	-0,116	0,066		
IL	0,129	0,467	0,668	0,139	0,243		
IW	0,724	0,026	0,153	0,381	0,162		
NS	0,887	0,169	0,004	0,036	-0,152		
LSt	-0,078	0,014	-0,142	0,018	0,944		
RH	0,007	-0,052	0,691	0,572	-0,084		
NSt	0,906	0,019	0,283	0,045	0,064		
Proper value	3,12	2,39	2,33	1,58	1,13		
Variance (%)	24,01	18,42	17,89	12,19	8,70		
Cumulative variance (%)	24,01	42,43	60,33	72,52	81,22		

CP2 extracted 18,42 % and was mainly represented by stem diameter, number of internodes and width of the limbo. However, the second indicator showed an inverse performance with regards to the other two.

In the other components, the least variable indicators were grouped in descending order: CP3 extracted a 17,89 % and was represented, with higher bearing, by stem and internode length. The limbo and stalk length were expressed in CP4 and CP5, with a variance of 12,19 and 8,70 %, respectively. This performance is maybe due to the fact that those accessions belong to the same species and, thus, have interspecific characteristics which can be very similar and little variable for some of the morphological indicators in particular.

When conducting the cluster analysis the formation of six groups was detected (table 3). Group I was formed by 10 accessions, which showed an average performance in the studied indicators. Group II, with two accessions, had the highest stalk length. Group III, with only one accession (CIAT-16197), showed the highest length and the highest number of internodes. This last indicator was also the most outstanding in group V (CIAT-16317, CIAT-26290), whose accessions showed high differentiation in most of the indicators (SL, IW, NSt, NS). Likewise, the accessions of groups IV and VI had a high differentiation in indicators WL and LL, related to the leaf limbo. When analyzing the values of the descriptors, it can be stated that the variability was relatively wide, although it was influenced by the external factors that conditioned their expression.

The results of some studies conducted in species of the genera *Brachiaria* (Keller-Grein *et al.*, 1998), *Sorghum* (Ayana and Bekelel, 1999) and *Bouteloua* (Morales *et al.*, 2008) coincide with the ones in this study, because they indicate that there was a high morphological variability among the individuals, and that it was influenced by environmental factors, such as the climate ones, etc.

The qualitative indicators (table 4) were mainly represented by the colors in the different parts of the plants; as well as the presence or absence of trichomes.

Regarding the color of the limbo, green prevailed, varying from light to dark. Similar results were reported by Roche *et al.* (1990) and Olivera *et al.* (2006), who made the observations without using the Pantone pattern (Pantone, 2004), and reported that in this species a range of shades can be found from light green to purple. The direct visual observation to determine the qualitative traits, without using pre-established patterns, has also been used in studies of other species such as uchuva (*Physalis peruviana*) and potato (*Solanum tuberosum*), according to the reports by Bonilla *et al.* (2008) and Castillo *et al.* (2010), respectively.

The color of the sheath was very similar in most of the accessions (16), which showed yellow-

Indicator	Group	I Group II		Group III G		Group	Group IV		Group V		Group VI	
	Х	SD	Х	SD	Х	SD	Х	SD	Х	SD	Х	SD
SD	0,29	0,05	0,31	0,04	0,24	-	0,51	-	0,34	0,08	0,30	0,11
SL	46,71	5,35	32,30	14,14	44,80	-	36,80	-	55,05	1,06	30,40	12,59
LL	35,16	3,17	26,35	0,64	13,80	-	35,50	-	38,50	1,84	39,26	3,27
WL	1,54	0,11	1,70	0,28	1,60	-	1,90	-	1,45	0,07	1,33	0,21
LIN	8,36	0,94	5,55	0,92	9,90	-	8,00	-	7,70	1,27	5,73	1,30
NI	5,79	0,59	5,50	0,71	6,00	-	3,00	-	6,00	0,00	5,00	1,00
IW	15,67	2,89	11,50	4,67	8,50	-	14,20	-	20,05	1,06	13,36	0,85
NSt	3,60	0,97	3,00	0,00	4,00	-	4,00	-	6,50	0,71	3,67	1,15
LS	8,25	1,26	11,10	2,12	4,20	-	8,50	-	7,65	2,05	7,90	0,72
NS	128,82	55,33	87,50	0,71	71,00	-	74,00	-	318,50	33,23	68,67	27,30
Group	Quantity of accessions	Accession										
Ι	10	CIAT-16300, CIAT-16322, CIAT-16819, CIAT-26646, CIAT-16809, CIAT-16128, CIAT-16132, CIAT-16303, CIAT-16448, CIAT-16469										
II	2	CIAT-16334, CIAT-26032										
III	1	CIAT-16197										
IV	1	CIAT-16332										
V	2	CIAT-16317, CIAT-26290										
VI	3	CIAT-1	.6485, C	IAT-163	35, 1539)						

Table 3. Distribution of the individuals, mean and standard deviation, according to the cluster analysis.

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Name	CL	CS	HF	HB
CIAT-16300	Light green	Yellowish green	Yes	No
CIAT-16322	Dark green	Yellowish green with purple shimmers	No	No
CIAT-16819	Light green	Yellowish green	Yes	No
CIAT-26646	Light green	Yellowish green	No	No
CIAT-16197	Light green	Yellowish green	Yes	Yes
CIAT-16317	Dark green	Yellowish green	No	No
CIAT-26032	Light green	Yellowish green	No	No
CIAT-16469	Dark green	Yellowish green	No	No
CIAT-16334	Light green	Yellowish green	Yes	Yes
CIAT-16809	Dark green	Yellowish green	No	No
CIAT-16128	Dark green	Yellowish green	No	No
CIAT-16303	Light green	Yellowish green	No	No
CIAT-16448	Light green	Yellowish green	No	No
CIAT-16485	Light green	Yellowish green	No	No
1539	Light green	Yellowish green	Yes	Yes
CIAT-26290	Dark green	Yellowish green with purple shimmers	No	No
CIAT-16332	Light green	Yellowish green	No	No
CIAT-16132	Dark green	Yellowish green with purple shimmers	No	No
CIAT-16335	Light green	Yellowish green	No	No

Table 4. Qualitative indicators.

ish green sheaths, except CIAT-16322, CIAT-26290 and CIAT-16132 that had the same color, but with purple shimmers.

Regarding the presence, or absence, of trichomes on the front and back, it was observed that there were accessions which showed hairs on one or both surfaces; but a trend was noticed towards the absence of this trait. Similar results were reported by Roche *et al.* (1990) and Olivera *et al.* (2006).

It is important to know the morphological characteristics of the germplasm to avoid interspecific confusions within this genus. In this sense, Roche *et al.* (1990), in a study to characterize several species of the genus *Brachiaria*, stated that the highest complexity for the identification was found between *B. decumbens* and *B. brizantha*, because many similar traits were observed in them, mainly in vegetative state. Both species include individuals with similar growth habit, leaf size, color and stem structure. In addition, both of them show ecotypes, from purely glabrous to densely pubescent.

This phenotypical variability shows that there is a germplasm which, in spite of belonging to the same species, has marked differences in many cases. It should be emphasized that the knowledge of such variability (mainly in the indicators IW, NS, SD, WL and NI) is very valuable, because this collection is preserved *in vivo* in the germplasm field of the Research Station of Cascajal, which contributes to maintain its purity.

Morphological characterization, by being a reliable tool to identify accessions with a higher or lower degree of morphological variability, allowed to prove that there is an acceptable genetic richness in this species, which showed outstanding forage attributes in this environment.

It is concluded that, in general, there is a *B. brizantha* germplasm which has an acceptable morphological richness, which should be known to prevent duplication. The characterization of this collection allowed to prove its relative variability, in which the highest degree of differentiation was found in the quantitative traits. It is suggested to perform this activity as initial stage of the evaluation process and to conduct essays in which its potential for agroproductive indicators of interest are evaluated, with selective purposes.

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