KARYOTIPIC ASYMMETRY OF BOTH WILD AND CULTIVATED SPECIES OF *PENNISETUM* ([1])

VÂNIA HELENA TECHIO ([2*]); LISETE CHAMMA DAVIDE ([2]); ALEXANDRO CAGLIARI ([3]); SANDRO BARBOSA ([4]); ANTÔNIO VANDER PEREIRA ([5])

ABSTRACT

This study aimed the establishment of the relation between karyotipic asymmetry values obtained for different accessions of both wild and cultivated species of *Pennisetum* from Germplasm Bank of Embrapa Gado de Leite/Juiz de Fora-Minas Gerais State, Brazil. Conventional cell cycle synchronization protocols and Feulgen staining method were used to obtain metaphases plates. The wild-type accessions corresponded to the species *P. setosum* (2n=6x=54), *P. nervosum* (2n=4x=36), and *P. orientale* (2n=4x=36), and the cultivated to *P. purpureum* (2n=4x=28) and *P. glaucum* (2n=2x=14). No significant difference was found for the total length of chromosomes ($p \geq 0.05$) among the species. The analysis of intra-chromosomal asymmetry (A1) and inter-chromosomal asymmetry (A2) has shown that *P. setosum* has a tendency to chromosome asymmetry. *P. nervosum, P. orientale, and P. purpureum* have presented an intermediary level of asymmetry and *P. glaucum*, low asymmetry. Considering Stebbins criteria, the karyotype of *P. glaucum* and those from the three wild species fitted into the category 1A-symmetrical. With regard to *P. purpureum*, karyotypes of the accessions BAGs 54, 65 and 91 fitted into the category 2B and the other two genotypes (BAGs 63 and 75) fitted into the 1A. Comparison between the karyotype classification according to the inter- and intra-chromosomal asymmetry and Stebbins methodologies revealed that this last one alone was not able to detect small variations between karyotypes of the taxa closely related.

Key words: *Pennisetum purpureum, Pennisetum glaucum,* germplasm, karyotype.

RESUMO

ASSIMETRIA CARIOTÍPICA DE ESPÉCIES SILVESTRES E CULTIVADAS DE *PENNISETUM*

O objetivo deste estudo foi estabelecer a relação entre os valores de assimetria cariotípica obtidos para diferentes acessos de espécies silvestres e cultivadas de *Pennisetum* do Banco de Germoplasma da Embrapa Gado de Leite em Juiz de Fora (MG). Para obtenção das metáfases, utilizaram-se os protocolos convencionais de sincronização do ciclo celular e coloração pelo método de Feulgen. Os acessos silvestres correspondem às espécies *P. setosum* (2n=6x=54), *P. nervosum* (2n=4x=36) e *P. orientale* (2n=4x=36) e os cultivados a *P. purpureum* (2n=4x=28) e *P. glaucum* (2n=2x=14). Para o comprimento total dos cromossomos não foram observadas diferenças significativas ($p \geq 0,05$) entre as espécies. Pela análise da assimetria intra (A1) e intercromossômica (A2) observa-se que *P. setosum* tem maior tendência à assimetria. *P. nervosum, P. orientale* e *P. purpureum* tiveram grau intermediário de assimetria e *P. glaucum*, menor assimetria. Considerando os critérios de Stebbins, os cariótipos de *P. glaucum* e das três espécies silvestres analisadas enquadraram-se na categoria 1A-simétrico. Para *P. purpureum*, os cariótipos dos acessos BAGs 54, 65 e 91 enquadraram-se na categoria 2B e os outros dois genótipos (BAGs 63 e 75), na 1A. A comparação entre as classificações dos cariótipos obtidas conforme as metodologias de assimetria inter e intracromossômica e a de Stebbins revelou que esta última não permitiu detectar pequenas variações entre os cariótipos de táxons proximamente relacionados.

Palavras-chave: *Pennisetum purpureum, Pennisetum glaucum,* germoplasma, cariótipo.

([1]) Recebido para publicação em 24 de setembro de 2009 e aceito em 9 de dezembro de 2009.

([2]) Departamento de Biologia, Universidade Federal de Lavras, 37200-000 Lavras (MG). E-mail: vaniatechio@yahoo.com.br; vhtechio@dbi.ufla.br (*) Autora correspondente.

([3]) Departamento de Genética, Universidade Federal do Rio Grande do Sul, 91501-970 Porto Alegre (RS).

([4]) Departamento de Ciências Biológicas e da Terra, Universidade Federal de Alfenas, 37130-000 Alfenas (MG).

([5]) Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Leite, 36038-330 Juiz de Fora (MG).

1. INTRODUCTION

The development of actions that promote a better conservation and use of genetic resources has become a necessity to equilibrate the rational management of germplasm and to make available the material for breeding programs and for biotechnology without causing problems of genetic erosion, vulnerability and losses of old varieties. In such situation, one of the main studied points has been the assessment of genetic diversity and its structure within a certain gene pool. Such question can be better understood through studies of domestication processes , the gene flow between different species and different gene pools and, more particularly, between the wild-type and cultivated forms. These studies may involve the analysis of parameters associated with recombination, forms of reproduction, adaptation and even the cytogenetic traits (KHALFALLAH et al., 1993).

The analysis of numeric or structural variation of karyotype in populations of one species – cytotypes or chromosomal race – or between species may be useful for determining the differences between taxons and infer patterns of divergence between the populations. Such information is essential for clarifying the possible role of chromosomal rearrangements in the evolution of organisms and in the speciation.

Regarding the genus *Pennisetum,* the species are classified into five sections – *Pennisetum, Heterostachya, Brevivalvula, Gymnothrix,* and *Penicillaria* – (STAPF and HUBBARD, 1934; BRUNKEN, 1977) gathering the cultivated and wild ones with population from exotic material introduced, adapted to different edaphic conditions, some apomictic with chromosomal basic number of 5, 7, 8, and 9 (SCHMELZER, 1997; JAUHAR and HANNA, 1998). The section *Pennisetum* gathers the cultivated species from the primary and secondary gene pool, millet (*P. glaucum,* 2n=2x=14) and elephant grass (*P. purpureum,* 2n=4x=28), respectively. The chromosomal races or cytotypes *P. orientale* (2n=18, 27, 36, 45, 54) and *P. pedicelatum* (2n=36, 45, and 54) are found among the perennial species of the tertiary gene pool (JAUHAR and HANNA, 1998).

Despite the existence of mechanisms that contribute for the maintenance of this genetic structure, such as linkage and gametophytic competition (ROBERT et al., 1991), some species are not sexually compatible. This possibility of interspecific combination is important for breeding as it allows the transference of alleles into species of agronomical importance. Previous authors have described the formation of the inter-specific hybrids between the millet and other species of *Pennisetum* like with the elephant grass (*P. purpureum*) (BURTON, 1944); with *P. orientale* (PATIL and SINGH, 1964; DUJARDIN and HANNA, 1983b), with *P. setaceum* (HANNA, 1979), *P. dubium* (GILDENHUYS and BRIX, 1958), and with *P. squamulatum* (PATIL et al., 1961; KAUSHAL et al., 2007). DUJARDIN and HANNA (1984) also described the formation of tri-specific hybrids originated from the breeding between the millet (*P. glaucum*), the elephant grass (*P. purpureum*) and *P. squamulatum.*

The triploid and hexaploid hybrids between the millet and the elephant grass have been considered as important sources of variation for the selection of superior clones. Characters of forage importance superior to those found in the parents such as the presence of larger leaves in greater number, more developed stem, smoother leaf hairs and less fibrous stems and greater level of dry matter are observed in triploid hybrids (BOGDAN, 1977; PEREIRA, 1998).

The efforts to assess the potential of allele transference between species of *Pennisetum* have been focused in the apomixis and in the perennial growth habit (DUJARDIN and HANNA, 1983a; DUJARDIN and HANNA, 1989; JAUHAR and HANNA, 1998). However, there are other desired attributes that may be introduced in the genome of cultivated species such as precocity in vegetative development, the increase of efficiency in nutrient use, the fairer distribution of dry matter production during the year, and the increase of fertile seeds. In this case, knowledge of the cytogenetic traits of wild species of *Pennisetum* and their relation and similarity with cultivated species may enhance the better use of exotic germplasm in breeding. This study aimed the establishment of the cytological relation between different accessions of both wild and cultivated species of *Pennisetum* available in the Active Germplasm Bank of Embrapa Gado de Leite, Juiz de Fora, Minas Gerais State, Brazil.

2. MATERIAL AND METHODS

Fifteen wild accessions and ten cultivated accessions (Table 1) of *Pennisetum* from the Active Germplasm Bank of Embrapa Gado de Leite, Juiz de Fora, Minas Gerais State, Brazil were assessed. These plants were sampled in different physiographic areas of Brazil and identified as BAG (Active Germplasm Bank) followed by the number of its record.

The cytogenetic analyses were carried in meristematic cells obtained from root edges. The material was submitted to cell cycle synchronization using a 2.5 mM hydroxyurea solution for 14h. The roots were pretreated with cycleheximid solution (25 mg/L) and 8-hidroxyquinoline (300 mg/L) (1:1) for 2h45min, fixed in Carnoy solution for 24h, and

submitted to enzymatic maceration with pectinase (SIGMA^{TM}) diluted in citrate buffer, pH 4.6 (5:1) for 2h45min (Techio et al., 2002). The staining was carried through the Feulgen method and the slides prepared according to the smear technique. The material herborized is conserved at the Herbarium ESAL of the Biology Department of the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais State, Brazil.

The chromosomal measures were performed using the software Image Tool® (Wilcox et al., 2002). The chromosomal data of the accessions of *P. glaucum* and *P. purpureum* were obtained from Barbosa et al. (2003). The centromeric index (IC=[BC/(BC+BL)]x100) was calculated for all accessions and the chromosomes were classified according to the centromere position in metacentric – m (IC between 50 and 37.5) – and submetacentric – sm (IC between 37.5 and 25) – according to Levan et al. (1964). The criteria proposed by Stebbins (1971) and Zarco (1986) were used for the karyotypic symmetry.

The analysis of variance followed by the test of Scott Knott (1974), employing the level of significance $p>0.05$, were used for comparison of the variation magnitude regarding to the chromatin total length (CTC).

3. RESULTS AND DISCUSSION

The plants analysed corresponded to the species *P. setosum* (accessions 3, 4, 5, and 6), with 2n=6x=54; *P. nervosum* (accessions 1, 7, 8, 9, 10, 12, 13, 14, 16, and 17), with 2n=4x=36, and *P. orientale* (accession 15), with 2n=4x=36 chromosomes (Table 1 and Figure 1). Barbosa et al. (2003) have confirmed for *P. purpureum* (BAG 54, 63, 65, 75, and 91) and for *P. glaucum* (BAG M 24, 35, 36, 38, and 44), 2n=4x=28 and 2n=2x=14 chromosomes, respectively (Figure 1).

Table 1. *Pennisetum* acessions of Active Germplasm Bank of Embrapa Gado de Leite. Juiz de Fora, Minas Gerais State, Brazil.

Accession	Scientific name	Common name	Place
BAG Selvagem 3	*P. setosum*	-	Distrito Federal
BAG Selvagem 4	*P. setosum*	-	Mato Grosso
BAG Selvagem 5	*P. setosum*	-	Mato Grosso
BAG Selvagem 6	*P. setosum*	-	Mato Grosso
BAG Selvagem 1	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 7	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 8	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 9	*P. nervosum*	Bufão	Goiás
BAG Selvagem 10	*P. nervosum*	-	Goiás
BAG Selvagem 12	*P. nervosum*	-	Unavailable
BAG Selvagem 13	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 14	*P. nervosum*	-	Rio Grande do Sul
BAG Selvagem 16	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 17	*P. nervosum*	-	Mato Grosso do Sul
BAG Selvagem 15	*P. orientale*	-	Mato Grosso do Sul
BAG 54	*P. purpureum*	Capim cana d'África	Unavailable
BAG 63	*P. purpureum*	Cuba 169	Unavailable
BAG 65	*P. purpureum*	Roxo Botucatu	Unavailable
BAG 75	*P. purpureum*	IJ 7136	Unavailable
BAG 91	*P. purpureum*	-	Unavailable
M24	*P. glaucum*	ICMB 90111	Unavailable
M35	*P. glaucum*	-	Unavailable
M36	*P. glaucum*	AFPOP88	Unavailable
M38	*P. glaucum*	AFPOP 90	Unavailable
M44	*P. glaucum*	-	Unavailable

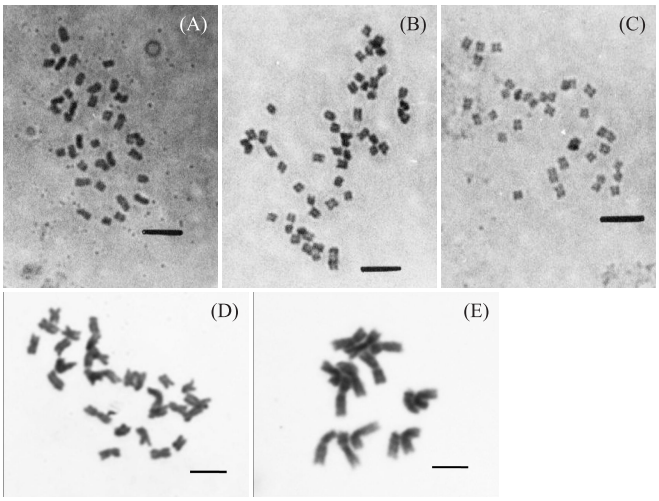

Figure 1. Mitotic metaphases A. *Pennisetum nervosum* (2n=4x=36); B. *Pennisetum setosum* (2n=6x=54); C. *Pennisetum orientale* (2n=4x=36); D. *Pennisetum purpureum* (2n=4x=28); E. *Pennisetum glaucum* (2n=2x=14). Bars correspond to 5 µm.

The analysis of variance followed by the test of Scott Knott confirmed the absence of significant difference (p≥0,05) for the total length of chromosomes between the wild accessions of *P. orientale* (29.41 µm±2.82), *P. nervosum* (38.72 µm±4.39) and *P. setosum* (49.43 µm±4.18) and the cultivated species *P. purpureum* (28.09 µm±1.69) and *P. glaucum* (28.36 µm±3.92).

Considering that in a nucleus the total mass of chromosomes is strictly correlated with the DNA content, Stebbins (1971) reported the possibility of inferences upon the DNA content of an organism through the chromosomal analysis. Based on this criterion, no substantial difference was found in the amount of DNA among those three wild species, whose basic chromosomal number is x=9, and cultivated species with x=7. Results obtained for the total chromosomal length of wild species coincided with the data of DNA quantification obtained through flow cytometry by Martel et al. (1997), who found that species of *Pennisetum* with x=9 showed similar amount of DNA per haploid genome, varying from 0.85 to 0.95pg, independent of the level of ploidy. The cultivated species of *P. glaucum* and *P. purpureum,* both with x=7, have 2.36 and 1.15 pg per haploid set respectively. In the triploid hybrid (*P. glaucum* x *P. purpureum*), Campos et al. (2009) observed that the amount of DNA per genome was 1.50 pg. In *Pennisetum*, cytologic and flow cytometry studies have evidenced that the size of genome and chromosomes are negatively related to the chromosomal basic number (Jahuar, 1981; Martel et al., 1997).

The accessions of *P. nervosum* have shown a mean length of 1.17 µm for the chromosome 1, which represents in average 3% of the complement, and for the shortest chromosome such values were 0.90 µm and 2.3%. In *P. orientale*, the length of the longest chromosome (1.09 µm) represents 3.7% of the complement and the shortest (0.54 µm), 1.8%. In the hexeploid accessions of *P. setosum,* the longest (1.15 µm) and the shortest (0.70 µm) have represented 2.3% and 1.4% of the complement, respectively. For the accessions BAG 54, 65, and 91 of *P. purpureum,* Barbosa et al. (2003) have described that the longest chromosomal pair represents, in average, 10.7% of the length of the haploid lot, being twice as long as the last pair, whose relative length represents, in average, 5% of the haploid lot. Among the accessions BAG 63 and 75, the relation between the longest and shortest chromosome has not reached the proportion 2:1. In *P. glaucum*, the authors have described that the longest pair represented, in average, 17.1% of the haploid lot, being 1.6 times longer than the last pair, whose relative length represented, in average, 10.9% of the haploid set.

The karyotypes of *P. orientale* and *P. glaucum* showed metacentric and submetacentric chromosomes, while the karyotype of *P. setosum, P. nervosum, and P. purpureum* have shown only metacentric chromosomes (Table 2). The high level of chromosomal condensation and the similarities in chromosomal sizes hindered the identification of morphologic traits, besides the centromere, being only possible the visualization of satellites in one pair of chromosomes in the accessions of *P. nervosum*. Those numbers are, probably, underestimated, since due to their terminal or interstitial positioning, the secondary constrictions are not easily detectable through conventional staining techniques. In the accessions of *P. glaucum* and *P. purpureum,* Barbosa et al. (2003) described the presence of satellites in one or two pairs of chromosomes.

Using the criteria proposed by Zarco (1986), the data analysis from chromosomal measurements suggest that karyotypes of the accessions/species, even considerably similar, may be distinguished with regard to chromosomal length and karyotypic asymmetry. The analysis of the intra-chromosomal asymmetry (A1) and the inter-chromosomal asymmetry (A2) have shown that *P. setosum* (x=6) presents more tendency to asymmetry, with the following mean indexes A1=0.968 and A2=0.161. The tetraploid accessions of *P. nervosum, P. orientale,* and *P. purpureum* have exhibited intermediary level of asymmetry and the karyotype of *P. glaucum* (x=7) presented the smallest asymmetry, represented by mean values of A1=0.898 and A2=0.162 (Table 1, Figure 1).

Table 2. Karyotypic traits of accessions of *Pennisetum nervosum, P. setosum,* and *P. orientale, P. purpureum* and *P. glaucum:* chromosomal number, karyotypic formulae, total length of the chromatin (CTC), chromosomal mean length (CMC); length of the longest (L) and shortest (S) chromosomes of each species; mean centromeric index (IC) and mean indexes of karyotypic asymmetry (A1) and inter-chromosomal asymmetry (A2), according to ZARCO (1986)

Species	Chromosomal number	Karyotypic formulae	CTC		CMC	L	S	IC	A1	A2
			2n	x						
					µm					
P. setosum	2n=6x=54	54 m	49.43	8.24	0.91	1.15	0.70	45.80	0.968	0.161
P. nervosum	2n=4x=36	36 m	38.72	9.68	1.07	1.17	0.90	46.68	0.953	0.164
P. orientale	2n=4x=36	32 m + 4 sm	29.41	7.36	0.82	1.09	0.54	43.16	0.954	0.145
P.purpureum	2n=4x=28	28 m ([1])	28.09	7.02	2.001	2.941	1.471	44.891	0.941	0.194
P. glaucum	2n=2x=14	10 m + 4 sm ([1])	28.36	14.18	4.02[1]	4.86[1]	3.06[1]	40.74[1]	0.898	0.162

([1]) BARBOSA et al. (2003).

Considering the criteria proposed by STEBBINS (1971) for the definition of symmetry, the karyotypes of the three wild species analysed fit into the category 1A-symmetrical, since the relation between the longest and shortest was lower than 2:1. Regarding *P. purpureum*, BARBOSA et al. (2003) suggested the inclusion of the karyotype of the accessions BAG 54, 65, and 91 in the category 2B and accessions BAG 63 and 75, in the category 1A. The karyotypes of the accessions of *P. glaucum* were included in the category 1A.

The proposal of STEBBINS (1971), supported by the concepts of the Russian School lead by LEVITSKY (1931), define that symmetry of the karyotype is characterized by the predominance of chromosomes similar in length and with centromere in metacentric and submetracentric positions. The increase of karyotypic asymmetry occurs due to the change of the centromere to terminal or subterminal positions, and to differences in the relative size of the chromosomes of the complement, making the karyotype more heterogeneous.

The comparison between the classifications of karyotypes obtained according to the methodology of ZARCO (1986) and STEBBINS (1971) revealed that this last one does not allow the detection of little variations among the karyotype closely related, making the classification inaccurate. Such finding ratified the critics stated by ZARCO (1986) and PASZKO (2006) regarding to the amplitude of categories proposed by STEBBINS (1971).

Generally, the species with more symmetric karyotypes and higher number of chromosomes are considered more primitive in a certain group of plant. For *Pennisetum*, however, it has been widely reported (AVDULOV, 1931; PANTULU and RAO, 1982; MARTEL et al., 2004;) that the ancestral traits of the genus belong to the group that presents a chromosomal basic number equal to nine (x=9), small chromosomes, apomictic mode of reproduction and perennial life cycle. The species with x= 5, 7 and 8 would be those that diverged more recently, indicating that the structure of the genome of *Pennisetum* must be involved in the reduction of chromosome number and increase of their size (MARTEL et al., 2004).

Observing the spatial arrangement between the analysed populations in the Figure 2, it is verified that polyploid species (*P. setosum, P. nervosum, P. orientale,* and *P. purpureum*) are limited to closer areas in the graphic of dispersions, indicating more karyotypic homogeneity between them and a better possibility of gene exchange, given that there is sexual compatibility. Studies regarding gene structure show that the distribution of genetic variability is not random within the populations and may be determined by characteristics such as geographic distribution of the species, gene flow, reproductive system, effective size of the populations and more, a set of evolutive factors, such as migration, mutation, natural selection and gene derivation (WEIR, 1996). In other forage species, *Lolium perene* (SWEENEY and DANNENBERGER, 1994), *Lolium multiflorum* (VIEIRA et al., 2004), and *Trifolium pratense* (KONGKIATNGAM et al., 1995), a higher intra-population variability rather than an inter-population variability was detected.

Considering the closer karyotypic proximity between *P. purpureum* and the other polyploid species, the promotion of breeding between them and the assessment of performance, agronomic behaviour, and level of fertility of these hybrids would be recommended in comparison to those obtained from the breeding with *P. glaucum*.

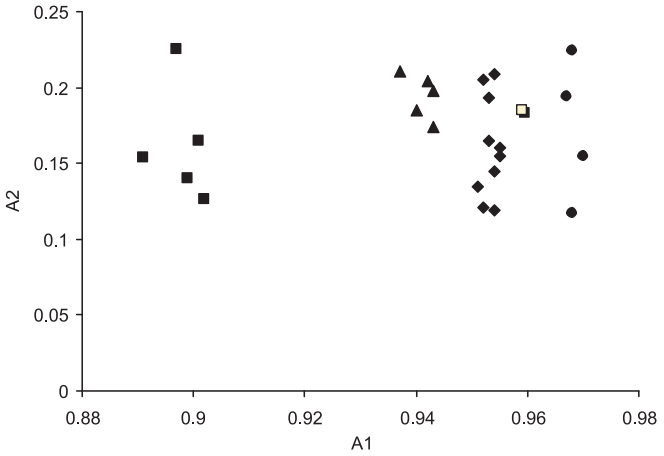

Figure 2. Karyotypic asymmetry according to ZARCO (1986). Accessions of *P. glaucum* - 2n=2x=14 (■); *P. purpureum* – 2n=4x=28 (▲); *P. nervosum* – 2n=4x=36 (◆); *P. orientale* - 2n=4x=36 (□) and *P. setosum* - 2n=6x=54 (●). A1= intra-chromosomal asymmetry and A2= inter-chromosomal asymmetry.

4. CONCLUSIONS

1. No difference was found for the total length of the chromosomes between the wild species (x=9) and the cultivated species (x=7).

2. The tetraploid accessions of *P. setorum, P. nervosum, P. orientale* and *P. purpureum* have presented an intermediary level of asymmetry and higher karyotypic homogeneity and the karyotype of *P. glaucum* (x=7), a lower asymmetry.

3. The indexes of both inter- and intra-chromosomal asymmetry allow the detection of low variations between the karyotypes of the taxa closely related, helping in the identification of the accessions of *Pennisetum* sp. in the germplasm banks.

ACKNOWLEDGMENT

Authors gratefully acknowledge the Fundação de Amparo à Pesquisa do Estado de Minas Gerais for the financial support, and the biologist Larissa Fonseca Andrade Vieira by contributions in obtaining the photomicrographs.

REFERENCES

AVDULOV, N. P. Karyosystematicshe Untersuchung der Familie Graamineen. **Bulletin Applied Botany of Genetics and Plant Breeding,** v.4, p.1-428, 1931.

BARBOSA, S.; DAVIDE, L. C.; PEREIRA, A. V. Cytogenetics of *Pennisetum purpureum* Schumak x *Pennisetum glaucum* hybrids and their parents. **Ciência e Agrotecnologia,** v.27, p.26-35, 2003.

BOGDAN, A. V. **Tropical pasture and fodder plants (grasses and legumes).** London: Longman, 1977. 241p.

BRUNKEN, J. N. A systematic study of *Pennisetum* Sect. *Pennisetum* (Gramineae). **American Journal of Botany**, v.64, p.161-176, 1977.

BURTON, G. W. Hybrids between napier grass and cattail millet. **Journal of Heredity,** v.35, p.227-232, 1944.

CAMPOS, J. M. S.; CALDERANO, C.A.; PEREIRA, A.V.; DAVIDE, L. C.; VICCINI, L. F.; SANTOS, M. O. Embriogênese somática em híbridos de *Pennisetum* sp. e avaliação de estabilidade genômica por citometria. **Pesquisa Agropecuária Brasileira**, v.44, p.38-44, 2009.

DUJARDIN, M.; HANNA, W. W. Apomictic and sexual pearl millet x *Pennisetum squamulatum* hybrids. **The Journal of Heredity,** v. 74, p. 277-279, 1983a.

DUJARDIN, M.; HANNA, W. W. Meiotic and reproductive behavior of facultative apomictic BC_1 offspring derived from *Pennisetum americanum-P. orientale* interspecific hybrids. **Crop Science,** v.23, p.156-160, 1983b.

DUJARDIN, M.; HANNA, W. W. Cytogenetics of double cross hybrids between *Pennisetum americanum-P. purpureum* amphiploids and *P. americanum x P. squamulatum* interspecific hybrids. **Theoretical and Applied Genetics,** v.69, p.97-100, 1984.

DUJARDIN, M.; HANNA, W. W. Crossability of pearl millet with *Pennisetum* species. **Crop Science,** v.29, p.77-80, 1989.

GILDENHUYS, P.; BRIX, K. Cytological abnormalities in *Pennisetum dubium.* **Heredity,** v.12, p.441-452, 1958.

HANNA, W. W. Interspecific hybrids between pearl millet and fountain grass. **Journal of Heredity,** v.70, p.425-427, 1979.

JAUHAR, P. P. **Cytogenetics and breeding of pearl millet and related species.** New York: Liss A. R., 1981. 289p.

JAUHAR, P. P.; HANNA, W. W. Cytogenetics and genetics of pearl millet. **Advances in Agronomy,** v.64, p.1-26, 1998.

KAUSHAL, P.; ROY, A. K.; KHARE, A.; MALAVIYA, D. R.; ZADOO, S. N.; CHOUBEY, R. N. Crossability and characterization of interspecific hybrids between sexual *Pennisetum glaucum* (pearl millet) and a new cytotype (2n=56) of apomictic *P. Squamulatum*. **Cytologia,** v.72, p.111-118, 2007.

KHALFALLAH, N.; SARR, A.; SILJAK-YAKOVLEV, S. Karyological study of some cultivated and wild stocks of pearl millet from Africa (*Pennisetum typhoides* Stapf et Hubb. And *P. violaceum* (Lam.) L. Rich. **Caryologia,** v.46, p.127-138, 1993.

KONGKIATNGAM, P.; WATERWAY, M. J.; FORTIN, M. G.; COULMAN, B. E. Genetic variation within and between two cultivars of red clover (*Trifolium pratense* L.): comparisons of morphological, isozyme, and RAPD markers. **Euphytica**, v.84, p. 237-246, 1995.

LEVAN, A.K., FREDGA, K., SANDBERG, A.A. Nomenclature for centromeric position on chromosomes. **Hereditas**, v.52, p.201-220, 1964.

LEVITSKI, G. A. The karyotype in systematics. **Bulletim Applied Botany Genetics in Plant Breeding,** v.27, p.220-240, 1931.

MARTEL, E.; DE NAY, D.; SILJAK-YAKOVLEV, S.; BROWN, S.; SARR, A. Genome size variation and basic chromosome number in pearl millet and fourteen related *Pennisetum* species. **The Journal of Heredity,** v.88, p.139-143, 1997.

MARTEL, E.; PONCET, V.; LAMY, F.; SILJAK-YAKOVLEV, S.; LEJEUNE, B.; SARR, A. Chromosome evolution of *Pennisetum* species (Poaceae): implications of ITS phylogeny. **Plant Systematics and Evolution,** v.249, p.139-149, 2004.

PANTULU, J. V.; RAO, K. Cytogenetics of pearl millet. **Theoretical Applied Genetics**, v.61, p.1-17, 1982.

PASZKO, B. A critical review and a new proposal of karyotype assymetry indices. **Plant Systematics and Evolution,** v.258, p.39-48, 2006.

PATIL, B. D.; HARDAS, M. W.; JOSHI, A. B. Auto-allopolyploid nature of *Pennisetum squamulatum*. **Nature,** v. 189, p. 419-420, 1961.

PEREIRA, A. V. Melhoramento de plantas forrageiras. In. AGUIAR, A. M.; ROSAL, C. J. S.; MENEZES, C. B.; RAPOSO, F. V.; CORTE, H. R.; FUZATTO, S. R. (Eds.) Simpósio sobre atualização em genética e melhoramento de plantas, 2, **Anais.** Lavras. UFLA/FAEPE, 1998. p.135-162.

ROBERT, T.; LESPINASSE, R.; PERNÈS, J.; SARR, A. Gametophytic competition as influencing gene flow between wild and cultivated forms of pearl millet (*Pennisetum typhoides*). **Genome,** v.34, p.195-200, 1991.

SCHMELZER, G. H. Review of *Pennisetum* section *Brevivalvula* (Poaceae). **Euphytica,** v.97, p.1-20, 1997.

SCOTT, A. J.; KNOTT, M. A cluster analysis method for group means in the analysis of variance. **Biometrics,** v.30, p.507-512, 1974.

STAPF, O.; HUBBARD, C.E. *Pennisetum*. In: PRAIN, D. (Ed.). *The flora of tropical Africa*. Kent: Reeve, 1934. p.954-1070.

STEBBINS, G. L. **Chromosomal evolution in higher plants**. London: Edward Arnold Publishers, 1971. 216p.

SWEENEY, P. M.; DANNEBERGER, T. K. Random amplified polymorphic DNA in perennial ryegrass: a comparison of bulk samples vs. individuals. **Hortscience**, v.29, n. 6, p.624-626,1994.

TECHIO, V. H.; DAVIDE, L. C.; PEREIRA, A. V.; BEARZOTI, E. Cytotaxonomy of some species and interspecific hybrids of *Pennisetum* (Poaceae, Poales). **Genetics and Molecular Biology,** v.25, p.203-209, 2002.

VIEIRA, E.A.; CASTRO, C. M.; OLIVEIRA, A. C. de;

CARVALHO, F. I. F. de; ZIMMER, P. D.; MARTINS, L. F. Genetic structure of annual ryegrass (*Lolium multiflorum*) populations estimated by RAPD. **Scientia Agricola**, v.61, p.407-413, 2004.

WEIR, B.S. **Genetic data analysis: methods for discrete population genetic data.** Sunderland: Sinauer Associates, 1996. 376p.

ZARCO, C. M. A new method for estimating karyotype asymmetry. **Taxon,** v.35, p.526-530, 1986.