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PACHYTENE ANALYSIS IN DIFFERENT ACCESSIONS OF *SETARIA ITALICA* (L.) BEAUV

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A detailed pachytene karyotype analysis was carried out in several accessions of *Setaria italica* (L.) Beauv. The pachytene chromosomes of different accessions showed well marked variations in the length of the long arms, short arms and of the total chromosomes. A significant variability was noticed in the amount and distribution pattern of chromomeres.

Key words : Chromomere, Idiogram, Karyotype, Pachytene.

Millets have socio-economic, food-feed, health and environmental impact on the resources of poor people of the world. These under researched crops are nutritious, valued culturally, adapted to harsh environments, and diverse in terms of their genetic, agro climatic and economic niches. Karyotypes are dynamic structures evolving through numerical and structural changes. For several decades, karyotype diversity has been a crux of plant evolution studies for two main reasons (Levin, 2002). First, chromosome rearrangements often result in partial or complete barriers to inter-specific gene flow and second, karyotypes may provide insights into the relationship between species. Usefulness of the pachytene stage of meiosis for studying detailed morphology of the chromosome was first demonstrated by McClintock (1931) and was subsequently emphasized by larger number of workers. The present paper deals with detailed analysis of the pachytene karyotype in nine accessions belonging to the cultivated species *Setaria italica.*

MATERIALS AND METHODS

Nine accessions, procured from National Bureau of Plant genetic Resources (NBPGR), of *S. italica* were worked out (Table 1). Young floral buds of different sizes were collected and fixed in Carnoy's fluid (6:3:1::absolute ethanol:chloroform:glacial acetic acid) for at least 48 hours and then stored in 70% ethanol in refrigerator. Anthers were smeared and squashed one at a time in 1.5% acetocarmine. The pachytene chromosomes were analysed from the photomicrographs taken by computerized Nikon Image Capturing system.

Table 1: List of *S. italica* accessions explored for pachytene karyotype analyseis.

Lab Code	Accession	Lab Code	Accession	Lab Code	Accession
S-1	GS-102	S-4	SIA-2870	S-7	Prasad
S-2	GS-872	S-5	TNAU-193	S-8	Narsimha Rayz
S-3	GS-931	S-6	SR-51	S-9	ISC-97

The parameters analysed included (a) Total length of the chromosome of a complement (TLCC), (b) Length of long and short arms and of the whole chromosome, (c) Arm's ratio (AR), (d) Total length of all short arms (TLSA), (e) Total length of all long arms (TLLA), (f) Centromeric index (ci), (g) Gradient index (GI), (h) Symmetry index (SI), (i) Total chromatin length (TCL %), (j) Relative length of the chromosome of a complement in relation to the longest chromosome of the cell (RL(A)) and all the cells studied (RL(B)), (k) Percent chromomere per arm (%Chrom.), and (l) Percent chromomere per chromosome (CPC%).

Arm's ratio, ci, GI, SI, TCL%, relative length

$$\text{Arm's ratio} = \frac{\text{Length of long arm of a chromosome}}{\text{Length of short arm of a chromosome}}$$

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$$ci = \frac{\text{Length of short arm of a chromosome}}{\text{Total length of the chromosome}} X100$$

$$GI = \frac{\text{Length of shortest chromosome of the complement}}{\text{Length of longest chromosome of the complement}} X100$$

$$SI = \frac{\text{Total length of all short arms}}{\text{Total length of all long arms}} X100$$

$$TCL\% = \frac{\text{Total length of a chromosome pair}}{\text{Total length of the gametophytic chromosome set}} X100$$

$$\text{Relative lengths} = \frac{\text{Length of a chromosome}}{\text{Length of the longest chromosome of the cell/all the cells studied}} X100$$

$$CPC\ \% = \frac{\text{Total amount of chromomere in long and short arm}}{\text{Total length of long and short arm}} X100$$

and CPC% were worked out using the below given formulae.

The pachytene chromosomes of the complement were arranged, on the basis of the total length, into four types, A-D (A>60.00 µm, B=45.00-60.00 µm, C=30.00-45.00 µm, D<30.00 µm). These chromosomes were further assorted into different types M= Median, m= metacentric, sm= submetacentric, st= subtelocentric, t= telocentric on the basis of the arm's ratio as per Levan et al. (1964). The pachytene karyotypes were also classified into various categories as per Stebbins (1958).

OBSERVATIONS

Data pertaining to various parameters related to pachytene karyotype are tabularized in Table 2. The photomicrographs of the pachytene chromosomes are presented in Figures 1-9. The idiograms showing the pattern of distribution and the amount of the chromomeres present in individual pachytene chromosomes of different accessions are presented in Figures 10-18. In most of the chromosomes of a compliment, the portions around the centromeres in both the arms were positively heteropycnotic, evidencing the aggregation of chromomeres around the centromeres. However, some chromosomes exhibited great inconsistency in the amount of chromomeres and also in the pattern of chromomere distribution. All the compliments possessed 'm' or 'sm' type of chromosomes as per Levan *et al.* (1964). As per Stebbins (1958), 5 accessions showed 1B type, 2 accessions showed 2B type and 2 accessions showed 1A type of karyotypes.

Table 2: Data related to pachytene karyotype of Setaria italica accessions.

Acc.	TLSA	TLLA	TLCC	GI	SI	Karyotype Formulae	KC
S-1	692.92	291.46	401.46	46.06	72.60	2B(m)+2B(sm)+10C(m)+2C(sm)+2D(m)	1B
S-2	789.08	320.71	468.37	36.02	68.47	2A(m)+2A(sm)+2B(m)+6C(m)+2C(sm)+2D(sm)	1B
S-3	690.41	280.51	409.90	34.98	68.43	4B(m)+4C(m)+8C(sm)+2D(sm)	1B
S-4	616.89	255.68	361.21	32.74	70.78	2B(m)+10C(m)+6D(m)	1B
S-5	701.80	304.06	397.74	30.93	76.45	6B(m)+8C(m)+2D(m)+2D(sm)	1B
S-6	635.14	262.75	372.40	37.89	70.56	4B(sm)+8C(m)+6D(m)	2B
S-7	659.04	274.56	384.49	47.32	71.41	2B(m)+8C(m)+2C(sm)+4D(m)+2D(sm)	2B
S-8	689.41	282.56	406.85	55.39	69.45	4B(m)+8C(m)+2C(sm)+2D(m)+2D(sm)	1A
S-9	612.22	247.71	364.51	62.11	67.96	10C(m)+4C(sm)+4D(m)	1A

TLSA=Total length of short arms (µm); **TLLA**=Total length of long arms (µm); **TLCC**=Total length of chromosome complement (µm); **GI**=Gradient index; **SI**= Symmetry index; **KC**= Karyotype Characterization (Stebbins, 1958).

DISSCUSSION

From a cytological standpoint the millets have not received much attention. Cytological studies in most of the genera are confined to the determination of chromosome numbers only. Various studies regarding this aspect have been conducted by several workers (Church 1929, Morinaga 1929, Rau 1929, Nakajima 1930, Avdulov 1931, Hunter 1934, Krishnaswamy and Ayyanger 1935, 1941, Kishimoto 1938, Krishnaswamy 1939, 1940, Burton 1942,

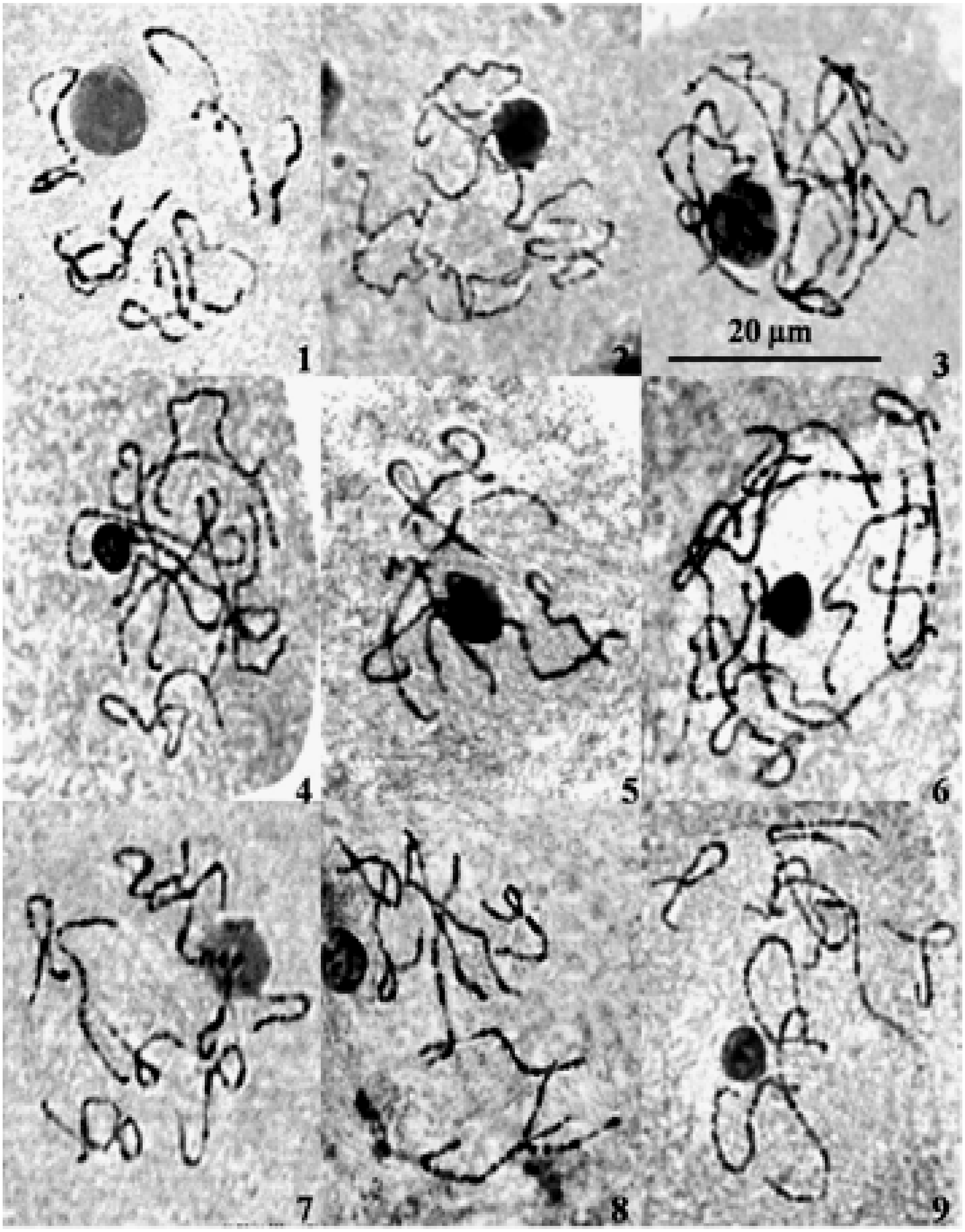
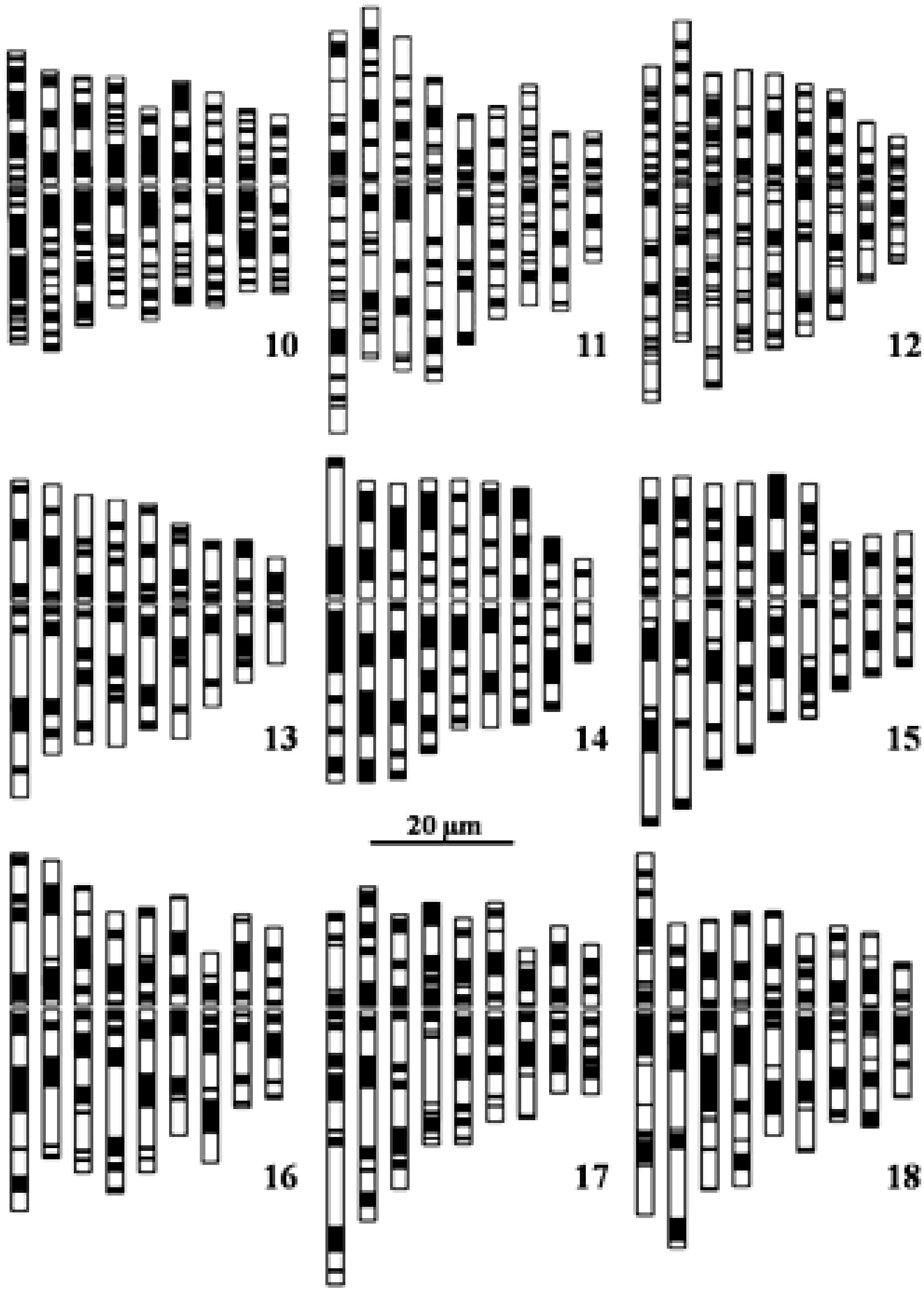


Plate-1 (Figures 1-9) : Pachytene Chromosomes of nine accessions of *Setaria italica*



Figures 10-18 : Pachytene karyotype of a accession of *Setaria italica*

Chandola 1959). During the present undertaking, the details of pachytene karyotypes were analyzed using the parameters as per Srivastava and Purnima (1990) and Srivastava and Kalara (1996). Variability within the individual chromosomes with respect to the size, shape and position of centromere, as well as, the amount and pattern of distribution of chromomeres was found to be well marked. The chromomeres (also called as heterochromatin knobs) are considered as an important landmark of a pachytene chromosome (Chen *et al.* 2000). As per McClintock *et al.* (1981) the size, number and chromosomal distribution of chromomeres vary between strains but are constant within strains. But, contrasting observations were made during the present investigation. A marked variation in the architecture of pachytene karyotype was observed between different accessions of S. italica indicating intra-specific karyotype polymorphism.

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