INTRODUCTION

Meat and milk production in tropical countries are maintained by the use of forage under grass, constituting the most economical way to feed the cattle.

In Brazil, forage plants used in livestock exploration are based on a narrow genetic base, reducing the number of varieties available for pasture establishment. The small existing variability turns pastures vulnerable to the attack of pests and diseases, besides limiting the development of varieties adapted to different soil and weather conditions and specific production systems (Valle and Souza, 1995). In this context, the genetic improvement can expressively contribute to increase pasture diversity, developing more productive and high quality forage plants and, consequently, increasing meat and milk productivity.

Species of genus *Brachiaria* are the most cultivated forage in Brazil, occupying 80% of the pastures planted in the nineties (Santos Filho, 1998). However, as highlighted previously, there are only a few species and varieties of this genus being cultivated. In addition, the incorrect classification of *Brachiaria* accessions also constitutes a problem.

There are seven important collections of *Brachiaria* in the world, all *ex situ*. They encompass a total of 987 accessions of 33 known species (Keller-Grein et al., 1998). Appropriate documentation on *Brachiaria* germplasms worldwide is an indispensable requirement to use it efficiently.

Some genotypes have been amply distributed with the incorrect name of the species, creating confusion in the published literature (Maass, 1998). Therefore, it is necessary to develop morphologic, agronomic and molecular detailed studies to establish the identity of these materials. Renvoize et al. (1998) propose the application of the morphological statistical analysis, allied to other information, to provide a reasonable system of classification for the genus *Brachiaria*.

Multivariate analysis are of great usefulness to breeding programs, once they allow the simultaneous evaluation of several agronomic, morphologic, physiologic and molecular characteristics, which are important to obtain genetic superior materials.

Although hardly used in genetic improvement, discriminant analyses contribute to the knowledge of the genetic divergence among accessions and species, bringing information on the inter-relationship of the species. Besides, the functions established are used to classify unknown individuals.

Genetic diversity studies are also of great importance to hybrid programs. However, when the number of genotypes is high, the selection of the genitors is a difficult task for the researcher and the identification of the best hybrid combinations through diallelic systems becomes practically unviable. Therefore, the...
choice of the genitors can be based on the genetic divergence and on the superiority of the genotypes (Cruz and Regazzi, 1997).

The objectives of this work were: to obtain information on the genetic divergence among species of *Brachiaria*, under the morphologic point of view; to establish discrimination functions among six species of *Brachiaria*; to verify the consistence of the established functions and to promote the elimination of the less important and redundant variables for the discrimination of the species in study.

**MATERIAL AND METHODS**

The present work was conducted with data obtained from the *Brachiaria* Germplasm Bank at “Embrapa Gado de Corte” (National Beef Cattle Research Center), located in the district of Campo Grande (MS), Brazil, at 20°27’ S, 54°37’ W and at an altitude of 530 m. The experiment was carried out without the use of fertilizers, in an acid and low fertility soil, classified as Dystrophic Red Latosol, on a flat topography, representative of the Brazilian savanna.

Three hundred and one accessions of *Brachiaria* species were evaluated in 5 m² plots, being 150 accessions of *B. brizantha*, 46 of *B. decumbens*, 36 of *B. humidicola*, 31 of *B. jubata*, 28 of *B. ruziziensis* and 10 of *B. dictyoneura*.

The experimental design was entirely randomized with 6 treatments (species), being each accession per species considered as a repetition for that treatment. Most characteristics were assessed using 5 plants per plot, and their average values were used during the analysis.

Twenty-four morphologic characters were evaluated. Assessments were made through visual observations or with a millimetric rule, according to the nature of the characteristics, as follow: plant height (PHT), plant growth habit (PGH), leaf length (LLT), leaf width (LWT), leaf growth habit (LGH), length of leaf sheath (LLS), length of floral stem (LFS), inflorescence length (ILT), number of racemes (NUR), length of basal raceme (LBR), number of spikelets on basal raceme (NUSR), spikelet insertion (SIN), rachis width (RWT), spikelet spot percentage (SSP), stigma colour (SCO), anther colour (ACO), density of rachis pubescence (DRP), length of rachis pubescence (LRP), density of leaf blade pubescence (DLB), length of leaf blade pubescence (LLB), margin of the leaf blade (MLB), density of leaf sheath pubescence (DSP), length of leaf sheath pubescence (LSP) and distribution of leaf sheath pubescence (USP).

The analyses of variance were carried out using the procedures available in the Statistical Analysis System (SAS), version 6.12 (Littell et al., 1991).

**Discriminant Analysis Based on Principal Components**

Discriminant analysis based on principal components was conducted considering 301 accessions and 24 variables. The 301 accessions were previously grouped, according to their species, forming 6 groups (Group 1 - *B. brizantha*, group 2 - *B. decumbens*, group 3 - *B. humidicola*, group 4 - *B. jubata*, group 5 - *B. ruziziensis* and group 6 - *B. dictyoneura*).

Principal components analysis was performed. However, mean values (center point) for the characters inside each group previously established were used.

The principal components technique, described in the books of Mardia et al. (1979), Cruz and Regazzi (1997) and Johnson and Wichern (1998), consists of transforming a group of *p* variables *x*₁₁, *x*₁₂, ..., *x*ᵢᵢ, belonging to *n* individuals, into a new group *PC*₁₁, *PC*₁₂, ..., *PC*ᵢᵢ, in which *PC*ᵢ’s are linear functions of the *x*’s and independent from each other. The mean of character *j* (j=1, 2, ...*p*) appraised in accession *i* (i=1, 2, ...*n*) is *x*ᵢᵢ.

Principal components were obtained utilizing the correlation matrix among the original means, since standardization of data was performed. The means for each variable *j*, inside each group *i*, were standardized in agreement with the expression:

\[ MP_{ij} = \frac{x_{ij}}{s_{xj}} \]

where

\[ MP_{ij} = \text{standardized mean of variable } j \text{ of group } i; \]

\[ x_{ij} = \text{arithmetic mean of variable } j \text{ of group } i; \]

\[ s_{xj} = \text{standard deviation among the means of the species in relation to the variable } j. \]

The first components should retain about 80% of the total variation, allowing graphic interpretation of the material under study (Cruz and Regazzi, 1997). Thus, dispersions of center point and accession scores were represented graphically.

**Anderson’s discriminant analysis**

The Anderson’s discriminant analysis was conducted with the same groups used in the previous analysis,
the 24 characteristics being included simultaneously.

Considering the n populations or groups \( \Pi_1, \ldots, \Pi_n \) \((n \geq 2)\), where a multivariate normal distribution is associated to each population and the equality of the covariance matrices are supposed, the discriminant functions are obtained in agreement with the expression (Anderson, 1958):

\[
D_i(x) = \left( \Sigma^{-1} \bar{x} \right)^t x - \frac{1}{2} \left( \Sigma^{-1} \bar{x} \right)^t \bar{x} + \ln(p_i)
\]

where

- \( D_i(x) \) = classification score of group \( i \);
- \( \Sigma^{-1} \) = inverse of covariance matrix;
- \( \bar{x}_i \) = means’ vector of group \( i \);
- \( x \) = vector of individual observations that one wants to classify;
- \( p_i \) = a priori probability that an individual belongs to population \( i \).

The \( p_i \) values were \( p_1 = p_2 = p_3 = p_4 = p_5 = p_6 = 1/6 \), which means the same classification probability of an individual in any one of the six groups.

The new individual is classified as belonging to the group for which it has the largest classification score, in other words, the unknown individual \( x \) will be classified in the group \( \Pi_i \) if and only if

\[
D_i(x) = \max \left[ D_1(x), D_2(x), \ldots, D_n(x) \right].
\]

Consistence analysis was performed, where data were submitted to a new classification. The probability of a bad classification for each group can be estimated by the expression:

\[
\hat{P_i} = \frac{m_i}{t_i}
\]

where

- \( m_i \) = number of observations bad classified in \( \Pi_i \) and;
- \( t_i \) = total number of observations in \( \Pi_i \).

Adding all unfavorable cases, the rate of apparent error is obtained, according to the expression:

\[
\text{TEAP} = \frac{\sum_{i=1}^{n} m_i}{\sum_{i=1}^{n} t_i}
\]

The described analyses were performed utilizing the computational program GENES (Cruz, 2001).

**RESULTS AND DISCUSSION**

In agreement with the analysis of variance for each characteristic, significant differences (\( P < 0.05 \)) among treatment effects for all the characters were verified, indicating the presence of genetic variability in the material assessed. The variation coefficients varied according to the characteristic, being “margin of the leaf blade” (17.5%), “length of rachis pubescence” (17.7%) and “length of floral stem” (21.6%) the variables with the lowest values found. The highest values were observed for the variables “spikelet spot percentage” (93.0%) and “length of leaf blade pubescence” (91.1%). These high values don’t necessarily mean low experimental precision, once repetitions refer to different accessions. High variation coefficients, in this case, indicate the presence of variation inside the species. However, variation among species was large enough to allow significant differences among treatment effects for all characteristics, as mentioned previously.

Means for the 24 characteristics for each species are in Table 1. All characteristics presented at least two means statistically different (\( P < 0.05 \)) among the six species, except for the variable “leaf growth habit”. It was not possible to classify the species according to different character groups (vegetative, reproductive and pubescence), based only on the morphologic characteristic means.

**Discriminant Analysis Based on Principal Components**

Discriminant analysis based on principal components showed that the first three components (PC1, PC2 and PC3) were sufficient to explain 85.37% of the observed variation. Graphic dispersions constituted by components PC1 and PC2 and by components PC1 and PC3 can be observed in Figure 1 and in Figure 2, respectively. In both graphics, the accessions of B. ruiziiensis form a more concise group around their center point (5). B. decumbens accessions also condense around their center point (2), although there is closer interrelation with accessions of B. brizantha. They are amply dispersed and it is possible to detect a subgroup more distant from its center point (1) and from other species accessions (Figure 1). Although B. jubata accessions mixes with B. brizantha accessions in Figure 1, they can be well visualized in
Table 1. Means of morphologic traits, evaluated in six *Brachiaria* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Traits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PHT</td>
</tr>
<tr>
<td><em>B. brizantha</em></td>
<td>72.41 a</td>
</tr>
<tr>
<td><em>B. decumbens</em></td>
<td>48.78 bc</td>
</tr>
<tr>
<td><em>B. humidicola</em></td>
<td>40.94 c</td>
</tr>
<tr>
<td><em>B. jubata</em></td>
<td>40.52 c</td>
</tr>
<tr>
<td><em>B. ruziziensis</em></td>
<td>65.29 a</td>
</tr>
<tr>
<td><em>B. dictyoneura</em></td>
<td>23.30 d</td>
</tr>
</tbody>
</table>

PHT: plant height; PGH: plant growth habit; LLT: leaf length; LWT: leaf width; LGH: leaf growth habit; LLS: length of leaf sheath; LFS: length of floral stem; ILT: inflorescence length; NUR: number of racemes; LBR: length of basal raceme; NUSR: number of spikelets on basal raceme; SIN: spikelet insertion; RWT: rachis width; SSP: spikelet spot percentage; SCO: stigma colour; ACO: anther colour; DRP: density of rachis pubescence; LRP: length of rachis pubescence; DLB: density of leaf blade pubescence; LLB: length of leaf blade pubescence; MLB: margin of the leaf blade; DSP: density of leaf sheath pubescence; LSP: length of leaf sheath pubescence; USP: distribution of leaf sheath pubescence. Means followed by the same letter, within columns, do not differ at 5% of probability by the Tukey test.

Figure 2, once they keep a reasonable distance from the others. This distance can be confirmed by the three-dimensional graph dispersion, where the three components are considered simultaneously. Areas of *B. humidicola* and *B. dictyoneura* accessions are intensely overlapped and their center point exhibits great proximity.

Group 5, which is constituted by accessions of *B. ruziziensis*, can be differentiated perfectly from Groups 3, 4 and 6, composed by *B. humidicola*, *B. jubata* and *B. dictyoneura* accessions, respectively. Group 2, represented by accessions of *B. decumbens*, also differs totally from Groups 3 and 6. Therefore, there is great diversity among the accessions and the
species studied, making intra as well as interspecific improvement possible. It is worth to point out that part of the material under study presents apomixis, being necessary the development of specific strategies that make the improvement of Brachiaria possible. Besides the divergence among the material to be used in breeding programs, the superiority of the accessions involved is also essential to obtain success in such programs.

Center points represented by points more distant from each other are more divergent than center points and accessions represented by closer points. Renvoize et al. (1998) grouped about 83 Brachiaria species into nine different groups, based mainly on the inflorescence morphology.

In this work, they included in the same group B. brizantha, B. decumbens and B. ruziziensis. According to the authors, the first two species are closely associated, becoming, many times, difficult to differentiate them. According to the dispersions presented, there is a tendency in establishing a group with these three species, however, with larger overlapped areas of B. brizantha and B. decumbens accessions, confirming the findings of Renvoize et al. (1998).

According to these same authors, B. jubata, B. humidicola and B. dictyoneura belong to the same group. The last two are closely related and they are frequently mixed up. Both graphics show the intense overlap among areas of B. humidicola and B. dictyoneura accessions, in which the center points are quite close. Mass (1998) also confirms the great proximity between the two species, which have been used as synonyms many times. By observing the

Table 2. Correct and incorrect classification of Brachiaria species obtained from Anderson’s discriminant analysis (%), considering 24 traits simultaneously.

<table>
<thead>
<tr>
<th>Species</th>
<th>B. brizantha</th>
<th>B. decumbens</th>
<th>B. humidicola</th>
<th>B. jubata</th>
<th>B. ruziziensis</th>
<th>B. dictyoneura</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. brizantha</td>
<td>86.67</td>
<td>10.67</td>
<td>0.67</td>
<td>0.67</td>
<td>0.00</td>
<td>1.33</td>
</tr>
<tr>
<td>B. decumbens</td>
<td>6.52</td>
<td>93.48</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>B. humidicola</td>
<td>5.56</td>
<td>0.00</td>
<td>72.22</td>
<td>2.78</td>
<td>0.00</td>
<td>19.44</td>
</tr>
<tr>
<td>B. jubata</td>
<td>0.00</td>
<td>3.23</td>
<td>0.00</td>
<td>96.77</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>B. ruziziensis</td>
<td>0.00</td>
<td>7.14</td>
<td>0.00</td>
<td>0.00</td>
<td>92.86</td>
<td>0.00</td>
</tr>
<tr>
<td>B. dictyoneura</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>0.00</td>
<td>90.00</td>
</tr>
</tbody>
</table>
dispersion of *B. jubata* accessions, it can be verified that these species tend to form a separate group.

**Anderson’s Discriminant Analysis**

Discriminant functions obtained from the 24 variables for each species are in Table 3. Probabilities *a priori* were the same for each group, considering that there are no evidences related to the studied data that justify a larger classification probability for a certain group.

From these functions, it is possible to classify an individual whose species is unknown and whose classification is unclear. It is necessary to assess the 24 characteristics and apply the values to each one of the established functions. Thus, six classification scores are obtained, each one corresponding to one of the six species studied. These scores are, then, compared to each other and the unknown accession is indicated as belonging to the species whose value was the highest among all.

**Comments on the Removal of Variables**

The knowledge of the association degree among the variables under study is important for the multivariate analysis, once characteristics highly correlated don’t bring any additional information, and can interfere in the results of the analysis. The time and amount of work spent on assessing an excessive number of characters has also motivated the reduction of number of analyzed variables, considering the possible redundancy among many of them. Thus, the simple correlations among the means of the twenty-four variables included in this work were calculated, and the results are in Table 4. There are highly correlated characteristics, and those above 0.80 (in absolute value) are underlined.

![Table 3. Anderson’s discriminant functions for six *Brachiaria* species, considering 24 morphologic traits.](image)

1/ *p = a priori* probability; PHT: plant height; PGH: plant growth habit; LLT: leaf length; LWT: leaf width; LGH: leaf growth habit; LLS: length of leaf sheath; LFS: length of floral stem; ILT: inflorescence length; NUR: number of racemes; LBR: length of basal raceme; NUSR: number of spikelets on basal raceme; SIN: spikelet insertion; RWT: rachis width; SSP: spikelet spot percentage; SCO: stigma colour; ACO: anther colour; DRP: density of rachis pubescence; LRP: length of rachis pubescence; DLB: density of leaf blade pubescence; LLB: length of leaf blade pubescence; MLB: margin of the leaf blade; DSP: density of leaf sheath pubescence; LSP: length of leaf sheath pubescence and USP: distribution of leaf sheath pubescence.

2002, Brazilian Society of Plant Breeding
The criterion for identifying variables that least contributed to the discrimination of populations or individuals, adopted by several plant breeding studies (Morais, 1992; Daher, 1993; Albuquerque, 1997; Ferrão, 1997; Shimmoy, 2000; Strapasson et al., 2000), is based on the fact that the relative importance of the principal components decreases from the first to the last, being the last components responsible for the explanation of the minimum fraction of the total available variation (Cruz and Regazzi, 1997).

However, this criterion was not used in the present study, as the rank of the variance and covariance matrix is the same or smaller than n-1 (Johnson and Wichern, 1998). It will be smaller than n-1 whenever the number of linear combinations of original data matrix exceeds the value p-(n-1). In these situations, the matrix is positive semidefinite and its determinant is zero. The number of positive and no eigenvalues is the same as that of the rank matrix.

In this work, five eigenvalues different from zero were obtained; so system dimension was defined as five. There were 19 eigenvectors associated to 19 eigenvalues equal to zero. It is necessary to define which are the least important to initiate the removal process. However, the 19 eigenvectors associated to the 19 eigenvalues equal to zero are different from each other, making the identification of the least important variables inconsistent, once the elements of the eigenvectors differ in the same value (null eigenvalue), indicating different variables susceptible to elimination.

**CONCLUSIONS**

There was genetic diversity among the accessions and species studied.

The study of the genetic divergence evidenced the formation of three different groups: the first, constituted by the *B. brizantha, B. decumbens* and *B. ruziizensis* species; the second by the *B. humidicola* and *B. dictyoneura*; and the last group by the *B. jubata* species.

The established discriminant functions were consistent, being recommended as an auxiliary approach to identify unknown individuals.

The least important variable criterion of identification, based on the analysis of the principal components and usually used in plant breeding, is inappropriate whenever the number of individuals or populations is inferior to the number of variables under study.

**ACKNOWLEDGEMENTS**

To CNPq, for the scholarships granted to the first author during her Master’s Degree course.
RESUMO

Divergência genética em espécies de *Brachiaria*

Foram analisados 301 acessos, pertencentes a seis diferentes espécies de *Brachiaria*, nos quais foram avaliadas vinte e quatro características morfológicas. Foram realizadas a análise discriminante baseada em componentes principais e a análise discriminante de Anderson para as seis espécies. A dispersão gráfica obtida proporcionou o conhecimento da divergência genética entre os acessos e entre as espécies. Foram identificados três distintos grupos. As funções discriminantes estabelecidas foram consistentes e apresentaram as seguintes taxas de classificação correta: *B. brizantha* (86,67%), *B. decumbens* (93,48%), *B. humidicola* (72,22%), *B. jubata* (96,77%), *B. ruziziensis* (92,86%) e *B. dictyoneura* (90,00%), podendo ser utilizadas como critério auxiliar na identificação de indivíduos desconhecidos.

REFERENCES


