

Cytogenetic Studies on Some Species of Genus *Pennisetum* L. (Rich) *Poaceae*

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Abstract: Six species of genus *Pennisetum* (poaceae) were subjected to mitotic and meiotic investigation in the present study. Diploid species (*P. glaucum* and *P. macrourum*) show 14 chromosomes almost lies in 7 pairs of metacentric type and 6 pairs and one pair of submetacentric chromosomes for the two species respectively. Regular meiosis, high chiasma frequency and pollen fertility were observed in tetraploids *P. divesum* and *P. orientale* show 36 mitotic chromosomes based on 9 as basic number. Karyotype formula of the former shows $32m + 4sm$ where the later possess structure of $28m + 4sm + 4st$. Meiotic behavior of the two species to some extent support mitotic finding as the former is of autopolyploid origin where the later shows allopolyploid nature. The two hexaploid species *P. polystachion* and *P. macrourum* show 54 chromosomes based on 9 as common basic number. Karyotype structure have 22 pair of metacentric, and 5 pairs of submetacentric type for the former and 19 pairs of metacentric, 7 pairs of sm and one pair of subtelocentric types for the later. Low values of chiasma frequency and pollen fertility recorded with degree of similarity suggest the autoallohexaploid nature of *P. polystachion* species. On the other hand irregular meiotic analysis and karyotype asymilarity suggest the allohexaploid nature of *P. macrourum*. [Journal of American Science 2010;6(9):193-200]. (ISSN: 1545-1003).

Key words: *Pennisetum*, mitosis, meiosis, chromosome association, chiasma, poaceae

1. Introduction

Genus *Pennisetum* belongs to family poaceae includes about 140 species distributed around world. (Chaudary 1989). The genus comprises many species of economic importance as grain crops (*P. americanum* and *P. typhoides*), common fodder (*P. orientale* and *P. clandestine*). Some others are of medical importance including *P. setaceous*, *P. villoseum* and *P. divesum* (Sujatha *et al* 1989).

The majority of species in this genus has been subjected to cytogenetic studies by many authors (Sujatha *et al* 1989, Sai Kumar *et al* 1987, Ferchichi *et al* 1994, Jauhar 1981, Burton 1942, Schmelzer 1997, Nagesh and Subarhmanyam 1996, Barbosa *et al* 2003, Akizama *et al* 2006, Pontes *et al* 2004). Majority of species in this genus constitute a heterogenous assemblage with different basic numbers ($x= 5,6,7,8$ and 9) and ploidy levels varying from diploids to octaploids (Martel 1997, Vania *et al* 2002). Species of this genus were not subjected to any of mitotic or meiotic studies in Egypt. This attracts the attention to carry out cytogenetic investigation on some available species.

In the present study, six species of genus *Pennisetum* were subjected to mitotic and meiotic investigation to gain insight into their cytogenetic structure and evolutionary relationship. Species were handled using modern criteria of investigation.

Parameters of karyotype structure, chromosome length, arm ratio, chromosome association, chiasma frequency, meiotic irregularity and pollen fertility were investigated. This work is a part of programme study cytogenetics of grass family (*Poaceae*).

2. Material and Methods

Materials of this study were collected from natural habitat during spring season for morphological description. Seeds and flowers of species were collected from plants for mitotic and meiotic studies. For mitotic study seeds were sown in small pots. Few days after germination lateral roots (1-2cm long) were treated with aqueous solution of 0.4% colchicine for 6h. treated roots were fixed in fresh prepared fixative solution (1:3 acetic alcohol) for 24 h., hydrolyzed in 1 N HCl for 10 min. and stained with Feulgen reagent. Well spread fully contracted c-metaphase cells were used for karyotype study.

For meiotic studies, young inflorescences were fixed at early morning in freshly prepared fixative solution of 1: 3 v/v acetic alcohol for 24 h. The fixed material was kept in 70% alcohol at 4°C till use. Anthers were squashed in 2% acetocarmine for pollen mother cell identification. Cells at diakinesis and metaphase 1 stages were used to observe chiasma frequency, chromosome

associations and meiotic irregularities. Pollen stainability as indication of fertility was assessed in mature anthers at time of anthesis using the same technique and calculated as a percentage.

3. Results and Discussion

I- Morphology

Morphological characters of six species investigated were carefully described based on flora of Egypt (Tackholm 1974) and weed flora of Saudi Arabia (Chaudhary 1989).

1- *Pennisetum glaucum*: Tall cultivated millet, culms robust up to 2 m tall, densely villous below the panicle. Leaf blade flat, large up to 1m long, 5cm wide cordate at base. Panicle stiff, very dense cylindrical, up to 50cm long, 2-2.5 mm long lemma and palea pubescent on the margins. Grain spherical, usually white at maturity.

2- *Pennisetum americanum*: Densely tufted, coarse, perennial grass, 20-80 cm tall. Leaf blade up to 30cm long, rigid, inrolled, 2-3 mm wide, the midrib thick on the upper surface. Panicle purplish, narrow, 6-20 cm long. The axis hispid-pilose, cylindrical with shallow angular ribs below the spikelet. Bristles 15-30 mm long. Spikelets lanceolate, 4.5-6 mm long; lower glume missing, or up to 1/3 the length of the spikelet. Lower lemma as long as the spikelet, acuminate. Upper lemma similar to lower one.

3- *Pennisetum divisum*: Perennial shrubby grass with short woody rootstock. Culms branched, woody up to 1 m tall. Leaf blade rigid, inrolled 2-7cm long. Sheaths more or less inflated, persistent at the nodes. Panicle oblong to more or less cylindrical, 5-12 cm long, around 10mm wide; axis with shallow angular ribs below. Spikelets enclosed singly by the involucre, the bristles glabrous 7-20 mm long spikelets narrow lanceolate 6-8 mm long, lower glume 1/2 to 3/4 the length of the spikelet. Upper glume almost as long as the spikelet, acute or acuminate, lower lemma similar to upper one.

4- *Pennisetum orientale*: Rhizomatous perennial often forming large clumps. Culms 20-60 cm to up 2m tall, branched from lower nodes. Leaf blade 5-20 cm long, and 4-5 mm wide, linear lanceolate. Panicle very much denser 8-30 cm long, axis with shallow angular ribs the scars of the spike clusters. The inner bristles of the involucre plumose, the longest bristles 15-30 mm long. Spikelets lanceolate 4-6 mm long, lower glume 1/4-to up to 1/2 as long as the spikelet, upper lemma similar to lower lemma.

5- *Pennisetum polystachion*: A perennial grass with culms 30cm to 2 m tall. Leaf blades 10-40 cm long, 3-16 mm wide. Panicle narrow cylindrical, 3-25 cm long, axis with sharp wings and glabrous involucre. Bristles scaberulous, not ciliate 5-25mm long, pale or purple. Spikelets lanceolate, 2-5 mm long, lower glume absent or very small, upper glume as long as the spikelet, lower lemma similar but staminate, upper lemma leathery, shiny, obtuse at maturity.

6- *Pennisetum macrourum*: A rhizomatous, perennial, reed like grass, 30 cm to 1 m tall. Leaf blade 10-15 cm long, rolled tapering to the tip. Panicle linear, 6-15 cm long, 5-10 mm wide; axis cylindrical with round ribs, involucre enclosing single spikelet; bristles glabrous 5-20 mm long. Spikelets narrowly ovate, 2-6 mm long, acute to acuminate, lower glume up to 1 mm long, upper one also small, 1/8- 1/4 the length of the spikelet, acute, acuminate or obtuse, lower lemma staminate more than 3/4 length of the spikelet, upper lemma as long as the spikelet, similar to the lower lemma in shape and texture.

II- Cytology

A- Mitosis

1- *P. glaucum*: Karyotype analysis of this species shows 14 chromosomes as diploid species ($2n = 14$) based on $X = 7$ as basic number ($2n = 2X = 14$). The basic numbers of 7 and 9 are common in this family as previously reported by Vania *et al* 2006, Jauhar & Hanna 1998, Minocha 1991, Haroun 1991, 1997, 2001).

Based on centromere position, karyotype structure (Figure 1-a) shows 7 pairs of metacentric chromosomes. Length of all chromosomes is very similar recorded mean value of 1.11 μ m (Table 1). High similarity in chromosome morphology medium centromere position and low SE, reflect the homogeneity between chromosomes within the complement and confirm the autopoloid nature of the species with AA suggested genome (Haroun 2001).

2- *P. americanum*: This species shows 14 chromosomes as diploid form ($2n = 14$), based on 7 as basic number ($2n = 2x = 14$). Mean chromosome length and arm ratio listed in Table (1), recording values of 1.29 and 1.25 respectively. SE values of both parameters consider to some extent high compared to *P. glaucum*. Karyotype formula shows $12m + 2sm$ chromosome (Figure1-b). Variation in lengths between chromosomes and centromere position pointed to degree of heterogeneity in the genome structure of the species. This hypothesis refer to that the species is widely distributed and cross pollinated, so there is a chance for crosses with another near by compatible species of grass family.

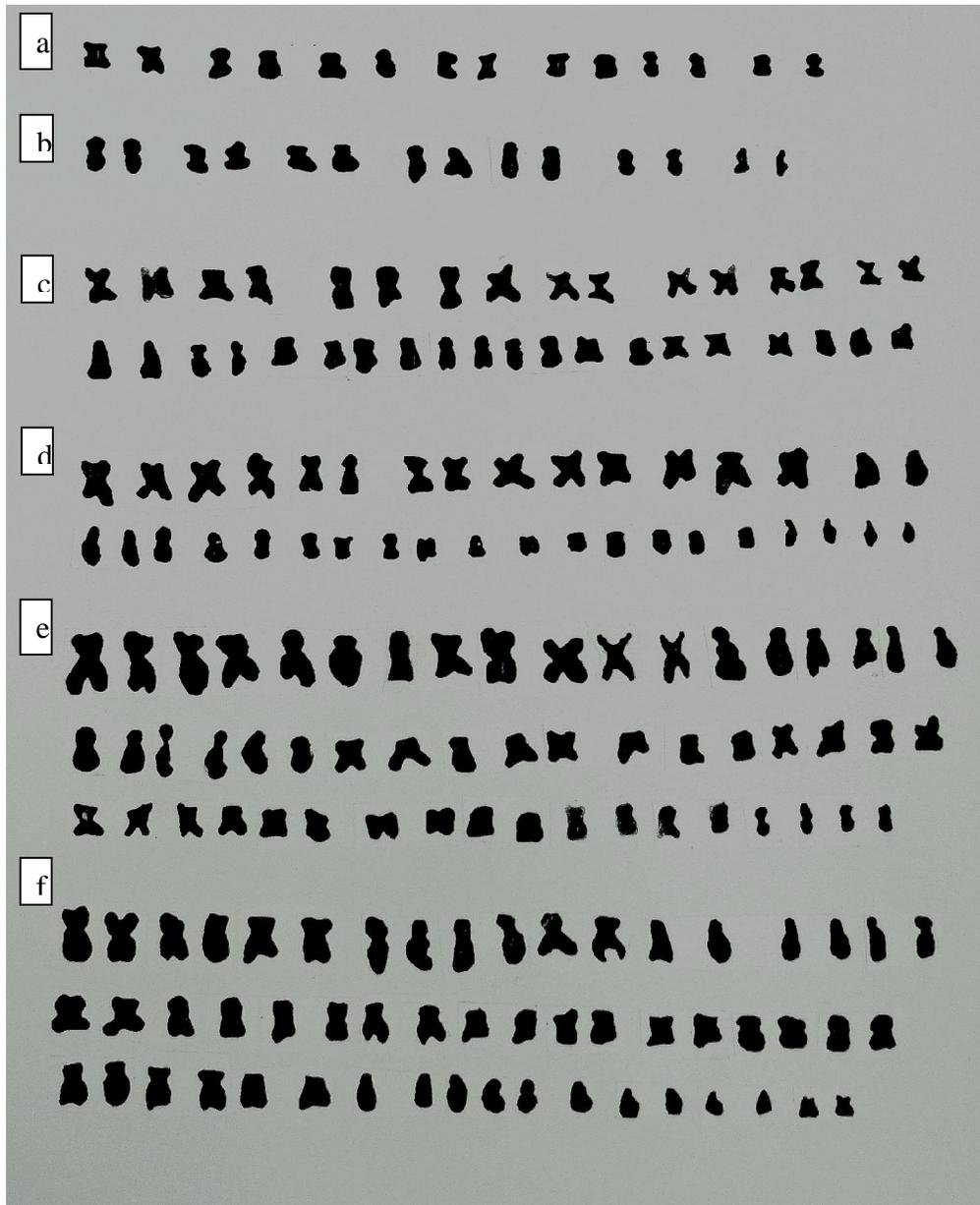


Fig. 1: karyotype structure of six species of genus *Pennisetum* L. a, *P. glaucum*, b, *P. americanum*, c, *P. divisum*, d, *P. orientale*, e, *P. polystachion*, f, *P. macrourum* (X: 1cm = 2µm)

This probably cause gradual changes in genetic material of the species after successive generations (Haroun 1997).

3- *P. divisum*: Based on centromere position the karyotype formula this species shows 32 m + 4 sm chromosomes recording 36 as tetraploid ploidy level (Figure 1-c). This number based on 9 as basic number is common in this family (barbosa, *et al* 2003, Haroun 2001, Swedlund & Vasil 1985 vania *et al* 2002). Values of 1.15 and 1.14 were recorded for mean chromosome length and arm ratio respectively. Low SE recorded for the two parameters reflect low variation between chromosomes within the set and suggest homologous genomes (AAAA) as autopoloid species.

4- *P. orientale*: This species shows 36 chromosomes as tetraploid based on 9 as basic number ($2n = 4X = 36$). Karyotype formula shows 28m + 4sm + 4st chromosomes (Figure 1-d). Mean chromosome length and arm ratio recording 1.22 and 1.29 respectively. Morphology of chromosomes indicate that the set has two main groups differ in morphology and length suggest the allopoloid nature of the species with genome structure of AABB. High value of SE for

karyotype measurements also support this hypothesis as previously stated by Haroun (1991 & 2001).

5- *P. polystachion*: This species shows 54 chromosomes as hexaploid based on 9 as basic number ($2n = 6X = 54$). This number is very common in this genus and family (Singh & Godward 1960, Haroun 1997, 2001). Based on centromere position karyotype formula shows 44m + 10sm types of chromosomes (Figure 1-e). Mean chromosome length and arm ratio recorded 1.31 and 1.07 respectively. Two pairs of satellite chromosomes were observed. Karyotype of the species lies in two main groups, 18 chromosomes of the set show high similarity in length and morphology, while the rest of the complement (36 chromosome) show same morphology. To some extent this indicate the autopoloid structure of the species AAAA, followed by outcross pollination with relative species BB forming autoallhexaploid with suggested genome of AAAA BB (Haroun *et al*, 1992). Submetacentric and satellite chromosome observed may also suggest some sort of translocation occurred between chromosomes within the complement.

Table 1: chromosome number, karyotype formula, mean chromosome length and arm ratio of six species of genus *Pennisetum*.

species	Chr. no	Kary..Form.	Basic no.	Ploidy level	X chr. Leng. ± SE	Arm ratio ± SE
<i>P.glaucum</i>	14	14m	7	2X	1.11 ± 0.08	1.17 ± 0.12
<i>P. americanum</i>	14	12m + 2sm	7	2X	1.29 ± 0.11	1.25 ± 0.18
<i>P. divisum</i>	36	32m + 4sm	9	4X	1.15 ± 0.09	1.14 ± 0.07
<i>P. orientale</i>	36	28m + 4sm + 4st	9	4X	1.22 ± 0.12	1.29 ± 0.31
<i>P. polystachion</i>	54	44m + 10sm	9	6X	1.31 ± 0.08	1.07 ± 0.13
<i>P. macrourum</i>	54	38m + 14sm + 2st	9	6X	1.25 ± 0.21	1.26 ± 0.15

m = metacentric, sm = submetacentric, st = subtelocentric, X = basic number

6- *P. macrourum*: Mitotic analysis of this species recorded 54 chromosomes as hexaploid species based on 9 as basic number. karyotype structure (Figure 1-f) composed of 38m + 14 sm + 2st chromosomes. Mean chromosome length and arm ratio recorded values of 1.25 and 1.26 respectively. High SE and variation in chromosome types mostly refer to a degree of heterogeneity in the complement. This indicate to some extent the allohexaploid nature of the species with suggested genome AABBCC. The open pollination system dominate in this family may support this hypothesis (Haroun 1991, 2001).

B- Meiotic analysis

1- *Pennisetum glaucum*: Meiotic chromosomes behavior of this species displayed a very regular

meiosis, 14 chromosomes formed 7 bivalents (Figure 2-a). This finding agrees with that previously recorded by Burton (1942) and Krashnaswamy (1951). Tetraploid form of this species ($2n = 28$) was previously recorded by Hanna *et al* (1993) and Barbosa *et al* (2003).

As a penalty of regular meiosis, high percentage of pollen fertility was recorded (93.7%), with no record of irregularities (Table 2). Chiasma frequency of this species is considered high compared with other species studied at different ploidy levels. For this species value of 13.01 is recorded as chiasma frequency per cell, which is very close to that previously recorded by Sujatha *et al* (1989) for some species of *Pennisetum*. Chromosome morphology and size during meiosis seems similar

and homologous. From mitotic and meiotic investigation it could be concluded that this species is

normal diplois and genetically stable.

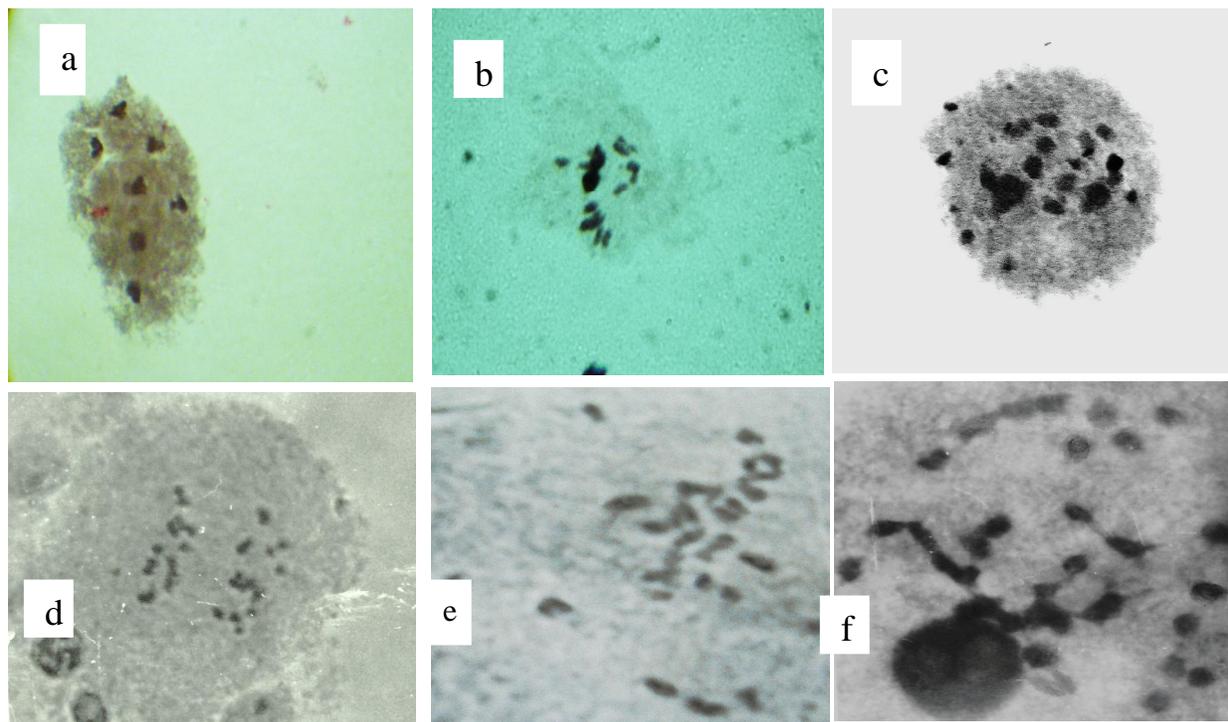


Fig. 2: a, diakinesis in *P. glaucum*, showing 7 bivalents (X = 2000), b- M1 in *P. americanum* showing univalents (X= 1400), c- diakinesis in *P. orientale* showing univalents and trivalent(X= 1600), d- normal segregation at anaphase I in *P. divisum* (X= 1600), e- diakinesis in *P. polystachion* showing multivalents (X= 2000), f- diakinesis in *P. macrourum*.

2- *Pennisetum americanum*: This species is natural diploid with $2n = 14$ as previously recorded by Sujatha *et al* (1989) and Sai Kumar *et al* (1983); (1987). Bivalents and univalents were observed in majority of pollen mother cells examined at diakinesis and metaphase I (Figure 2-b) recording (1.2 I + 5.7 II). Mean value of 12.1 was recorded for chiasma frequency at metaphase I. this value is close to that recorded by Sujatha *et al.* (1989) for the same species from India (13.5). Although few univalents was observed in first meiosis, a considerable high percentage of pollen fertility was recorded (90.5%). This value is lower than that recorded by Sujatha *et al.* (1989). Meiotic observation of this species support to some extent the mitotic finding as degree of heterogeneity probably exist between chromosomes.

3- *Pennisetum divisum*: This species showed 36 as tetraploid form based on 9 as common basic number

within this genus. Bivalents and quadrivalents association (1.2) were recorded at diakinesis and

metaphase I (Table 3). The number of bivalents recorded considered high compared to other tetraploid and diploid species in the present study. This to some extent pointed to the homogeneity of genome structure of the species. Univalents recorded (5.3) may take part in some of laggards and irregularities observed. Our data regarding this species agree with that previously recorded by Sujatha *et al.* (1989) for the same species. High value of chiasma frequency recorded (25.1) per cell is considerably high compared to the other tetraploid species.

Regular meiosis, normal segregation at anaphase I (Figure 2-d) and quadrivalents association recommend the autotetraploid origin of this species as previously stated by Sujatha *et al* (1989) and Mehra (1973). High percentage of pollen fertility (87.6%) seems to be a penalty of regular meiosis. Meiotic behavior seems to be most likely support mitotic analysis hypothesis as the species is genetically stable and autotetraploid as previously suggested by Techio *et al* (2005) and Pagliarini (2000).

Table 2: chromosome number (2n), basic number, ploidy level, pollen size, percentage of pollen fertility and irregularity of six species of *Pennisetum* L. Rich.

Species	2n	Basic no.	Ploidy level	Poll. Size ± S.E	%fert. ± SE	% irreg.
<i>P. glaucum</i>	14	7	2x	29.1 ± 1.5	93.7±1.4	0.0
<i>P. americanum</i>	14	7	2x	26.1 ± 0.9	90.5±1.8	0.0
<i>P. divisum</i>	36	9	4x	31.9 ± 1.0	87.6±2.1	5.3
<i>P. orientale</i>	36	9	4x	30.7 ± 2.0	55.3±1.6	8.7
<i>P. polystachion</i>	54	9	6x	38.5 ± 2.5	61.3±1.9	7.8
<i>P. macrourum</i>	54	9	6x	42.3 ± 1.4	50.4±2.3	9.1

4- *Pennisetum orientale*: This species also had 36 chromosomes as tetraploid associated to form bivalents (6.9), trivalents (2.5) and tetravalents (1.2) at diakinesis and metaphase I (Table 3). Sujatha *et al.* (1989) and Patil *et al.* (1961, 1962) had recorded the same chromosome number ($2n = 4x = 36$) for this species. Diploid number $2n = 18$ for this species was recorded by Nagesh and subrahmayam (1996). Chromosome association in forms of univalents and trivalents (Figure 2-c) recording 4.6 and 2.5 respectively. Pollen fertility recorded 55.3% which is considered high than that previously recorded by Sujatha *et al.* (1989) for the same species. Low chiasma frequency (22.73), irregular meiosis (8.7%) and odd number of chromosome configuration to some extent suggest the allopolyploid nature of the species (Pontes *et al.* 2004, Sujatha *et al.* 1989 and Narayan (1962)). On the other hand this finding is contrary to that previously recorded by Rahngaswamy (1972) as the species is autopolyploid origin.

5- *Pennisetum polystachion*: This species is hexaploid has 54 chromosomes based on 9 as basic number. The number was previously recorded by Sujatha *et al.* (1989), Singh and Godward (1960) and Tateoka (1965). Schmelzer (1997) had recorded different ploidy levels for this species ($2n = 18$ & 36) as diploid and tetraploid.

Few univalents and bivalents were observed recording 3.7 and 9.3 respectively. Multivalents in forms of trivalents (1.6), tetravalents (3.5) and pentavalents (0.7) were observed (Figure 2-e). Chiasma frequency 33.9 per cell is very close to that previously recorded by sujatha *et al.* (1989) and Akizama *et al.* (2004, 2006) for same species and ploidy level. Pollen fertility recorded 61.3% seems indirectly affected by odd number of

chromosome association and reflect degree of heterogeneity within the complement indicate the allopolyploid nature of the species as previously suggested by Sujatha *et al.* (1989). The presence of quadrivalents in considerable value in the present study strongly suggest that species has at least one genome present in autopolyploid form. So we could conclude that the species has autoallopolyploid genome structure.

6- *Pennisetum macrourum*: This species is hexaploid recording 54 chromosomes, based on 9 as basic number ($2n = 6x = 54$). This finding was previously recorded by Burton (1942) and Sujatha *et al.* (1989). Associations in forms of univalents, bivalents and trivalent were observed more frequent compared to *P. polystachion*. Associations higher than quadrivalents were observed in forms of pentavalents and hexavalents (Figure 2-f), recording 1.1 and 0.6 respectively (Table 3).

Low chiasma frequency (30.3), pollen fertility (50.4%) and high percentage of irregularity (9.1%) listed in Table (2) indicate heterogeneous genome structure and pointed to allopolyploid nature of the species as previously stated by Sujatha *et al.* (1989).

In general diploid species display a very regular meiosis. Irregularities are absent or rare. Chiasma frequency recorded seems to be evolutionary response to the challenge of fertility. The tetraploid *P. divisum* shows high level of chiasma per bivalent but not as diploids. Regular meiosis and fertility reflect the autopolyploid origin of this species. This in contrast to that recorded for tetraploid *P. orientale* as allopolyploid nature. Tetraploids show high level of irregularities and low level of fertility compared to diploids. Nevertheless there is a negative relationship between the percentage of meiotic irregularities and pollen fertility.

Table 3: Mean values of chromosome association and chiasma frequency of six species of *Pennisetum* L. Rich.

Species	Chromosome association						Chais. Freq./ cell \pm S.E
	I	II	III	IV	V	VI	
<i>P. glaucum</i>	0.0	7.0	0.0	0.0	0.0	0.0	13.01 \pm 0.21
<i>P. americanum</i>	1.2	5.7	0.0	0.0	0.0	0.0	12.84 \pm 0.32
<i>P. divisum</i>	3.1	12.8	0.8	1.2	0.0	0.0	25.11 \pm 0.43
<i>P. orientale</i>	4.6	6.9	2.5	1.2	0.0	0.0	23.73 \pm 0.27
<i>P. polystachion</i>	3.7	9.2	1.6	3.5	0.7	0.0	33.93 \pm 0.47
<i>P. macrourum</i>	4.4	11.5	2.9	1.7	1.1	0.6	30.41 \pm 0.41

Hexaploids show low levels of chiasma frequency and pollen fertility compared either diploids or tetraploids (Table 4). In contrast hexaploids show high irregularities compared to the

other two ploidy levels. These finding suggest high degree of allohexaploid structure of the two species. But the degree of homogeneity recorded for *P. polystachion* pointed to its autoallohexaploid nature.

Table 4: mean values of pollen size (um), percentage of fertility and irregularity and chiasma frequency for three ploidy levels of *Pennisetum* species.

Ploidy level	Pollen size	% fert.	% irreg.	Chiasma freq.
2X	27.6	92.1	0.0	12.9
4X	31.6	74.5	7.0	24.2
6X	40.4	60.8	8.5	29.7

Not surprisingly, that hexaploids have greater pollen size than diploids and tetraploids. However, it seems that it follows the ploidy level or chromosome number. Based on data in the present study concerning pollen size, the level of polyploidy may be predict as follow: pollen over 30um could represent tetraploids whereas pollens over 38um represent hexaploid. Sometimes there is overlapping between diploids and teraploids in pollen size as stated by Haroun *et al* (1992).

Finally, we should not ignore the variation in basic chromosome number recorded for diploids (7) and tetraploids and hexaploids (9). Such variation to some extent indicates the occurrence of intraspecific chromosome races in genus *Pennisetum* and in the present studied species as previously stated by Swaminthan and Nath (1956) and Joshi *et al* (1959).

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5/12/2010