Brachiaria access germplasm distinction using SDS PAGE

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ABSTRACT. *Brachiaria* is a very important grass used as forage in tropical countries. However, improvement of *Brachiaria* species is slow and time consuming, being made essentially by introduction of the foreign genetic material. To verify the difference among accesses of different species of *Brachiaria*, five access of six species, were submitted to SDS-PAGE. The accesses of *Brachiaria* presented variations in almost all the species. *Brachiaria jubata* showed little variation, meaning that received samples are very homogeneous genetically. The other species present enough variations to separate wild accesses.

Key words: soluble proteins, genetic access, electrophoresis, genetic diversity, *Brachiaria.*

RESUMO. Separação de acessos de *Brachiaria* usando-se SDS-PAGE. *Brachiaria* é uma forrageira muito importante para países tropicais, usada largamente para pastejo. Porém, o melhoramento genético das espécies desse gênero é lento, sendo feito essencialmente por introdução do material genético. Para verificar a diferença entre acessos de espécies diferentes de *Brachiaria*, cinco acessos de seis espécies, foram submetidos a eletroforese em SDS-PAGE. Os acessos de *Brachiaria* apresentaram variações em quase todas as espécies. Os acessos de *Brachiaria jubata* são geneticamente muito homogêneos. As outras espécies apresentam variações suficientes para separar os acessos silvestres.

Palavras-chave: proteínas solúveis, acessos genéticos, eletroforese, diversidade genética, *Brachiaria.*

Introduction

The savannah ecosystem covers approximately 250 million hectares and it is occupied with *Brachiaria* grass in over 40 million hectares, especially in Colombia, Paraguay, Venezuela and Brazil. The largest territorial area extension used for grazing is in Brazil, and it is generating a great interest for alternative pastures, different from those native that do not fill all the nutritional requirements, originating low yields in animal breeding.

According to Keller-Grein *et al*. (1996): "The genus *Brachiaria*, tribe Paniceae, includes 100 species which occur in the tropical and subtropical regions of Africa, Asia and America, but mostly in the eastern and western hemispheres". Seven perennial species of African origin have been used, *B arrecta, B.brizantha, B.decumbens, B.dictyoneura, B.humidicola, B.mutica* and of *B.ruziziensis* as forage plants, particularly in tropical America.

The improvement of the *Brachiaria* species is slow and time consuming, being made essentially by introduction of the foreign genetic material, frequently collected in tropical Africa countries, and distinction between accesses could allow the improvement of germplasm banks as the monitoring the breeding process.

Electrophoresis could be a useful tool for the determination of the molecular weight of the proteins and characterisation of nucleic acids (DNA, RNA). The recognition of proteins and nucleic acid using electrophoresis is necessary and valuable for taxonomy studies in plants (van den Berg *et al*., 2000), animals, micro-organisms and the viroids (Alfenas, 1998).

In several cultures the electrophoretic pattern can vary according to the type of protein. Storage proteins are the most stable ones, being constant with variations in environmental conditions (Cooke, 1995). Proteins are the most direct expression of the genes, and the main advantage of its use, in comparison to the classic descriptive techniques, it the direct determination of an organism genetic expression, independent of environmental influences, considering that the patterns of the seed proteins are highly stable (Sanches-Yelamo *et al*., 1992).

Seed protein profiles were used to verify polymorphism in seed protein, being an easy and

more useful method for cultivar distinction (Nehra *et al*., 1991; Nevo *et al., 1*993), environmental stress answer (Marmiroli *et al*., 1989; Siegel, 1993), as a method for identification of species, subspecies and at variety levels in *Trifolium* (Badr, 1995), *Lotus* and *Vicia* (Pryzybylska and Zimniak-Pryzybylska, 1997), *Phaseolus* beans (Gomes *et al*., 1984; Romero-Andreas and Bliss, 1985; Romero-Andreas *et al*., 1986; Gepts *et al*., 1986; Hussain *et al*., 1986; Gepts, 1990; Castineiras *et al*., 1991; Echeverrigaraay *et al*., 1993; Driedger *et al*., 1994); mungbean (Tomooka *et al*., 1990), rice (Hirano *et al*., 1991), species of *Sida* (Vieritiz, 1992), *Pennisetum purpureum* mutants (Cruz *et al*., 1993) and *Arachis* (Lanham *et al*., 1994).

The objective of this work was to verify the possibilities of identification of *Brachiaria* species accesses using SDS-PAGE.

Material and methods

Lots of six species of *Brachiaria* seed were donated by Embrapa - CNPGC (Empresa Brasileira de Pesquisa Agrepecuária - Centro Nacional de Gado de Corte) (Table 1).

All seed lots were stored in a cold storage room at 15ºC in the Seed Analysis Laboratory - Unoeste - Presidente Prudente, state of São Paulo.

Electrophoresis was carried out in the Plant Tissue Culture Laboratory - Unoeste and image capture was made using the Chemilmager (4000i-v 4.04) at Cytogenetics and Molecular Biology Laboratory - Unoeste.

Table 1. Ecotypes of *Brachiaria* donated by Embrapa-CNPGC. Numbers in the upper line are the codes of Cenargen (Centro National de Recursos Genéticos)-Embrapa; in the under line codes of Embrapa-CNPGC

Specie	Accession number				
B. brizantha	BRA001945	BRA003336	BRA002844	BRA003107	BRA003719
	B-23	B-67	B-112	B-127	B-158
B.decumbens	PI355744	BRA004472	BRA004499	BRA000191	BRA000116
	D-53	D-7	D-9	D-58	D-59
B. ruziziensis	BRA005541	BRA005649	BRA005584	BRA005631	BRA002291
	R-100	R-106	R-108	R-109	R-128
B.humidicola	BRA004952	BRA004979	BRA005011	BRA001937	BRA002208
	H-10	H-12	H-13	H-108	H-112
B.nigropedata	BRA001123	BRA005916	CIAT16911	CIAT16921	CIAT16923
	N-190	N-191	N-197	N-202	N-203
B. jubata	BRA05380	BRA005291	BRA005461	BRA005533	BRA005223
	J-30	J-4	J-8	J-13	J-17

A dehulled seed sample (200 mg) of each access or species was ground and introduced into a test tube with 2.0ml of extraction buffer composed of 0.625M Tris-HCl pH 6.8; 2% SDS, 5% 2-mercaptoethanol and 20% glycerol. The tubes were shaken and kept for 1 h at room temperature and then boiled for 3min, after which the solution was centrifuged at 9500xg for 10min. The pellet was processed again. The resulting supernatants were mixed and frozen in Eppendorf tubes. Protein was quantified according Bradford (1976).

Electrophoresis was carried out according to Laemli (1970) in a system composed of the running gel containing 10% acrylamide-bisacrylamide (30:0.8), pH 8.8, and a stacking gel with 2.5% acrylamide-bisacrylamide, pH 6.8, at 50V for 30 min and 20mA for 4h. Aliquots of 30 μg of protein were loaded per well. The running buffer was composed of Tris (25mM) :Glycine (38mM): SDS (0.7mM), pH 8.8. The gels were fixed with isopropanol:acetic acid:water (4:1:5) for 30min and stained in the same solution containing 2% Coomassie Blue R250 until the protein bands appeared. If the coloration was too dark, the gel was destained in 10% acetic acid.

The evaluation was made observing the presence/absence of bands of the same apparent molecular weight, and a binary matrix constructed. The grouping of each species was made by PC-ORD (2.0 MJM Software Design) using the nearest neighbour group linkage procedure.

Results

Figure 1 shows us that there is difference among accesses of *B. ruziziensis*. In most of the materials the presence of bands, indicated by the white arrows, allows to differentiate the materials, access R-100 (line 3/3D) presented just a band of ~77.5KDa, and the absence of others, R-106 (line 4/4D) exhibited a pattern of four bands 77.5, 60.2, 47.7 and 40.9KDa, but did not exhibit the 66KDa or the 32.8KDa; access R-108 (line 5-6/5-6D) presented six bands 77.5, 60.2, 58.8, 52.8, 40.9 and 32.6KDa, R-109 (line 7/7D) showed a four banding pattern 77.5, 66, 52.8 and 40.9KDa and R128 access bands of 77.5, 52.8, 40.9 and 34.2KDa. Access R100 exhibited the highest divergence (Figure 7) from the other accesses; and accesses R109 and R128 were closely related.

There are differences among accesses *of B. humidicola* as shown in Figure 2. H-10 showed bands with 73.1, 50.5, 42.5, 35.4, 19.6, 18.4, 13.9, 13.4KDa; H-12 bands averaging 53.4, 45.9, 33.1, 24.0, 22.0, 18.4, 13.9, 13.4KDa; H-13 with 46.8, 25.3, 18.8, 14.2KDa; H-108 showed the most complex pool of proteins (116.0, 88.0, 73.9, 57.8, 51.9, 46.8, 37.4, 42.5, 34.8, 25.0, 20.6, 15.4, 14.2, 13.5KDa) and H-112 the less complex pool of proteins (84.4, 24.4, 20.8KDa). Accesses H110 were more closely related with H112 and H113 than with the other two accesses (Figure 7).

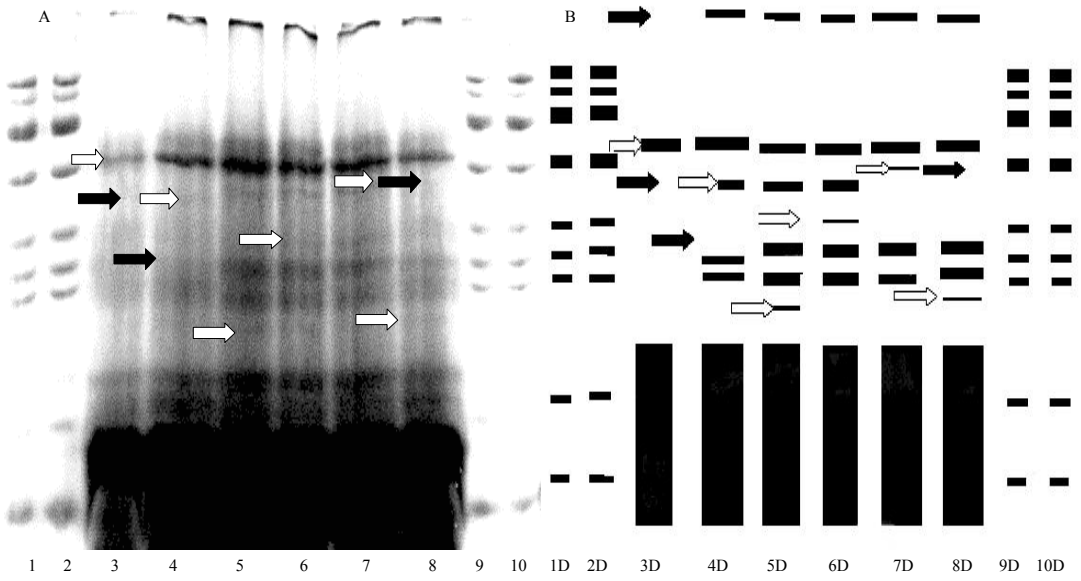

Figure 1. Protein electrophoresis of five accesses of *Brachiaria ruziziensis.* Line 1, 2, 9 and 10, Molecular Sigma Markers Wide range (205 up to 6,5 kDa) line 3 access R-100, line 4 access R-106, lines 5 and 6 access R-108, line 7 R-109 and line 8 access R-128. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

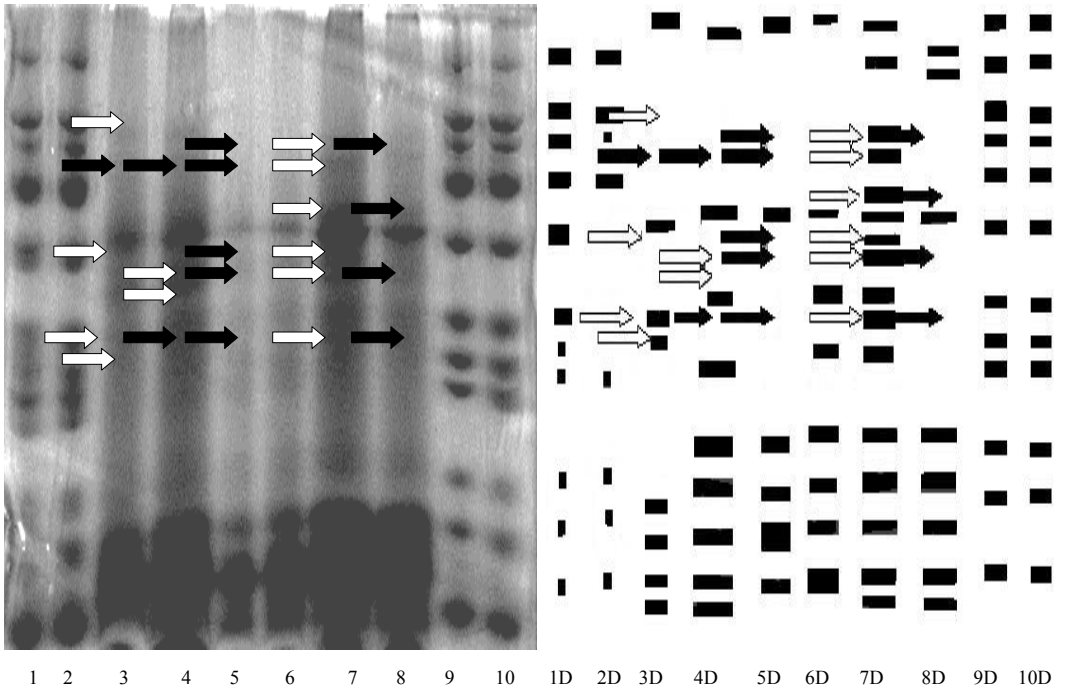

Figure 2. Protein electrophoresis of five accesses of *Brachiaria humidicola.* Line 1, 2, 9 and 10, Molecular Sigma Markers Wide range (205 up to 6,5 kDa) line 3 access H-10, line 4 access H-12, lines 5 and 6 access H-13, line 7 H-108 and line 8 access H-112. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

The Figure 3 presents differences among accesses of *B. brizantha*. The absence of bands, indicated by the black arrows, in the genotypes B-23 (58.4, 25.1, 21.5KDa) and B-112 (53.2, 48.8, 29.0, 21.8, 20.2KDa), exhibited protein diversity, differentiating these materials. The protein profiles in the accesses B-67 (100.6, 75.6, 64.8, 58.4, 53.2, 46.9, 41.4, 24.3, 21.8, 20.2, 18.7KDa), B-127 (60.4,

52.4, 48.8, 44.6, 36.0, 29.0, 24.3, 21.8, 20.2, 18.7KDa) and B-158 (50.2, 44.6, 36.0, 29.0, 24.3, 21.8, 20.2, 18.7KDa) differs for the presence of extra bands (white arrows). Species grouping (Figure 7) showed that at least four accesses were different, being the accesses B127 and B158 the most similar.

In the Figure 4 it is shown the differentiation among accesses of *B. decumbens*. In most of the materials the absence of bands, indicated by the black arrows, allows to differentiate each material from another, the accesses D-7, with band of 100.4, 95.4, 86.0, 70.1, 56.1, 44.4, 34.8, 32.5, 27.4KDa, and D-9 with bands of 100.4, 95.4, 86.0, 49.5, 46.2, 43.8, 39.0, 35.2, 32.5, 27.4KDa presented low divergence. The accesses D-58 (95.4, 84.0, 58.0, 48.2, 33.7, 27.4KDa) and D-59 (100.4, 95.4, 33.7, 27.4KDa), as the access D-53 (73.2, 52.6, 47.5, 33.7, 27.4KDa), differ for the presence / absence of bands. Accesses D58 and D59 are not differentiated by group analysis (Figure 7) and access D53 was the most divergent.

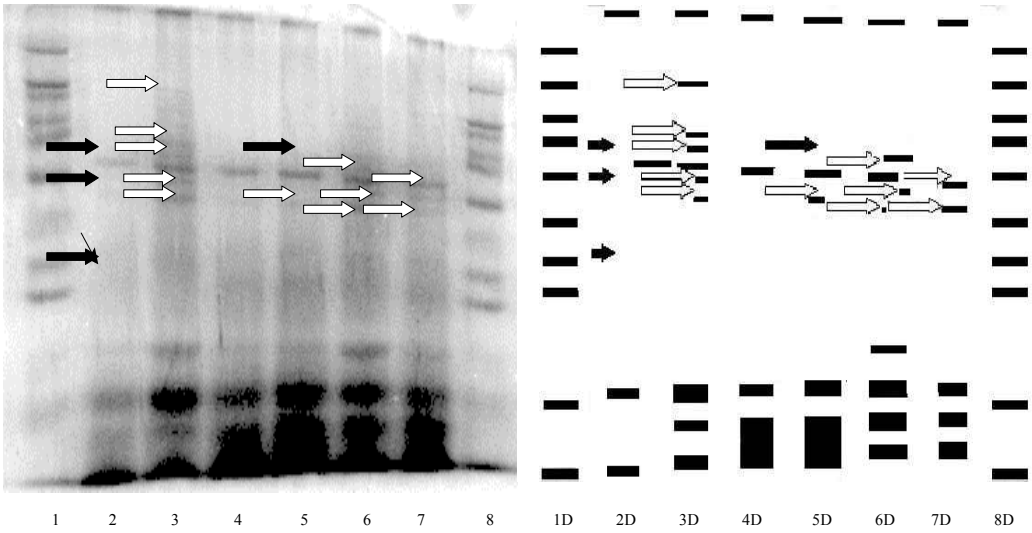

Figure 3. Protein electrophoresis of five accesses of *Brachiaria brizantha*. Line 1 and 8, Molecular Sigma Markers Wide range (205 up to 6,5 kDa) line 2 access B-23, line 3 access B-67, lines 4 and 5 access B-112, line 6 B-127 and line 7 access B-158. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

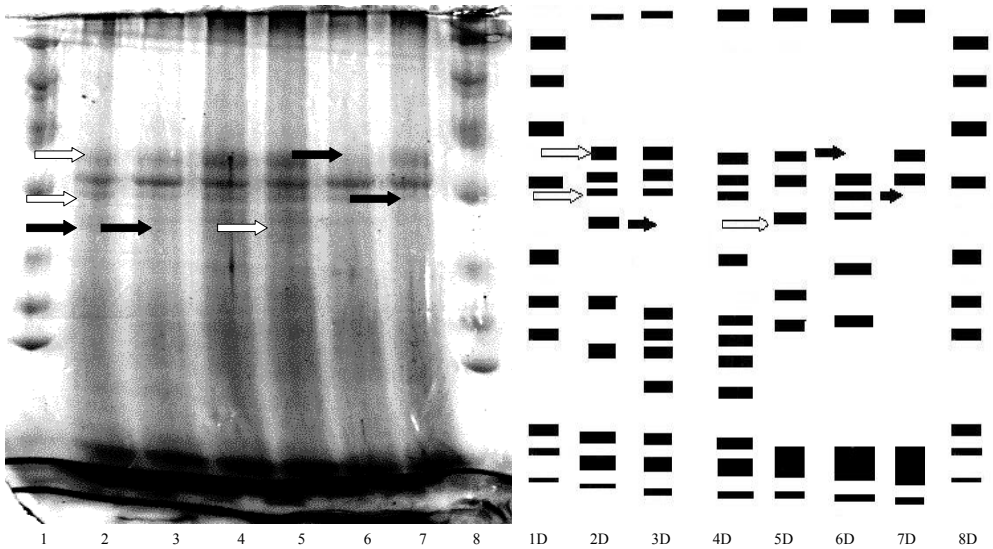

Figure 4. Protein electrophoresis of five accesses of *Brachiaria decumbens*. Line 1 and 8, Molecular Sigma Markers Wide range (205 up to 6,5 kDa) line 2 access D-7, line 3 access D-9, lines 4 and 5 access D-53, lines 6 access D-58, line 7 D-59. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

In Figure 5 it should be noted that there is differentiation among accesses of *B. jubata*. In most of the materials the absence of bands, indicated by the black arrows, did not allow us to differentiate the materials very well, but the access J-4 is the most different material of all, suggesting high genetic distance between this material and the others, which were very similar, as is shown in Figure 7.

The Figure 6 shows that there is differentiation among accesses of *B. nigropedata*. The absence of bands, indicated by the black arrows, allowed us to differentiate the materials, one from another, access N-190 showing the bands with 43,8, 40,7, 33,1KDa, N-191 (84,0, 52,6, 55,0, 40,4KDa) are the most divergent materials, suggesting a bigger genetic distance among these materials. The patterns of N-197 (66,0, 55,0, 33,1KDa), N-202 (82,4, 60,0, 55,0, 41,7, 33,1KDa) and N-203 (60,0, 55,0, 33,1KDa) presented, for this technique, a lot of similarity between them, exhibiting a very similar banding pattern. Access N191 (Figure 7) showed the highest divergence among the analysed samples and N203 and N202 that could not be differentiated were closely related with N197.

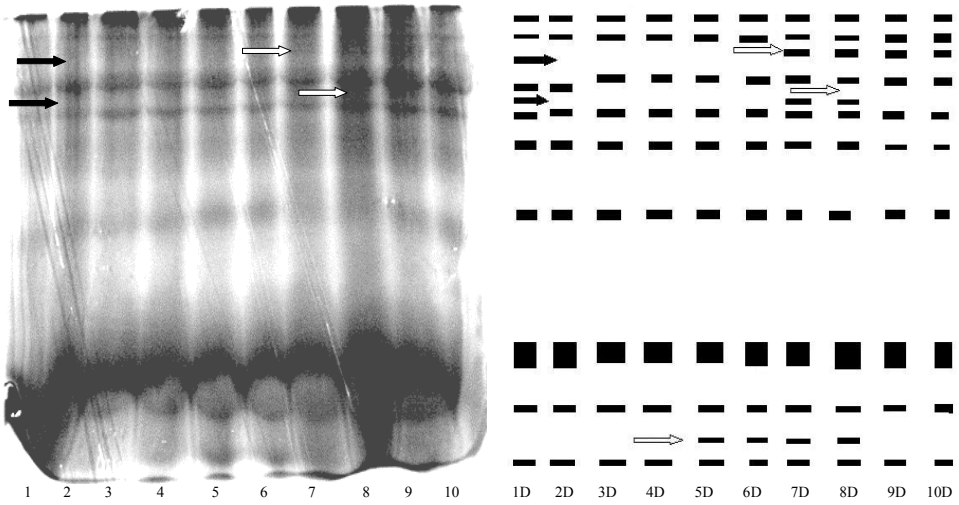

Figure 5. Protein electrophoresis (photography and diagram) of five accesses of *Brachiaria jubata.* Lines 1/1D and 2/2D access J-4, lines 3/3D and 4/4D access J-8, lines 5/5D and 6/6D access J-13, lines 7/7D and 8/8D access J-17, lines 9/9D and 10/10D access J-30. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

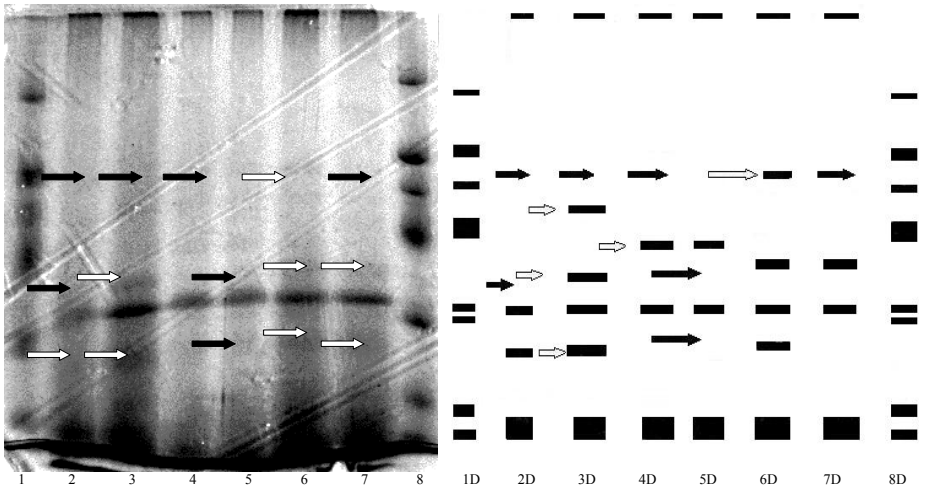

Figure 6. Protein electrophoresis (photography and diagram) of five accesses of *Brachiaria nigropedata.* Line 1/1D and 8/8D, Molecular Sigma Markers Wide range (205 up to 6,5 kDa) line 2/2D access N-190, line 3/3D access N-191, lines 4 /4D and 5/5D access N-197, line 6/6D access N-202, line 7/7D N-203. White arrows indicating differentiating bands for the materials, black arrows showing absence of bands

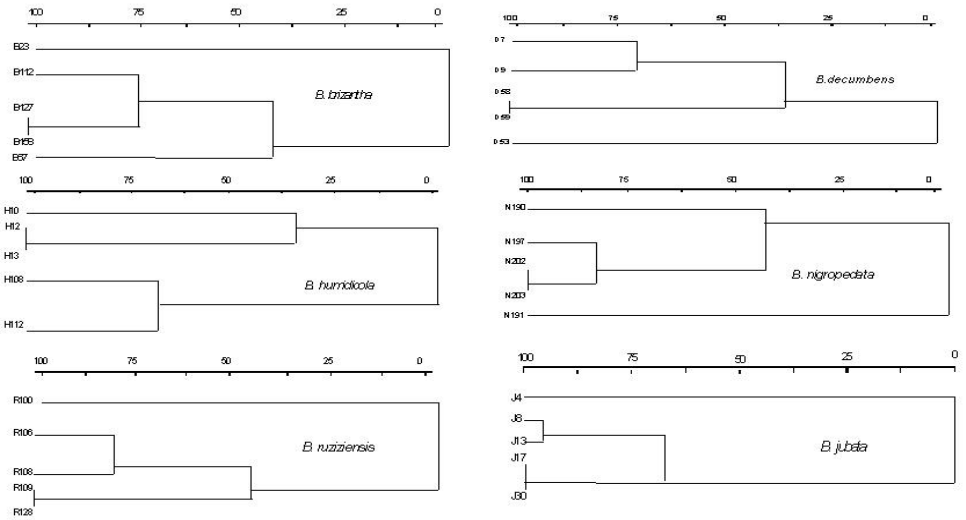

Figure 7. Grouping of six accesses of five species of *Brachiaria* according to their protein patterns

Discussion

A revision made by Cooke (1995) compared different electrophoresis types and different sources of proteins, used for identification and separation of accesses of the most diverse species. Such types of pattern analysis are made by international organisms as census of cultivar identification like for ISTA (International Seed Testing Association).

The differentiation of the materials is possible to be made, using the technique of SDS-PAGE, as well as variations can be identified in mungbean races (Tomooka *et al*., 1990), *Pennisetum purpureum* (Cruz *et al*., 1993), beans (Romero-Andreas and Bliss, 1985; Gepts *et al*., 1986; Osborn *et al*., 1986; Romero-Andreas *et al*., 1986; Gepts, 1990; Driedger *et al*., 1994); *Arachis* (Lanham *et al., 1*994), rice (Hirano *et al., 1*991) and *Sida* species (Vieritiz, 1992). Still, the refinement of the technique, as for example using different fractions of proteins, becomes necessary, permitting more frequent use of those techniques (Cooke, 1995). Accesses separation could allow the best exploitation of genetic variability. As seed banks are relatively expensive structures to be maintained, the knowledge of what is being conserved is necessary, knowing the physiological and agricultural characters of the stored seeds and the molecular patterns will permit a better identification of each access and the storage of a very good amount of genetic diversity as a core collection.

The accesses of *Brachiaria* presented variations in almost all the species. *Brachiaria jubata* showed little variation, meaning that received samples are genetically very homogeneous, in this case more expeditions for collecting material would be done to amplify the variation stored.

The other species present enough variations to separate wild accesses. Although of *B. brizantha* and *B. decumbens* are presented as apomitic species, there are some sources of variability, as the collection of new materials, or some escape of the apomixy, which would allow small variation rate. Valle and Savidan (1996) reported that although *Brachiaria* are considered apomitic, some degree of sexuality is present and varies in the species studied as the following proportion *B. jubata* (0-47%*); B. nigropedata* (5-11%); *B. decumbens* (3-56%); *B. brizantha* (0-74%) and *B. humidicola* (3-66%), which should explain part of the variability found in these accesses. It could be inferred observing the patterns of *Brachiaria ruzizienzis*, the most non apomitic species in this work. So, the higher is the apomixy escape probability, the higher is the recombination rate between genes and elevated the changes in alteration of protein pattern profiles. If it is admitted that collected material was submitted to evolutionary forces, several mutations and some recombination could be inferred, so the variation was just selected and maintained in germplasm banks, and those variations could be easily assessed by SDS-PAGE.

Conclusion

The protein variation between accesses of the same species, shown by the SDS-PAGE, could easily

differentiate the access examined and also estimate the genetic variation of the germplasm used.

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Received on January 29, 2002.
Accepted on April 30, 2002.