Cytotaxonomy of some species and of interspecific hybrids of *Pennisetum* (Poaceae, Poales)

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Abstract

Cytotaxonomic studies were carried out on 26 accessions of *Pennisetum* spp. originating from the Active Germplasm Bank of Embrapa Gado de Leite-Juiz de Fora, Minas Gerais, Brazil. This study presents cytogenetics and reproductive morphological data for each one of these accessions, which allowed groupings and suggest botanical identifications to be established. The metaphases of the accessions characterized as *P. purpureum* confirmed the presence of 2n = 28 chromosomes that have already been described for this species. For the hybrids between *P. purpureum* and *P. glaucum* and for the hexaploids, resulting from the chromosome duplication of these hybrids, 2n = 21 and 2n = 42 chromosomes were confirmed, respectively. The wild accession identified as *P. setosum* showed metaphases with 2n = 54 chromosomes, while those characterized as *P. nervosum* had cells with 2n = 36 chromosomes. The wild accession 15 was different from the others for the morphological characters, with 2n = 36 chromosomes, and was classified as *P. orientale*. Two statistical procedures were used (canonical variables and cluster analysis on the basis of the Mahalanobis distance), and the results confirmed the conclusions obtained from the cytogenetic and morphologic analysis.

Key words: Pennisetum, cytogenetic, taxonomy, germplasm, genetic divergence.

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Introduction

The genus *Pennisetum* Rich. contains about 140 species, comprising such important cultivated species as napiergrass, pearl millet and kikuyu grass (Brunken, 1977; Kativu and Mithen, 1987). The species belonging to this genus constitute a heterogeneous assemblage with different basic chromosome numbers (x = 5, 7, 8 and 9), ploidy levels varying from diploid to octoploid, sexual or apomictic reproductive behavior and annual, biennial or perennial life cycle (Martel *et al.*, 1997).

Napiergrass (*Pennisetum purpureum* Schumach.), due to its high productive potential, carrying capacity and nutritient quality, has been highlighted as one of the most important tropical forages for dairy grazing system improvement in the tropics. (Pereira, 1994). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a species with a dual purpose; grains are used for human consumption and for cattle forage (Minocha, 1991).

Napiergrass and pearl millet germplasm offers great diversity in types of plants as well as wide genetic variability for the great majority of the characters of forage importance. Also, among the wild species of *Pennisetum,* there is great variation for these characteristics, with some of them having potential for napier grass breeding. However, the use of these species depends on the genetic compatibility with napiergrass.

Because of the great diversity of types and forms of *Pennisetum* and the possibility of substitution of the cultivar's common names in the introduction processes, the characterization and taxonomic classification has become confusing, imprecise and even controversial at times. Some studies have used eletrophoretic separation through isozyme patterns, cytogenetics analysis and flow cytometry to characterize cultivars of numerous species, including those belonging to the *Pennisetum* genus (Manara, 1973; Hanna, 1981; Dujardin and Hanna, 1983 and 1985; Hanna and Dujardin, 1986; Daher, 1993; Passos and Vidigal, 1994; Passos *et al.*, 1994). However, little or no relationship has been shown between the results of these studies and taxonomy.

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The objective of this study was to characterize cyto-genetically and morphologically some accessions of *Pennisetum* belonging to the Active Germplasm Bank at Embrapa Gado de Leite.

Material and Methods

Twenty-six *Pennisetum* spp. accessions belonging to the Active Germplasm Bank at Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil were evaluated including six *P. purpureum* accessions, five *P. purpureum* and *P. glaucum* interspecific hybrids and fifteen *Pennisetum* spp. accessions. The *Pennisetum* spp. accessions were collected in several different areas of Brazil and have not yet received any identification type or post-collection characterization. The accessions were identified as BAG (Active Germplasm Bank). The root tip cells were used for cytogenetic analysis. The material was submitted to cell cycle synchronization (Lee *et al.*, 1997) using 2.5 mM hydroxyurea solution for 14 h. The roots were pretreated with 25 mg/L cyclohe-ximide and 300 mg/L hydroxyquinoline (1:1) solution for 2h45', fixed in Carnoy solution for 24 h, and submitted to enzymatic maceration in pectinase. They were stained with Schiff reagent and the slides prepared by the smear technique. Twenty-one of the 26 accessions were morphologically evaluated considering the following characteristics: panicle length and width, spikelet number and size, number of bristles, length of the longest bristle, length and number of ribs of the 1st, 2nd and 3rd glume, length and number of ribs of the palea, length of the lemma and length of the anthers. The accessions BAG 27, BAG 54, F92-167-2 and wild accessions 5 and 7 were not evaluated morphologically because they had no inflorescences during the collection and analysis periods. For the other materials, voucher specimens were prepared and kept in the herbarium of the Department of Biology (Universidade Federal de Lavras-UFLA, Lavras, Minas Gerais, Brazil). The experimental units, in which the evaluations were made, corresponded to the inflorescences of a same plant, the representative of the accession in the Germplasm Bank. Thus, a completely randomized design with 3 replications was considered, allowing the decomposition of the sums of squares and of products between and within accessions. Two statistical procedures were used (canonical variables and cluster analysis on the basis of Mahalanobis distance) to study the genetic divergence among the accessions and to establish relationships among the results obtained from the application of these two methods and those derived from the association between the cytogenetic and morphologic characters.

Results and Discussion

Groupings could be established among the accessions from the results of the cytogenetic and morphological analysis based on the degree of similarity. Each group was
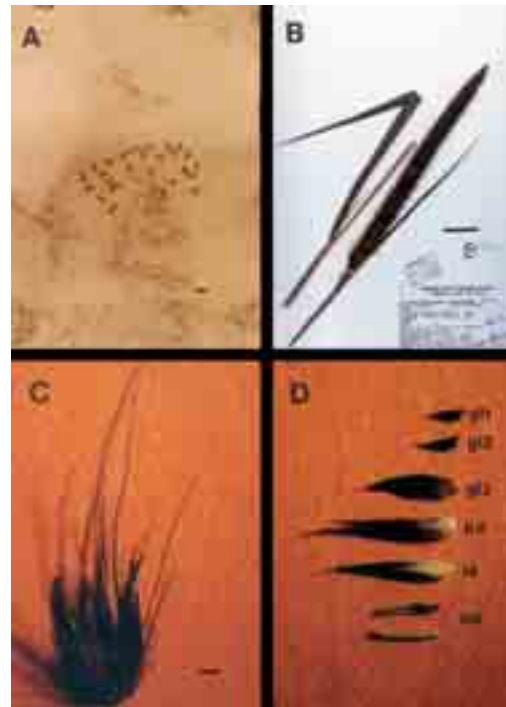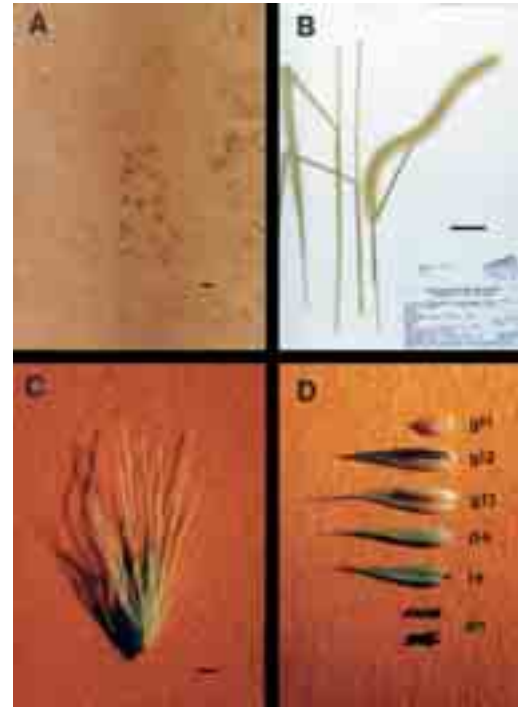

Figure 1 - BAG 65 (*P. purpureum*). A. Mitotic metaphase with 2n = 28 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl3 (3rd glume), gl2 (2nd glume), gl1 (1st glume). The bars represent: A. 5 μm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.
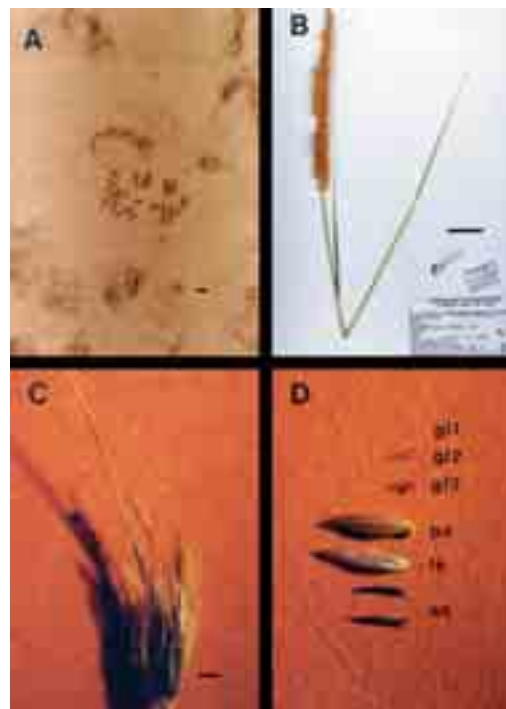

Figure 2 - BAG F92-176-1 (Interspecific hybrids between *P. purpureum* and *P. glaucum*). A. Mitotic metaphase with 2n = 21 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl3 (3rd glume), gl2 (2nd glume), gl1 (1st glume). The bars represent: A. 5 μm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.
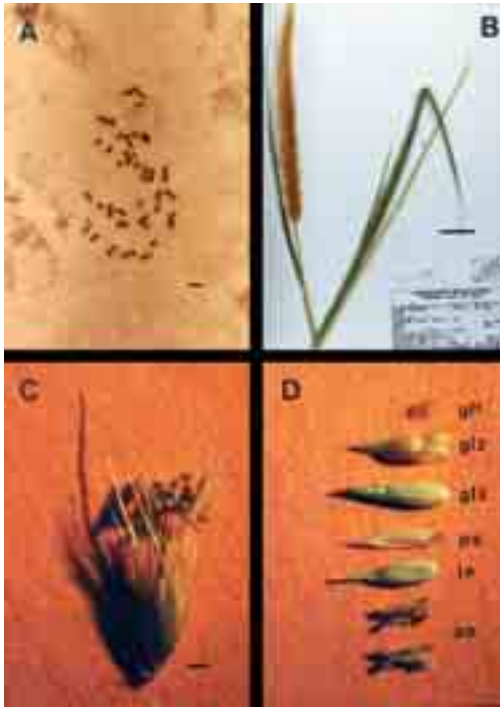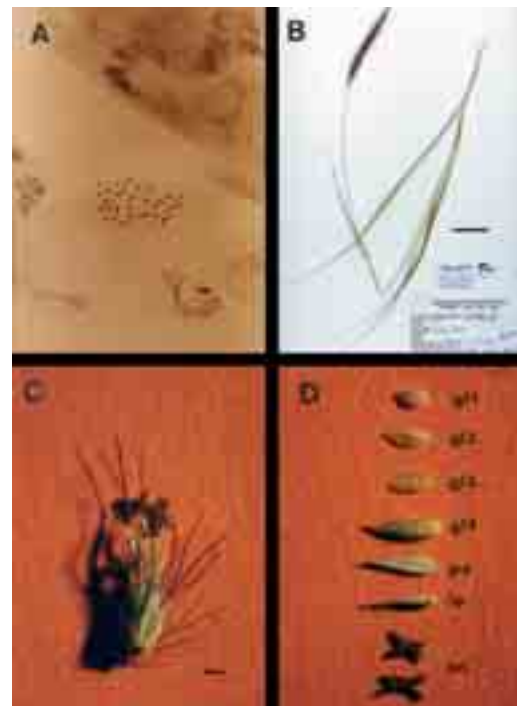
Figure 3 - BAG Hexaploid 204. A. Mitotic metaphase with 2n = 42 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl3 (3rd glume), gl2 (2nd glume), gl1 (1st glume). The bars represent: A. 5 µm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.

Figure 5 - Wild BAG 12 (*P. nervosum*). A. Mitotic metaphase with 2n = 36 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl3 (3rd glume), gl2 (2nd glume), gl1 (1st glume). The arrows indicate satellites and the bars represent: A. 5 µm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.
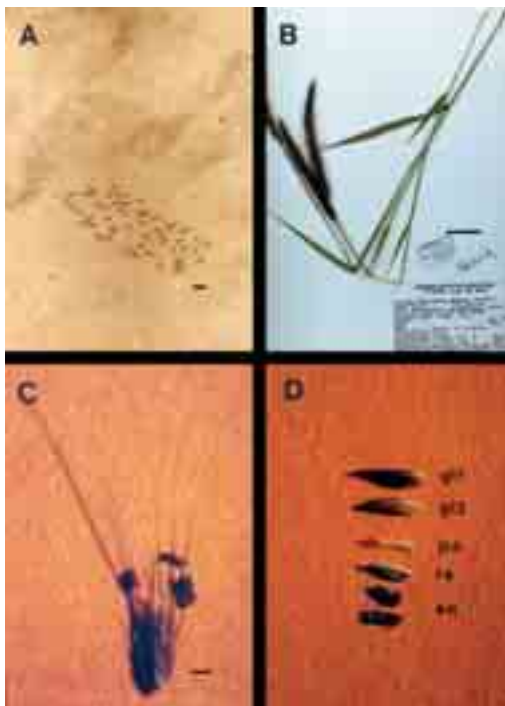
Figure 4 - Wild BAG 3 (*P. setosum*). A. Mitotic metaphase with 2n = 54 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl2 (2nd glume), gl1 (1st glume). The bars represent: A. 5 µm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.

Figure 6 - Wild BAG 15 (*P. af. orientale*). A. Mitotic metaphase with 2n=36 chromosomes; B. Specimens; C. Spikelet; D. Constituent of the spikelet: an (anthers), le (lemma), pa (palea), gl4 (4th glume), gl3 (3rd glume), gl2 (2nd glume), gl1 (1st glume) The bars represent: A. 5 µm; B. 5.6 cm; C and D. 2.3 mm. UFLA. Lavras, MG, Brazil, 1998.

Table I - Chromosome number and identification of the accessions of *Pennisetum* spp. of the Active Germplasm Bank of Embrapa Gado de Leite. UFLA. Lavras, Minas Gerais, Brazil, 1998.

Accessions	Chromosome number	Former identification	Actual identification
BAG 27	28	*P. purpureum*	*P. purpureum*
BAG 45	28	Interspecific hybrid	*P. purpureum*
BAG 46	28	Interspecific hybrid	*P. purpureum*
BAG 54	28	*P. purpureum*	*P. purpureum*
BAG 65	28	*P. purpureum*	*P. purpureum*
BAG 103	28	*P. purpureum*	*P. purpureum*
F92-167-2	21	Interspecific hybrid	Interspecific hybrid
F92-167-1	21	Interspecific hybrid	Interspecific hybrid
F92-176-1	21	Interspecific hybrid	Interspecific hybrid
Hexaploid 199	42	Interspecific hybrid	Interspecific hybrid
Hexaploid 204	42	Interspecific hybrid	Interspecific hybrid
Wild BAG 3	54	*P. pedicellatum*	*P. setosum*
Wild BAG 4	54	*P. polystachyon*	*P. setosum*
Wild BAG 5	54	*P. polystachyon*	*P. setosum*
Wild BAG 6	54	*P. polystachyon*	*P. setosum*
Wild BAG 1	36	*P. nervosum*	*P. nervosum*
Wild BAG 7	36	*P. nervosum*	*P. nervosum*
Wild BAG 8	36	*P. nervosum*	*P. nervosum*
Wild BAG 9	36	*P. pedicellatum*	*P. nervosum*
Wild BAG 10	36	*P. polystachyon*	*P. nervosum*
Wild BAG 12	36	not identified	*P. nervosum*
Wild BAG 13	36	*P. nervosum*	*P. nervosum*
Wild BAG 14	36	*P. latifolium*	*P. nervosum*
Wild BAG 16	36	*P. nervosum*	*P. nervosum*
Wild BAG 17	36	*P. nervosum*	*P. nervosum*
Wild BAG 15	36	*P. nervosum*	*P.* af. *orientale*

represented by a photograph of an accession (Figures 1 to 6). Botanical identifications could also be suggested and compared to those already existing in the Active Germplasm Bank (Table I).

Group 01: BAGs 27, 45, 46, 54, 65 and 103

The cytogenetic analysis performed for these materials revealed a chromosome number of 2n = 28 (Figure 1) that is in line with the literature, that corresponded to the chromosome number of *P. purpureum*, which was determined for the first time by Burton (1942) and confirmed by Manara (1973), Brunken (1977), Jauhar (1981) and Dujardin and Hanna (1985).

The presence of satellites was observed in two chromosomes in the metaphases of BAG 27 and BAG 54. Manara (1973) had previously recorded the presence of these structures in *P. purpureum*, although in only one of the homologs of the chromosome pair, with variation in number from 1 to 2.

Morphologically, accessions 45, 46, 65 and 103 all agreed with the botanical description of *P. purpureum* (Hitchcock, 1935; Bogdan, 1977; Brunken, 1977 and Kativu and Mithen, 1987), especially in relation to panicle and spikelet color and length and number, length and color of the bristles.

Group 02: BAGs F92-176-1, F92-167-1 and F92-167-2

The number 2n = 21 chromosomes (Figure 2) confirmed the hybrid origin of these accessions, resulting from different crossings between *P. purpureum* and *P. glaucum*. In the analyzed metaphases it was possible to ascertain the presence of 7 larger chromosomes originating from *P. glaucum* and the other from *P. purpureum*. This observation agrees with the results obtained by Burton (1942), who studied the cytological behavior of the hybrids between these two species.

There is little bibliographical information available concerning measurements of the structures that compose the inflorescence for hybrids between *P. purpureum* and *P. glaucum*. Bogdan (1977) reported the superiority of some hybrids over napiergrass after comparing characters such as leaf dimension and number, developed culms, softer leaf hairs and less fibrous stems, and yield.

The simultaneous examination of the characteristics mentioned above for accessions 45, 46, 65 and 103, defined as *P. purpureum*, and for hybrids F92-167-1 and F92-176-1, although restricted to inflorescence data, showed similarity among them. Although no analogy has been observed for *P. glaucum*, it was possible to observe many structures characteristic of *P. purpureum* , such as panicle and spikelet shape, size and color appearing in the hybrids. This information agrees with the report of Gonzales and Hanna (1984) justifying the greatest genetic contribution and dominance of the B genome of *P*. *purpureum* over the A genome of *P*. *glaucum.*

Group 03: BAGs hexaploids 199 and 204

Group 03 accessions constituted chromosome races originating from the University of Florida and obtained by the chromosome duplication of hybrids between *P. purpureum* and *P. glaucum* (Pereira, 1994). Metaphases with 2n = 42 chromosomes were confirmed for these materials (Figure 3).

As was the case with hybrids, botanical data were not found in the literature for measurements of the reproductive structures for the hexaploids. The morphologic characteristics did not differ significantly from those of the *P. purpureum* varieties.

Group 04: Wild accessions 3, 4, 5 and 6

The chromosome number (2n = 54) enabled these four wild accessions to be grouped (Figure 4). The inflorescence morphological characteristics of these accessions were also equivalent (Figure 4).

The phenotypic data were matched to those reported for the *P. setosum* species. (Swartz.) L. Rich. (synonymy *P. polystachyon* Schult.) for Hrishi (1952), Burger (1980), Kativu and Mithen (1987). This was consistent with the key to identification proposed by Hafliger and Scholz (1980), with special reference to the length, width and color of the panicle, spikelet density per cm, presence of solitary spikelets in the involucre, number and size of the bristles and structures of the rachis.

Results described by Hrishi (1952) and Rangasamy (1972) indicating 2n = 6x = 54 chromosomes for *P. setosum*, confirm the classification of the accessions on the basis of the chromosome number information. Other au-

thors mentioned by Schmelzer (1997) also report 2n = 18, 36, 45, 48, 52 and 63 chromosomes for this species.

Group 05: Wild accessions 1, 7, 8, 9, 10, 12, 13, 14, 16 and 17

An important phenotypic characteristic that justified the grouping of these accessions was the absence of longer bristles in the involucre that is typical of the other materials evaluated. The other structures and characteristics of the panicle and spikelet also resembled each other, except the color. In accessions 10, 12 and 14 they were green and in the others they were mainly purple (Figure 5). This information agrees partially with data proposed by Hitchcock (1935), Gould (1975) and Renvoize (1984) for *P. nervosum* (Ness.) Trin. According to Hitchcock (1935), *P. nervosum* was already found in Brazil, Ecuador and Argentina.

The chromosome number reported by Gould (1975) for *P. nervosum* is 2n = 36, also agreeing with the results obtained in this study for the accessions above. The analysis of the metaphases of these accessions indicated the presence of satellites in 2 chromosomes. The fact that satellites were not observed in all the accessions can be attributed either to the stage of chromosome condensation in the metaphases or to the proximity and overlap of the metaphases, or possibly to the detachment and loss of the satellite. This problem was encountered by Guerra *et al.* (1997) when analyzing metaphases in *Citrus* accessions. According to these authors, the observation of both primary and secondary constrictions was impaired by the largest chromossome condensation caused by the pretreatment.

Group 06: Wild accession 15

Different from the other accessions, especially for the morphological characters, wild accession 15 was considered morphologically similar to the species *P. orientale* Rich. Its botanical description matched the data of Hrishi (1952) for this species, mainly in relation to the length of the panicle, spikelet density per cm, spikelet number in the involucre and glume number (Figure 6). There is also agreement with the chromosome number observed (2n = 36) and the number reported by this author for the species. However, citations exist describing 2n = 18, 27, 45 and 54 chromosomes (Dujardin and Hanna, 1983).

For the species *P. orientale*, which originated in Africa, neither records of natural occurrence nor of its introduction exist in Brazil. In the Germplasm Bank this accession was introduced as *P. nervosum*, making up a group containing accessions 1, 7, 8, 13, 16 and 17. The botanical description for the species *P. nervosum* (Gould, 1975; Renvoize, 1984), however, does not resemble the morphological characters recorded in this work. Also there is not similarity among the phenotypic data of the accessions described above with those described for wild access 15, which accounted for its classification as *P. nervosum.*
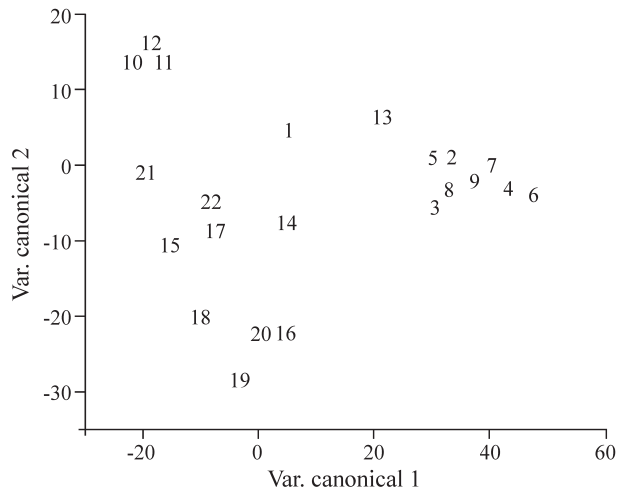
Figure 7 - Graphic dispersion of the scores of the two more important canonical variables for the 21 accessions of *Pennisetum* spp. of the Active Germplasm Bank of Embrapa Gado de Leite. The numeration corresponds to the accessions: 1. wild 15; 2. wild 12; 3. wild 10; 4. wild 14; 5. wild 9; 6. wild 16; 7. wild 13; 8. wild 17; 9. wild 1; 10. wild 6; 11. wild 4; 12. wild 3; 13. wild 8; 14. BAG 45; 15. Hexaploid 204; 16. Hexaploid 199; 17. F92-167-1; 18. F92-176-1; 19 BAG 65; 20. BAG 46; 21. BAG 103. UFLA. Lavras, MG, Brazil, 1998.
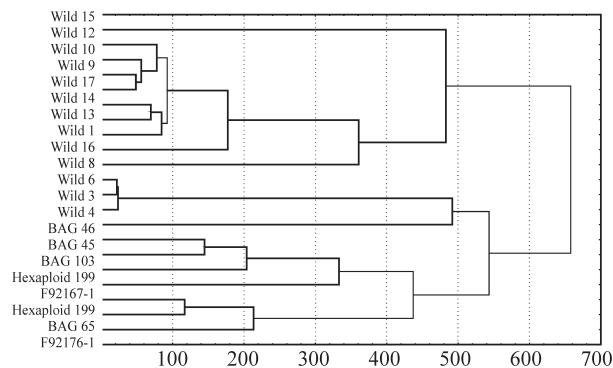
Figure 8 - Dendrogram obtained by the closest neighboring method, from the Mahalanobis distance, for 21 accessions of *Pennisetum* spp. of the Germplasm Bank of Embrapa Gado de Leite. UFLA. Lavras, MG, Brazil, 1998.

Analysis of genetic divergence

The results obtained by the application of the two multivariate methodologies through the comparative visualization of the dendrogram corresponding to the cluster analysis and of the graphic dispersion (Figures 7 and 8) showed a good agreement. One exception was F92-167-1, which in the former method was grouped with other accessions, while in the graphic analysis of canonical variables remained as an isolated group. However, it should be noted that its dissimilarity values in relation to the accessions BAG 45, BAG 103 and hexaploid 199, were close to the cut-off value used. It is notable that there is agreement of the analysis when using canonical variables and cluster analysis.

The use of statistical methods corroborated some of the conclusions obtained on the basis of cytogenetic and morphological analysis. Prominence may be given to the groupings among wild accessions 3, 4 and 6 and among wild accessions 1, 9, 10, 12, 13, 14, 16 and 17 that, except for wild accession 8, were in accordance for all analysis (cytogenetic and morphologic, canonical variables and of cluster on the basis of Mahalanobis distance).

Some differences were expected in the formation of the groups involving the hybrids, the hexaploids and the accessions of *P. purpureum*. In the latter case, the differences observed can be explained by the existence of a number of varieties of *P. purpureum* carrying some nearly undistinguishable reproductive morphological characteristics. Depending on the approach adopted, those differences may be important parameters. Considering the access BAG 46, characterized as *P. purpureum*, it may be observed that the glumes that compose the spikelet differ, in number, when compared to those of the other accessions of *P. purpureum*: BAG 45, BAG 65 and BAG 103. Because of the greater importance of morphological information with this method (absence of 2 glumes), the cluster analysis and canonical variables considered it to be an isolated item and different from the others.

In the cluster analysis (Figure 8), if the cut-off point for differentiation among the groups is established in about 35% of the largest distance, the formation of 10 groups is observed. Some accessions, separated in the cluster analysis, resulted in membership of the same group according to the approaches to the cytogenetic and morphologic analysis. For example, in the cluster analysis, accessions BAG 45 and BAG 103 formed a group distinct from the access BAG 46. These three materials, however, were analyzed according to the cytogenetic and morphological analysis. In such cases the contradiction among the approaches are not representative, but reflect the fact that multivariate analysis was more conservative in the formation of groups. The only significant contradiction was the occurrence of hexaploid 204 and access BAG 65 in a same group (Figure 8), which were separated according to the cytogenetic and morphological approaches. Although they resemble each other morphologically, it is more reasonable to maintain them in different groups since they contain different ploidy levels. Especially in the case of the wild materials, the agreement was pointed out among the methodologies, suggesting the viability of its associated use in future studies.

Acknowledgments

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