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Brachiaria germplasm dissimilarity as shown by RAPD markers

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ABSTRACT - *The main objective of this work was to study the genetic dissimilarity among germplasm accesses of six Brachiaria species and to compare the most important cultivars through the use of RAPD markers with the studied material. DNA was extracted from seeds of five different accesses of* B. decumbens, B. ruziziensis, B. nigropedata, B. humidicola, B. jubata *and* B. brizantha *from the EMBRAPA – CNPGC, 12 commercial cultivars, and two accesses each of* P. maximum *and* B. arrecta. *From 120 primers tested, 10 polymorphic were selected, amplifying a total of 114 polymorphic bands. Dissimilarity indexes were estimated for all samples analysed, and ranged from 0.262 to 0.907, grouped by UPGMA, and four groups were clearly distinct. All accesses and cultivars of Brachiaria could be grouped through RAPD markers. Dissimilarity within a species was found. The distribution in African species was narrower in* B. decumbens *and* B. ruziziensis *showed low levels of dissimilarity, and naturally widely-spread species showed high dissimilarity levels.*

Key words: molecular markers, genetic variability, DNA, pasture.

INTRODUCTION

Brachiaria grasses are responsible for opening new opportunities for tropical cattle breeding due to their wide environmental adaptability, ample forage production, high nutritional qualities and easy propagation.

Grass forage is the base for beef production in Brazil with 115 million hectares of grasses being cultivated, predominantly with *Brachiaria* species (Zimmer and Euclides 2000). The intensification of beef cattle production has generated a demand for superior grass cultivars that must combine high production capacity and ecological plasticity in a continuous process of introduction, evaluation and selection.

The success of any plant breeding or conservation program is highly dependent on the knowledge of the amount of variation existent in the target species. There are seven important *Brachiaria* collections in the world, containing 987 accesses of 33 known species (Keller-Grein et al. 1996). Among *Brachiaria* species, there is a huge natural variability, and because of that the identification of characteristics that can uniquely identify a species is difficult (Assis et al. 2003, Renvoize et al. 1996).

Morphological and agronomical characteristics used to evaluate genetic diversity in some populations have shown a low power to discriminate very similar taxonomic groups due to environmental factors. To solve problems like this, molecular techniques are being used to evaluate genetic variability (Parker et al. 1998).

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According to Vilela-Morales and Valois (2000) germplasm accesses are a source of variability for creating genetic improvements, higher environmental and ecological plasticity and resistance to biotic and abiotic stress, finely matching the need for sustainable agricultural development.

The knowledge about the genetic variability available in germplasm banks is very important for breeding plans, collection expeditions or germplasm exchange in order to acquire specific characteristics. The use of native or exotic germplasm according to Chiari et al. (2007) is strategic in the development of new cultivars of forage and is dependent on the identification of superior parents.

The success of any breeding program lies in the working population variability (Cruz 2006), and the recommendation for establishing a base population is to inter-cross elite and divergent cultivars evaluated by agronomic, morphological and molecular characteristics. Much information, about each genetic material, a cultivar or an access, is expressed together in a dissimilarity measure, which represents the diversity arisen from the studied pool.

The need for genetic resources in agriculture implies that there is an increasing of modern methods and biotechniques to reach success in sustainable agriculture, and from these processes molecular markers have been used Vilela-Morales and Valois (2000).

Among the markers available today, the detection of RAPD (Random Amplified Polymorphic DNA) markers is distinct for its simplicity, fast execution, low cost, the small quantity of DNA necessary, and the possibility of studies of species for which there is no previously known genetic information. It also has its limitations; RAPD are dominant markers that do not allow for the differentiation of heterozygous individuals, and there is also low repeatability for some bands (Lacerda et al. 2002). Yet, the use of RAPD markers is viable (Simmons 2007).

The dominant characteristics of RAPD markers cause some inconveniences, especially in the statistical analysis overview. Clark and Lanigan (1993) and Stewart and Excoffier (1996) have attempted to develop methodologies of data analysis that get around the dominance problem. In relation to the low repeatability of some bands, several studies have highlighted the fact that concentrations of magnesium chloride, genomic DNA, primer and Taq polymerase are the principal causes of the artifactual bands. Thus, there is a consensus that for studies with RAPD, primers known to work in the targeted species, optimal reagent concentrations, PCR program adjustments and care in protocol execution reduce the production of low repeatability bands (Heun and Helentjaris 1993, Virk et al. 1995).

RAPD have been used for different number of species, independent of the strategy of reproduction, with success as in *Coffea arabica* autogamous (Diniz et al. 2005); *C. canephora* allogamous (Ferrão et al. 2009), *Aspidosperma* allogamous (Torezan et al. 2005), *Zea mays* self-fertilized (Souza et al. 2008), *Catharantus roseus* (Shaw et al. 2009), Cassava allogamous (Ferreira et al. 2008).

Chiari et al. (2007) highlighted the importance of knowing the genetic variability in the *Brachiaria* germplasm bank accesses for successful plant breeding plans, planning of divergent crosses and guidance of new collections or germplasm exchange. The application of molecular marker technology in breeding programs of *Brachiaria* is to determine the available genetic variability to help parental selection for inter and intra-specific hybridization.

Recent work has shown the high genetic diversity within *B. humidicola* accesses in the germplasm bank at Embrapa Gado de Corte, and that this is valuable material for breeding plans and the selection of new cultivars (Chiari et al. 2007).

The aim of this work was to study the genetic dissimilarity among germplasm accesses of six *Brachiaria* species and to check the proximity of the most important commercial cultivars of this genera with the germplasm analysed, through the use of RAPD markers.

MATERIAL AND METHODS

The experiment was conducted in the UNOESTE's laboratory of Molecular Genetics and Cytogenetics in Presidente Prudente – SP.

For DNA extraction, the method described by Doyle and Doyle (1987) was used, with modifications. Seeds of five accesses from six species of *Brachiaria* (*B. brizantha, B. decumbens, B. ruziziensis, B. jubata, B. nigropedata* and *B. humidicola)* donated by EMBRAPA – Gado de Corte, 11 commercial cultivars of *B. brizantha, B. decumbens, B. humidicola, B. ruziziensis*, one sample of the hybrid *Brachiaria* cv

'Mulato', two of *P. maximum ('Mombaça'* e *'Tanzânia'*) and leaves of *B. arrecta* comprising a total of 44 samples were used (Table 1).

Tissue was ground in a mortar with a pestle under liquid nitrogen. To the powder was added buffer composed of Tris-EDTA pH 8.0, 500 mM CTAB and 2% ß-mercaptoethanol at 65 ºC at a 1:10 ratio (w:v), and the mix was incubated at that temperature for 40 min. After that, chloroform:isoamyl alcohol (24:1) was added and mixed carefully for 10 min. The mixture was centrifuged

Table 1. *Brachiaria* accession list with codes, identification, register and country of origin according Embrapa Gado de Corte

Accesses of Embrapa CNPGC	Code	Identification	Register	Origin/Country
B. ruziziensis	B. ruziz 1	R100	BRA005541	Trans Nzoia/Kenya
B. ruziziensis	B. ruziz 2	R106	BRA005649	Bujumbura/Burundi
B. ruziziensis	B. ruziz 3	R108	BRA005584	Cibitoke/Burundi
B. ruziziensis	B. ruziz 4	R109	BRA005631	Ruyigi/Burundi
B. ruziziensis	B. ruziz 5	R128	BRA002291	———
B. decumbens	B. decum 1	D53	PI355744	Bogota/Colombia
B. decumbens	B. decum 2	D7	BRA004472	South Nyanza/ Kenya
B. decumbens	B. decum 3	D9	BRA004499	Nakuru/ Kenya
B. decumbens	B. decum 4	D58	BRA000191	Embrapa- CPATU/Brazil
B. decumbens	B. decum 5	D59	BRA000116	Embrapa CNPMF/Brazil (cv 'Ipean')
B. brizantha	B. briz 1	B158	BRA003719	Bungoma/ Ethiopia
B. brizantha	B. briz 2	B23	BRA001945	Embrapa CNPGC/Brazil
B. brizantha	B. briz 3	B67	BRA003336	Ilubabor/Ethiopia
B. brizantha	B. briz 4	B112	BRA002844	Welega/ Ethiopia
B. brizantha	B. briz 5	B127	BRA003107	Gamo Gofa/ Ethiopia
B. jubata	B. jubata 1	J17	BRA005223	Idamo/ Ethiopia
B. jubata	B. jubata 2	J13	BRA005533	Yumba/Rwanda
B. jubata	B. jubata 3	J8	BRA005461	Trans Nzoia/ Kenya
B. jubata	B. jubata 4	J4	BRA005291	Ungoma/ Kenya
B. jubata	B. jubata 5	J30	BRA05380	Ericho/Kenya
B. nigropedata	B. nigro 1	N203	CIAT16923	Masvingo/Zimbabwe
B. nigropedata	B. nigro 2	N190	BRA001123	———
B. nigropedata	B. nigro 3	N191	BRA005916	Hwange/Zimbabwe
B. nigropedata	B. nigro 4	N202	CIAT16921	BIKITA/Zimbabwe
B. nigropedata	B. nigro 5	N197	CIAT16911	Urungwe/Zimbabwe
B. humidicola	B. humi 1	H10	BRA004952	Inyanga/Zimbabwe
B. humidicola	B. humi 2	H12	BRA004979	Inyanga/Zimbabwe
B. humidicola	B. humi 3	H13	BRA005011	Masvingo/Zimbabwe
B. humidicola	B. humi 4	H108	BRA001937	Embrapa CNPGC/Brazil
B. humidicola	B. humi 5	H112	BRA002208	CSIRO/Australia
Commercial Materials				
B. brizantha	B.briz MG4	MG4	02256	unkonown
B. brizantha	B.briz Mar	Marandu	02250	Marondera/ Zimbabwe
B. brizantha	B.briz Laliber	La Libertad		unkonown
B. brizantha	B.briz Xaraés	Xaraes	04509	CIbitoka /Burundi
B. brizantha	B.briz MG5	MG5	04509	CIbitoka /Burundi
B. decumbens	B.dec Basilisk	Basilisk	02277	Uganda
B. humidicola	B. hum - a	Tully	04189	Pretoria / South Africa
B. humidicola	B. hum - b	Tully	04189	Pretoria / South Africa
B. ruziziensis	B. ruzi - a	Kennedy	02043	Ruzi Valley / Zaire
B. ruziziensis	B. ruzi - b	Kennedy	02043	Ruzi Valley / Zaire
B. arrecta	B. arrecta			
Brachiaria Hybrid	Mulato		09669	CIAT / Colombia

at 12,000 rpm for 15 min. The aqueous phase was collected and cooled in an ice bath. 7.5M ammonium acetate and isopropyl alcohol were added to a final concentration of 6% and 54%, respectively. The mixture was placed at -20 ºC for 24 h. A new cycle of centrifugation was done and the liquid phase discarded. Pellets were washed twice with 70% ethanol at 65 ºC for one minute each and air-dried in an air flow cabinet. DNA was dissolved in TE buffer, pH 8.0, and quantified by spectrophotometer at 260/280 nm and adjusted to 10 $\mu g\,\mu L^{-1}$.

Amplifications were done as in Williams et al. (1990) with modifications. Genomic DNA was amplified in a reaction volume of 25 µL containing 10% of Tris KCl buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl), 1.5 mM $MgCl_2$, 0.4 µM of primer, 0.2 µM of each dNTP, one Taq unit and template DNA (25 and 50 ng). RAPD amplifications were performed in a thermocycler with the following steps: 94 ºC for three minutes for initial denaturation, followed by 43 cycles of one min at 94 ºC, one min at 37 °C for primer annealing and 30 s at 72 ºC for chain elongation. One extra step of five minutes at 72 ºC was done at the end for a final elongation. Only bands present in amplifications of both 25 and 50 ng of template DNA were considered.

Amplification products were separated by gel electrophoresis in a 1.5% agarose gel with ½X TBE buffer. The gel was stained with ethidium bromide and pictures were captured by a CCD machine (Alpha-Inmotech) and by ChemiImager software.

One hundred twenty Operon primers were evaluated. Initially, they were scanned in *B. brizantha* cv 'MG4' with a large amount of template DNA. Forty-three primers were selected since they were polymorphic and exhibited more than two strong bands. After that, those primers were scanned against samples of *B. ruziziensis* and *B. jubata*. Using the same criteria, ten primers were finally elected for RAPD reactions (Table 2), but they were not polymorphic for all the species.

Bands were used to construct a similarity matrix based on the Jaccard coefficient, coding 1 as present and 0 as absent. The grouping analysis was done using the UPGMA algorithm (Unweighted Pair-Group Method Using an Arithmetic Average) performed with the software Genes (Cruz 2006).

Molecular variance analysis (Amova) was calculated by total decomposition of its components among and between accesses using the square distances (Excoffier et al. 1992) with the Arlequim program (Excoffier *et al*. 2006).

Table 2. Nucleotide sequence, number of bands and number of polymorphic bands of each primer used for RAPD-PCR analyses of *Brachiaria* samples

Primers	Nucleotide Sequence (5' –> 3')	Number of amplified sites	Number of polymorphic sites	Fragment size range (pb)
A5	AGG GGT CTT G	10	10	260 to 1000
A9	GGG TAA CGC C	10	10	200 to 1000
D11	AGC GCC ATT G	10	10	300 to 900
G5	CTG AGA CCG A	12	12	200 to 1000
G9	CTG ACG TCA C	11	11	300 to 1300
G10	AGG GCC GTT C	8	8	300 to 1000
G17	ACG ACC GAC A	16	16	200 to 1300
X15	CAG ACA AGC C	12	12	300 to 1450
X17	GAC ACG GAC C	13	13	300 to 1000
Y1	GTG GCA TCT C	12	12	300 to 1300

Species	Polymorphic sites per specie	% Polymorphism
Brachiaria decumbens	69	60.52
Brachiaria ruziziensis	74	64.91
Brachiaria brizantha	89	78.07
Brachiaria humidicola	76	66.70
Brachiaria jubata	64	56.14
Brachiaria nigropedata	49	42.98

RESULTS AND DISCUSSION

The 42 samples of several species of the *Brachiaria* genus and the two samples of *Panicum maximum* were amplified with the ten selected primers, generating 114 bands with an average of 11.4 bands per primer, all of them polymorphic. The fragment size ranged from 200 to 1450 bp; similar values were found by Chiari et al. (2007) between 58 accesses of *B. humidicola*. The number of bands, according Telles et al. (2001), is more important than the primer number, an idea that is in accordance with several authors regarding genetic estimates by the RAPD technique. Dias et al. (2004) argued that the better results for RAPD use may be done, according the literature, with at least 160 bands. However, Chen et al. (2006) showed that the genetic variability and clone diversity of three rare natural populations of *Caldesia grandis*, by means of RAPD study, could be accessed by 7 primers, out of 60, in 342 samples with highly reproducible bands. These primers generate 61 bands with 82.25% of polymorphism. The AMOVA showed that 81.5% of the variation is inside the populations. In this work 68.7% of the variation is inside the species and just 31.3% were between species (Table 3).

The dissimilarity index was estimated in all analysed samples (Tables 4 and 5). Excluding *P. maximum,* the observed dissimilarity index between samples ranged from 0.262 to 0.907, exhibiting a significant genetic variability among accesses and cultivars in the *Brachiaria* genus. In some other grasses evaluated by RAPD markers, the results were similar. Dong et al. (2003) showed that in *Vetiveria zizanioides* values ranged between 0.005 to 0.495 and Chandra et al. (2004) showed that in *Dichantium annulatum* they ranged from 0.02 to 0.62.

It was not possible to establish a relationship between origin place and dissimilarity values. Chiari et al. (2007) showed that by grouping accesses of *B. humidicola*, there was also no relationship between origin and accession.

The dissimilarity genetic value ranged from 0.262 to 0.907 among all samples (Table 5). The lowest was between two *B. decumbens*, BRA000191 (*B. decum* 4) and BRA000116 (*B. decum* 5). The highest was between *B. humidicola* BRA002208 (*B. humi* 5) and *B. jubata* BRA005223 (*B. jubata* 1).

High variability was also found by Chiari et al. (2007) in *B. humidicola* from the Germplasm Bank of Embrapa – Gado de Corte. Of the 58 accesses, the genetic

Table 3. AMOVA Results of *Brachiaria* species grouped by RAPD markers

Source of variation	DF	Sum of Square	Components of Variation	Percentage of variation
Between species	7	367.21	6.79	31.21
Inside species	38	568.63	14.96	68.79
Total	45	935.84	21.75	

Table 4. Wright's measure of population differentiation (Fst) using RAPD and highest and lowest values of genetic dissimilarity between accesses of each studied species and country of origin

		Highest dissimilarity			Lowest dissimilarity		
Species	Fst	Accession	Origin	Value (%)	Accession	Origin	Value (%)
B. jubata	0.314	BRA005533	Rwanda	70.2	BRA005291	Kenya	40.5
		BRA05380	Kenya		BRA05380	Kenya	
B. ruziziensis	0.303	BRA005649	Burundi	55.6	BRA005541	Kenya	34
		BRA005584	Burundi		BRA005649	Burundi	
B. decumbens	0.312	BRA000191	Brazil	53.9	BRA000191	Brazil	26.2
		PI355744	Colombia		BRA000116	Brazil	
B. nigropedata	0.344	CIAT16911	Zimbabwe	57.7	BRA005916	Zimbabwe	34.8
		CIAT16923	Zimbabwe		CIAT16911	Zimbabwe	
B. humidicola	0.437	BRA002208	Australia	63.6	BRA001937	Brazil	40.5
		BRA004952	Zimbabwe		BRA002208	Australia	
B. brizantha	0.276	BRA003336	Ethiopia	72.3	BRA001945	Brazil	46
		BRA003107	Ethiopia		BRA003719	Ethiopia	

Table 5. Dissimilarity matrix between accesses of germplasm bank from Embrapa – CNPGC and the most used cultivars of *Brachiaria*

	Brachiaria jubata					*Brachiaria ruziziensis*					*Brachiaria decumbens*					*Brachiaria nigropedata*					*Brachiaria humidicola*					*Brachiaria brizantha*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Brachiaria jubata	2	0.807	0.662	0.727	0.729	0.667	0.340																							
Brachiaria ruziziensisis	3	0.778	0.708	0.764	0.696	0.704	0.380	**0.556**																						
Sample a		0.722	0.629	0.732	0.794	0.783	0.708	0.714	0.746	0.694	0.667	0.586	0.565	0.661	0690	0.638	0.721	0.762	0.730	0.672	0.678	0.814	0.754	0.719	0.780	0.722	0.667	0.746	0.782	0.761	0.707
Sample b		0.790	0.685	0.754	0.754	0.803	0.600	0.579	0.564	0.554	0.597	0.698	0.672	0.683	0.754	0.661	0.700	0.762	0.710	0.773	0.721	0.814	0.754	0.763	0.737	0.790	0.769	0.726	0.759	0.723	0.750
Brachiaria decumbens	4	0.833	0.732	0.774	0.772	0.782	0.721	0.684	0.741	0.707	0.702	**0.539**	0.463	0.362																
	5	0.821	0.690	0.741	0.741	0.750	0.714	0.678	0.690	0.632	0.695	0.509	0.407	0.298	0.262															
'Basilisk'		0.810	0.769	0.797	0.813	0.763	0.727	0.672	0.705	0.650	0.667	0.586	0.587	0.518	0.593	0.589	0.762	0.721	0.750	0.714	0.742	0.679	0.643	0.648	0.667	0.620	0.621	0.661	0.759	0.683	0.500
Brachiaria nigropedata	5	0.839	0.704	0.712	0.820	0.790	0.730	0.695	0.790	0.758	0.754	0.700	0.629	0.683	0.737	0.707	**0.577**	0.458	0.348	0.362										
Brachiaria humidicola	4	0.796	0.754	0.755	0.778	0.740	0.767	0.776	0.768	0.797	0.750	0.759	0.726	0.648	0.706	0.673	0.764	0.667	0.727	0.754	0.692	**0.636**	0.565	0.600						
	5	0.880	0.831	0.840	0.875	0.800	0.776	0.764	0.755	0.786	0.712	0.698	0.667	0.600	0.660	0.653	0.726	0.674	0.736	0.741	0.674	0.610	0.568	0.571	0.405					
Sample a		0.729	0.743	0.833	0.848	0.796	0.754	0.783	0.754	0.783	0.759	0.679	0.650	0.684	0.692	0.685	0.772	0.772	0.839	0.803	0.772	0.708	0.583	0.556	0.546	0.644	0.642	0.660	0.659	0.684	0.660
Sample b		0.825	0.795	0.768	0.767	0.754	0.719	0.683	0.625	0.683	0.700	0.730	0.721	0.672	0.767	0.695	0.754	0.643	0.742	0.766	0.754	0.667	0.604	0.709	0.654	0.660	0.762	0.738	0.796	0.791	0.719
Brachiaria brizantha	2	0.778	0.743	0.764	0.763	0.750	0.672	0.655	0.712	0.678	0.574	0.726	0.613	0.597	0.623	0.593	0.707	0.750	0.797	0.781	0.750	0.686	0.623	0.654	0.673	0.653	0.460				
	5	0.760	0.797	0.815	0.810	0.821	0.778	0.767	0.737	0.724	0.719	0.684	0.698	0.667	0.698	0.759	0.817	0.776	0.803	0.746	0.776	0.789	0.647	0.653	0.700	0.681	0.623	0.691	**0.723**	0.643	
'Marandu'		0.827	0.811	0.811	0.786	0.774	0.712	0.741	0.732	0.719	0.691	0.655	0.650	0.611	0.640	0.685	0.750	0.772	0.691	0.696	0.727	0.681	0.612	0.617	0.638	0.581	0.588	0.709	0.745	0.585	0.477
'MG4'		0.821	0.792	0.764	0.763	0.814	0.734	0.721	0.712	0.655	0.717	0.661	0.613	0.571	0.623	0.593	0.810	0.729	0.738	0.700	0.707	0.660	0.596	0.704	0.722	0.653	0.672	0.643	0.769	0.689	0531
'MG5'		0.828	0.798	0.750	0.771	0.800	0.779	0.769	0.742	0.750	0.746	0.773	0.706	0.698	0.729	0.700	0.738	0.625	0.705	0.710	0.738	0.810	0.750	0.800	0.817	0.807	0.746	0.700	0.800	0.633	0.604
'La libertad'		0.803	0.750	0.750	0.769	0.738	0.625	0.629	0.593	0.607	0.576	0.590	0.450	0.500	0.597	0.617	0.719	0.677	0.688	0.672	0.677	0.702	0.597	0.600	0.643	0.569	0.500	0.593	0.685	0.641	0.537
'Xaraes'		0.820	0.771	0.804	0.862	0.811	0.724	0.685	0.673	0.608	0.704	0.737	0.564	0.648	0.680	0.647	0.807	0.741	0.772	0.754	0.764	0.776	0.706	0.740	0.784	0.771	0.704	0.722	0.761	0.719	0.726
B. arrecta		0.816	0.882	0.889	0.818	0.830	0.783	0.871	0.807	0.853	0.869	0.797	0.870	0.875	0.898	0.885	0.845	0.845	0.869	0.889	0.883	0.907	0.839	0.830	0.827	0.885	0.790	0.867	0.857	0.714	0.745
Mulato		0.750	0.756	0.820	0.778	0.787	0.629	0.698	0.689	0.739	0.672	0.683	0.714	0.688	0.738	0.750	0.803	0.838	0.791	0.776	0.821	0.754	0.695	0.655	0.696	0.750	0.650	0.689	0.786	0.667	0.510
Mombaça		0.814	0.788	0.780	0.758	0.807	0.692	0.719	0.750	0.719	0.714	0.703	0.714	0.765	0.778	0.788	0.766	0.766	0.843	0.812	0.785	0.836	0.868	0.807	0.823	0.814	0.714	0.769	0.848	0.817	0.712
Tanzânia		0.814	0.772	0.780	0.758	0.787	0.692	0.698	0.750	0.739	0.714	0.683	0.714	0.765	0.758	0.750	0.726	0.746	0.809	0.794	0.766	0.817	0.815	0.787	0.841	0.793	0.672	0.730	0.807	0.800	0.712

In bold is the highest value and in the lowest of dissimilarity between accesses into species.

similarity ranged from 0.14 to 0.97, suggesting that even with the high morphological similarity there is a large amount of genetic variability in this group.

Examining the average dissimilarity among accesses within species, the lowest value was 0.405 for *B. decumbens*, followed by 0.445 in *B. ruziziensis*. The highest values of dissimilarity were found in *B. brizantha* and *B. jubata* (0.631 and 0.567, respectively). Another important information taken from these data was the population fixation index (Wright's FST) that exhibited the fragment fixation inside the species. *B.humidicola* had the highest level meaning that amplified fragments were frequently present in the populations studied. On the other hand, *B.brizantha* has the lowest value (Table 4) indicating a high variability of this species. In time, these values, for all species, exhibited an allogamy behaviour as the main reproduction mode.

These data support the observations of Valle (1990), who, using multivariate analysis in *B. brizantha, B. decumbens and B. ruziziensis*, argued that *B. brizantha* have a high level of variability in the studied characteristics and the other two species were limited to small areas of distribution, which indicated a high level of homogeneity in the analysed components.

In this study, a good correlation between the average dissimilarity of the accesses and the continental distribution in the African continent was achieved. Even *Brachiaria decumbens* and *B. ruziziensis* have a narrow natural distribution and low genetic dissimilarity. The *B. decumbens* germplasm was collected in the west of Kenya, Rwanda and Burundi between lat 4º21'S and long 1º09'N. *B. ruziziensis* was also collected in Burundi, Rwanda and Kenya between lat 4º 05' S and long 2º 54' S. On the other hand, *B. brizantha* have been found in nearly all of tropical Africa between lat 25º 05' S and long 12º 36' N, and the distribution of *B. jubata, B. humidicola* and *B. nigropedata* is similar (Keller-Grein et al. 1996). On the basis of these collected data, it could then be inferred that *Brachiaria* species with a broad distribution have evolved in different conditions due to their high genetic variability.

The same *Brachiaria* accesses used in this work were used by Machado Neto et al. (2002) to measure intra-species differences using seed protein electrophoresis (SDS-PAGE). Distinctions among each accession were found in nearly all species, and *B. jubata* was more genetically homogeneous than the others. In the present work *B. jubata* similarity was higher than in *B. decumbens, B. ruziziensis, B. nigropedata and B. humidicola*.

UPGMA analysis was performed on the dissimilarity values displayed in Table 4. The data is graphically represented as a tree (Figure 1). Four groups can be easily distinguished in the tree: group I with three samples, *P. maximum* (two cultivars) and *B. arrecta*; they form the outgroup or the more genetically distinct species. Group II, distinct from the others, groups all accesses of *B. jubata.* Group III contains all *B. nigropedata* accesses evaluated, and group IV contains all accesses and commercial cultivars of *B. ruziziensis, B. decumbens, B. brizantha* and *B. humidicola.*

Valle (1990) using principal component analysis of morphologic characteristics, also found that *B. jubata* was distinct from *B. ruziziensis, B. decumbens* and *B. brizantha*. The data obtained here also support the classification proposed by Renvoize et al. (1996) which separated *Brachiaria* in a series of group species with the most important species belonging to groups 2, 3, 5 and 6. That work isolated *B. arrecta*, a species from group 3, from the other *Brachiaria species. B. jubata* and *B. nigropedata*, classified into groups 6 and 2, respectively, were separated from the others, *B. decumbens, B. brizantha* and *B. ruziziensis,* that were put into group 5. However, in this work, *B. humidicola*, which has been grouped with *B. jubata* in group 6 by Renvoize et al. (1996) was closely related with the group 5 species, group IV in this work (Figure1).

A wide range of dissimilarity among accesses and cultivars was determined by the use of RAPD markers in the studied *Brachiaria* species.

There was genetic variability in each *Brachiaria* species studied, showing that the maintenance of the accesses in a germplasm banks is justified.

A positive relation between the average dissimilarity of a species and its distribution on the African continent was made. Species with lower genetic dissimilarity, such as *B. decumbens* and *B. ruziziensis,* have a narrow distribution. On the other hand, species with high genetic dissimilarity have a wide distribution across the African continent.

Brachiaria decumbens 'Basilisk' was grouped with *B. brizantha* instead of the accesses of the first species, which enforces that, is a true *B. brizantha*.

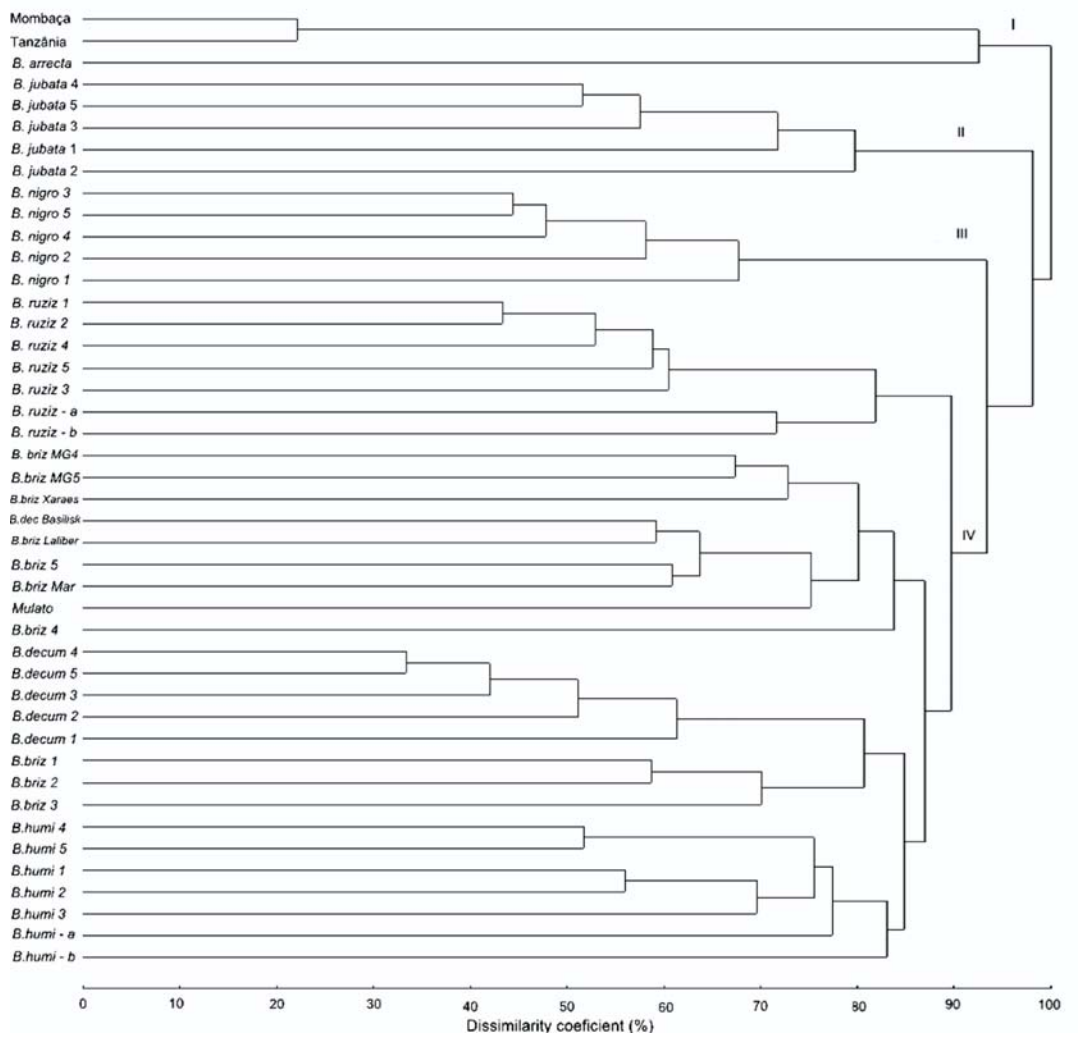

Figure 1. UPGMA tree based on the genetic dissimilarity among 42 samples of *Brachiaria* and two *Panicum* cultivars

Dissimilaridade em germplasma de *Brachiaria* por marcadores RAPD

RESUMO - *O objetivo deste trabalho foi estudar a dissimilaridade genética entre germoplasma de acessos de seis espécies de* Brachiaria *e os cultivares mais importantes por marcadores RAPD. DNA foi extraído de sementes de cinco acessos de* B. decumbens, B. ruziziensis, B. nigropedata, B. humidicola, B. jubata *e* B. brizantha *da EMBRAPA-CNPGC, 12 cultivares comerciais, dois* P. maximum *e* B. arrecta. *De 120 primers testados foram selecionados e amplificados 10, produzindo 114 bandas polimórficas. Os índices de dessemelhança foram calculados para todas amostras analisadas variando de 0,262 a 0,907. Eles foram agrupados por UPGMA e quatro grupos puderam ser claramente distintos. Foi possível agrupar todos os acessos e cultivares de* Brachiaria *por marcadores RAPD. Foi verificada a dissimilaridade dentro de espécies e a distribuição na África. Espécies com uma variação estreita como* B. decumbens *e* B. ruziziensis *mostraram baixos níveis de dissimilaridade e espécies amplamente distribuídas naturalmente mostraram altos níveis de dissimilaridade.*

Palavras chave: marcadores moleculares, variabilidade genética, DNA, pastagens.

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