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# Pachytene Analysis in the Genus *Pennisetum* (L.) Rich.

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Pachytene karyotype is undoubtedly the most useful stage for characterization of the chromosomal complement in plant species where the somatic chromosomes can not be identified individually. When the diploid complement of a species is identified, the way is open for identification of trisomic lines, inversions, translocation stocks and genomic analysis which are useful tools in establishing gene chromosome relationships.

The genus *Pennisetum* (L.) Rich which belongs to tribe Panaceae includes many forage plants and an useful grain crop *P. glaucum*. (L.) R. Br. The origin and evolution of the genus is associated with polybasic chromosome numbers of 5, 7, 8 and 9 (Swaminathan and Nath 1965) and in each basic chromosome number group, different species have been evolved by hybridization and polyploid (Sree Rangaswamy 1965).

Since the first report of McClintock (1929) standardized the morphology of maize chromosome at pachytene stage, a number of workers have reported detailed idiograms on the basis of pachytene analysis in various crop species including *Pennisetum glaucum* (L.) R. Br. having basic chromosome number $x=7$ with $2n=14$. Pachytene studies are not available in species with other basic chromosome numbers in the genus *Pennisetum*. The present paper reports cytomorphological characteristics of the pachytene chromosomes of three species with different basic chromosome numbers in diploid state viz. *P. ramosum* (Hochst.) schweinf. ($x=5$), *P. glaucum* (L.) R. Br. ($x=7$) and *P. hohenackeri* Hochst. ex. steud. ($x=9$).

## Materials and methods

The Genetic Resources unit, ICRISAT centre, Patancheru maintains 371 accessions of 20 wild species of *Pennisetum*. Each species is grown in 10 m rows spaced 150 cm apart in field conditions.

Young inflorescences from plants grown in the field were fixed in carnoy's solution (Johanson 1940) and stored in 70% alcohol for pollen mother cell (PMC) squashes. For chromosome staining, one anther from a floret of appropriate size was squashed in one drop of 2% acetocarmine solution and photomicrographs were taken using temporary slides. Repeated staining, addition of 45 percent acetic acid, gentle heating and application of pressure on cover glass helps in spreading the chromosomes. Al least 10 cells at pachytene stage were analyzed and measurements were taken for total length, location of the centromere and arm ratio (length of short arm/long arm). After identification of the chromosomes individually, those were serially numbered in the descending order of the length. Mean and standard error (S.E.) were calculated. The heterochromatin and euchromatin were shown as broad and narrow lines respectively. The photographs have a magnification of $\times 3200$. The photographs were provided for only two species viz. *P. ramsoum* and *p. hohenackeri* since these are being reported for the first time.

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## Results

The chromosomes were individually recognisable at pachytene stage by their relative lengths and position of the centromere. The deep staining regions on either side of the centromere and its achromatic nature help to identify it easily. The total length, arm ratio, position of the centromere and relative lengths of each of the chromosomes in each species is presented in Table 1.

*P. ramosum* (Hochest) Schweinf.:

This species belongs to the section Gymnothrix. In the $x=5$ basic chromosome number, the only species available is *P. ramosum* with $2n=2x=10$. These 10 chromsomes invariably form into five ring bivalents at diakinensis (Fig. 1). The length of individual chromosomes ranged from 78.44 $\mu$m to 26.28 $\mu$m with a mean length of 41.13 $\mu$m. The arm ratio varied from 0.86 to 0.29 (Table 1). Of the five pairs, two are median (M), two are submedian (SM) and one is a subterminal (ST). Chromosome number 3 is the nucleolar organizing chromosome (Figs. 2, 5). The length of the biggest chromosome (No. 1) is 78.44 $\mu$m while the shortest one (No. 5) is 26.28 $\mu$m.

*P. Glaucum* (L.) R. Br.:

This is a cultivated species belonging to the section Penicillaria having $x=7$ basic chromosome type with $2n=2x=14$. The total length of haploid chromosome complement was recorded to be 240.36 $\mu$m, the longest measuring 56.60 $\mu$m and the shortest 22.63 $\mu$m. The mean length of the complement is 34.38 $\mu$m. The arm ratio of the complement is varied from 0.89 to 0.21 (Table 1). The complement possesses one median (M), five submedian (SM) and one subterminal (ST) which is the smallest in the complement was the nucleolar organizing one. The length of the longest chromosome was 56.60 $\mu$m while its shortest counterpart was 22.63 $\mu$m in length. The photographs were not provided since the present study is very much coinciding the studies of Vari and Bhowal (1987) and Venkateswarlu and Pantulu (1968) wherein good photographs were made available.

*P. hohenackeri.* Hochest. ex Steud.:

It belongs to the section Gymonothrix with basic chromosome number $x=9$ ($2n=2x=18$) (Fig. 3). The nine pairs of pachytene chromsomes recorded a total length of 197.16 $\mu$m, the longest one showing 31.80 $\mu$m and the shortest with 12.72 $\mu$m. The mean length of the complement is 21.91 $\mu$m. The arm ratio varied from 0.96 to 0.30 (Table 1). The chromosomes are grouped into four median (M), three submedian (SM) and two subterminal (ST) based on the arm ratio and centromeric location. The fourth chromosome was the nucleolus organising chromosome (Figs. 4, 6).

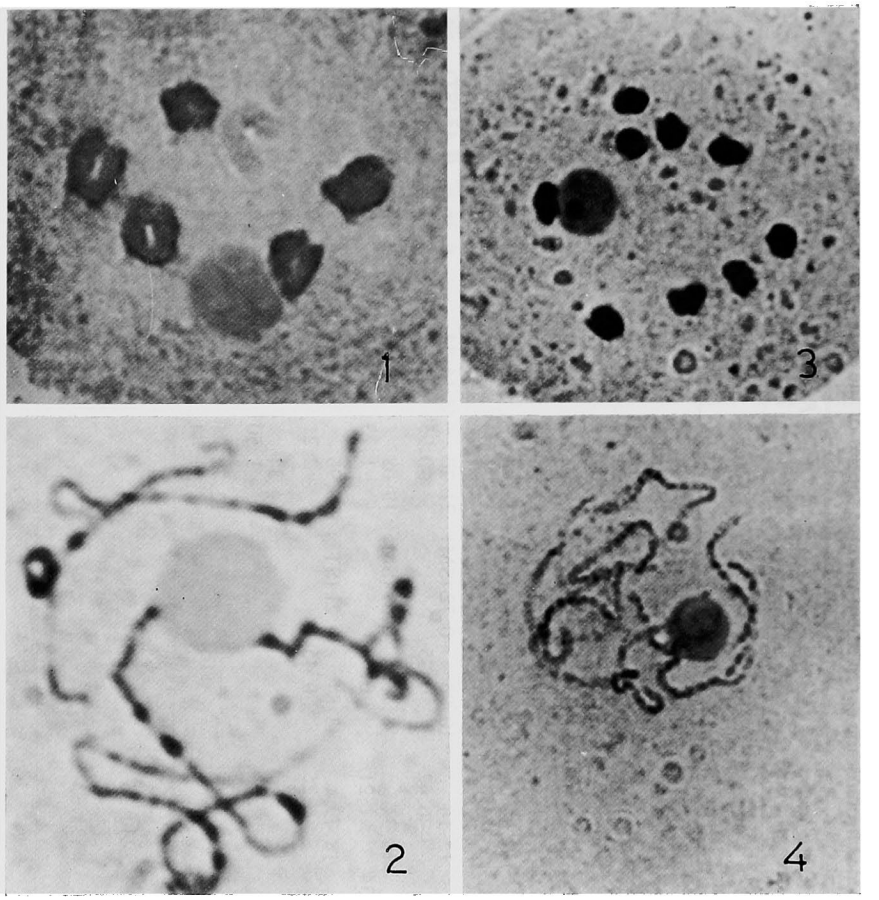
## Discussion

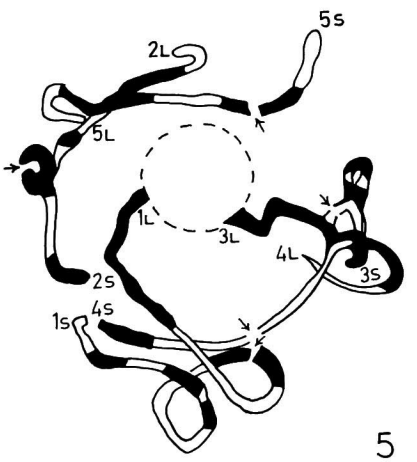
A comparative study on pachytene chromosomes of the diploid *P. ramosum* ($2n=10$), *P. glaucum* ($2n=14$) and *P. hohenackeri* ($2n=18$) showed differences in the size, shape, and position of the centromere (Table 1). The pachytene karyotypes of *P. glaucum* (=*P. americanum*) have been studied earlier by Labana and Gill (1973), Vari and Bhowal (1987) and Venkateswarlu and Pantulu (1968). The present study is also in agreement with those of the above authors but there are different opinions regarding the position of the centromere of different chromosomes which might have been due to the difficulty in locating them. The pachytene analysis of the species *P. ramosum* and *P. hohenackeri* has been reported for first time and

Table 1. Chromosome lengths and arm ratios of pachytene chromosome complement of three species of the genus *Pennisetum*

| Chromosomes | *P. ramosum* Length ($\mu$m) | | | Arm ratio (centromere position) | *P. glaucum* Length ($\mu$m) | | | Arm ratio (centromere position) | *P. hohenackeri* Length ($\mu$m) | | | Arm ratio (centromere position) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | long arm | short arm | | Total | long arm | short arm | | Total | long arm | short arm | |
| 1. | 78.44 ±1.82 | 42.21 ±1.84 | 36.23 ±1.01 | 0.86 (M) | 56.60 ±1.69 | 29.93 ±0.27 | 26.67 ±0.53 | 0.89 (M) | 31.80 ±1.63 | 24.46 ±0.38 | 7.34 ±0.39 | 0.30 (ST) |
| 2. | 40.28 ±0.48 | 26.66 ±1.25 | 13.62 ±0.45 | 0.51 (SM) | 48.36 ±3.21 | 27.64 ±0.19 | 20.72 ±0.68 | 0.75 (SM) | 29.68 ±2.42 | 16.48 ±1.82 | 13.20 ±0.74 | 0.80 (M) |
| 3. | 33.07 ±1.46 | 25.72 ±0.62 | 7.35 ±0.40 | 0.29 (ST*) | 35.40 ±2.63 | 23.17 ±1.62 | 12.23 ±0.52 | 0.53 (M) | 25.44 ±1.82 | 16.96 ±2.28 | 8.48 ±0.29 | 0.50 (SM) |
| 4. | 27.56 ±2.12 | 16.96 ±0.81 | 10.60 ±2.12 | 0.63 (SM) | 27.60 ±0.78 | 18.42 ±0.42 | 9.18 ±0 84 | 0.50 (SM) | 25.44 ±1.69 | 15.32 ±1.73 | 10.12 ±0.16 | 0.66 (SM*) |
| 5. | 26.28 ±0.93 | 14.36 ±1.76 | 11.92 ±2.13 | 0.83 (M) | 26.06 ±2.46 | 15.16 ±0.42 | 10.90 ±0.35 | 0.72 (SM) | 21.20 ±0.95 | 14.84 ±1.69 | 6.36 ±1.22 | 0.43 (ST) |
| 6. | | | | | 24.07 ±1.34 | 15.34 1.21 | 8.73 ±0.63 | 0.57 (SM) | 19 08 ±1.73 | 9.72 ±0.88 | 9.36 ±0.19 | 0.96 (M) |
| 7. | | | | | 22.63 ±2.26 | 18.79 ±0.25 | 3.84 ±0.45 | 0.21 (ST*) | 16.96 ±2.21 | 10.17 ±0.95 | 6.79 ±0.37 | 0.67 (SM) |
| 8. | | | | | | | | | 14.34 ±1.50 | 8 07 ±1.73 | 6.77 ±0.14 | 0.84 (M) |
| 9. | | | | | | | | | 12.72 ±1.44 | 6.75 ±0.33 | 5.97 ±0.32 | 0.88 (M) |
| Total length (n)** ($\mu$m) | | 205.63 | | | | 240.36 | | | | 197.16 | | |
| Ave. length ($\mu$m) | | 41.13 | | | | 34.33 | | | | 21.91 | | |

* Nucleolar orgnizing chromosome. ** $\chi^2$ value = 4.83.

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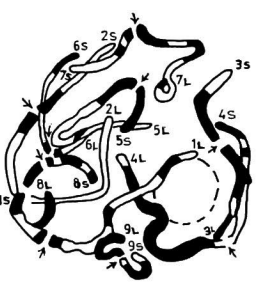


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Figs. 1–4. Meiotic stages in the genus *Pennisetum* (×2300). 1, diakinesis in *P. ramosum* showing five bivalents. 2, pachytene chromosomes of *P. ramosum*. 3, diakinesis in *P. hohenackeri* showing nine bivalents. 4, pachytene chromsomes of *P. hohenackeri*. 5, interpretive drawing of the pachytene cell of Fig. 2 (×2000). 6, interpretive drawing of the pachytene cell of Fig. 5 (×2000). The arrows indicate centromeric position. The numbers 1 to 9 stands for serial number of chromosomes. S-Short arm, L-Long arm.

hence no comparative data is available.

Irrespective of the basic chromosome numbers, the total length of the haploid complement remained almost same in the three species. $\chi^2$ analysis of the total length of the chromosomal complement amongst the three species indicated that the differences are not significant.

Being the lowest chromsome number in the genus, the average length of chromosomes of *P. ramosum* is approximately 16.41% longer than those of *P. glaucum* and 46.72% longer than those of *P. hohenackeri*. In contrast, the species with higher chromosome number (2n=18) in *P. hohenackeri*, have strikingly samller chromosomes than either of *P. ramosum* or *P. glaucum* in which 2n was observed to be 10 and 14 respectively. The trend of species with low chromosome numbers to have much longer chromosomes is evident in several other plant groups. In *Sorghum*, for example the average length of chromosomes of *S. versicolor* (2n=10), *S. vulgare* (2n=20) and *S. halepense* (2n=40) were 4.86, 2.24 and 1.98 $\mu$m respectively (Karper and Chisholm 1936). Similarly Garber (1944) noted much greater chromosome lengths in the parasorghum (2n=10) group compared with eusorghum (2n=20).

Phylogenetic divergence in large number of taxa has occurred because of increase or decrease of the basic chromsome numbers. Some notable examples are *Fritillaria pudica* (Darlington 1936) in which $x=13$ has arisen from $x=12$; Eurasian species of *Allium* ($x=8$), which originated from the primitive North American Species ($x=7$) as was reported by Levin (1935), and *Zea mays* (Molina *et al.* 1987) in which $x=10$ was concluded to be a derivative of $x=5$. A decrease in basic chromosome number has also been observed in many plants (Stebbins 1964). The studies of Jauhar (1970), Saideswara Rao *et al.* (1989) in the genus *Pennisetum* concluded that the $x=5$ is the primitive basic chromosome number in the genus *Pennisetum* and other basic chromosome numbers have been evolved from it. During the process of evolution, some structural changes in the chromosome with deletions and duplications might have taken place which has contributed for the differences in the chromosome number as well as the sizes between different species observed in the present study. However, the karyomorphological study with microdensitometric measurements of DNA would give better idea about the process of evolution.

## Summary

Pachytene chromosome analysis was studied in three species with different basic chromosome numbers ($x$) in the genus *Pennisetum* viz. *P. ramosum* (2n=2x=10), *P. glaucum* (2n=2x=14) and *P. hohenackeri* (2n=2x=18). The studies pointed out chromosome differentiating characteristics in between different species as well as within the same species. It was concluded that the length of the chromosomal complement remains almost same irrespective of differences of basic chromosome number. Appearance of longer chromosomes was observed with decrease of chromosome numbers in three species.

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