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SOMATIC KARYOTYPE ANALYSIS IN BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT

Detailed somatic karyotype analysis carried out in 13 varieties of barley revealed significant intervarietal differences in total chromatin length and chromatin volume. Total chromatin length of a haploid complement ranged from 67.23 to 46.02 μm while the chromatin volume varied from 36.80 to 22.09 μm^3. Somatic karyotypes, in general, carried two pairs of sat-chromosomes. The karyotypes are symmetrical, mostly with metacentric chromosomes and belong to 1A category of Stebbins. Relative length, S% (relative length of shortest chromosome compared to the longest) and TF% (proportion of short arms in the total chromatin length) estimations also indicate that the level of asymmetry is low in barley. Based on chromosome morphology, four chromosomes (1, 5, 6sat and 7sat) in the haploid complement can be readily identified individually. However, individual identification of the other chromosomes is somewhat difficult due to similarities in length and arm ratio.

Key words: Karyotype analysis, *H. vulgare*, chromatin length, chromatin volume, sat-chromosomes.

An accurate idiogram representing the chromosomes is the base of cytology and cytogenetics. Detailed chromosomal studies are helpful in successful planning of crop improvement programmes. Barley, a model crop for many research programmes, has been extensively used in genetic studies. However, there is still need to carry out detailed karyotype analysis in barley. The first accurate measurements on the somatic chromosomes of barley was done by Tjio and Hagberg [1]. After that, although many other workers studied the karyotypes of barley [2, 3], there were some disagreements among these results. Hence, the present study on karyotype analysis in thirteen varieties of barley.

MATERIALS AND METHODS

Seeds of thirteen varieties of barley procured from the barley germplasm collections maintained at the Department of Agricultural Botany, Meerut University, Meerut, were

used in the study. Healthy root tips, about 2–3 cm long, were pretreated with paradichlorobenzene for 3h at low temperature (15–20°C), washed, fixed in acetic-alcohol (1:3) for 24h and then transferred to 70% alcohol. For making a cytological preparation, the fixed root tips were kept overnight in 2% acetocarmine and then squashed in 45% acetic acid. Photomicrographs were taken mostly from fresh preparations on Nicon Optiphot microscope with HFX automatic microphotographic attachments. ORWO 35 mm black and white film was used. The chromosomes were measured with the help of Olympus micrometer utilizing good metaphase cells. In chromosome numbering, the standard pattern of giving numbers 1 to 5 to the nonsatellited chromosomes in a descending order of length and 6sat and 7sat to the satellited chromosomes was followed. Among the two satellited chromosomes, the one with large satellite and more median centromere was considered as chromosome 6. In the variety where only one pair of satellited chromosomes was recorded, the nonsatellited chromosomes were given numbers 1–6 on length basis while the satellited one was designated as 7sat. For satellited chromosomes the long and the short arms and satellite were measured separately.

Karyograms were prepared by cutting the photographs of chromosomes and arranging them according to their number in the haploid complement. Following Levan *et al.* [4], the chromosomes were classified into metacentric (arm ratio of 1.0–1.70), submetacentric (1.71–3.00) and subtelocentric (3.01–7.0). Relative length, arm ratio, TF% (proportion of short arms in the total chromatin length), S% (relative length of shortest chromosome compared to the longest) and chromatin volumes were calculated using the following formulae:

$$\text{Relative length} = \frac{\text{Chromosome length}}{\text{Total chromatin length of the haploid complement}} \times 100$$

$$\text{Arm ratio} = \frac{\text{Length of long arm}}{\text{Length of short arm}}$$

For calculating arm ratio in case of satellited chromosomes, the length of satellite is excluded.

$$\text{TF\%} = \frac{\text{Sum of short arm lengths of haploid complement}}{\text{Total chromatin length of haploid complement}} \times 100$$

$$\text{S\%} = \frac{\text{Length of shortest chromosome}}{\text{Length of longest chromosome}} \times 100$$

Chromatin volume = $\pi r^2 L$,

where L—length of chromosome, and r—radius of chromatid at metaphase.

RESULTS AND DISCUSSION

Chromosome number in all the varieties of barley studied was constant with $2n = 14$. The Chromosomes, in general, were long and metacentric (Fig. 1, 2). Chromosome measurements revealed significant differences in total chromatin length and chromatin volume among different varieties (Table 1). The total chromatin length of a haploid complement varied from as high as 67.23 μm in the variety Vijay to as low as 46.02 μm in K-169 (Table 1) with the average chromosome length in a complement ranging from 9.60 to 6.57 μm. Chromatin volume, a better estimate of chromatin content than chromatin length as it takes into account not only length but also width of the chromosomes, also exhibited a trend similar to chromatin length. It ranged from 36.80 μm^3 in var. DL-265 to 22.09 μm^3 in var. K-169 (Table 1). Although artifacts, different levels of condensation and environmental influences on differential contraction of chromosomes [5] may account for part of this variation, a major portion of the differences in chromatin lengths must be real as is evident from a comparison with the estimates of chromatin volume, which may be attributed to structural changes and loss or gain of heterochromatin material. Barley is one

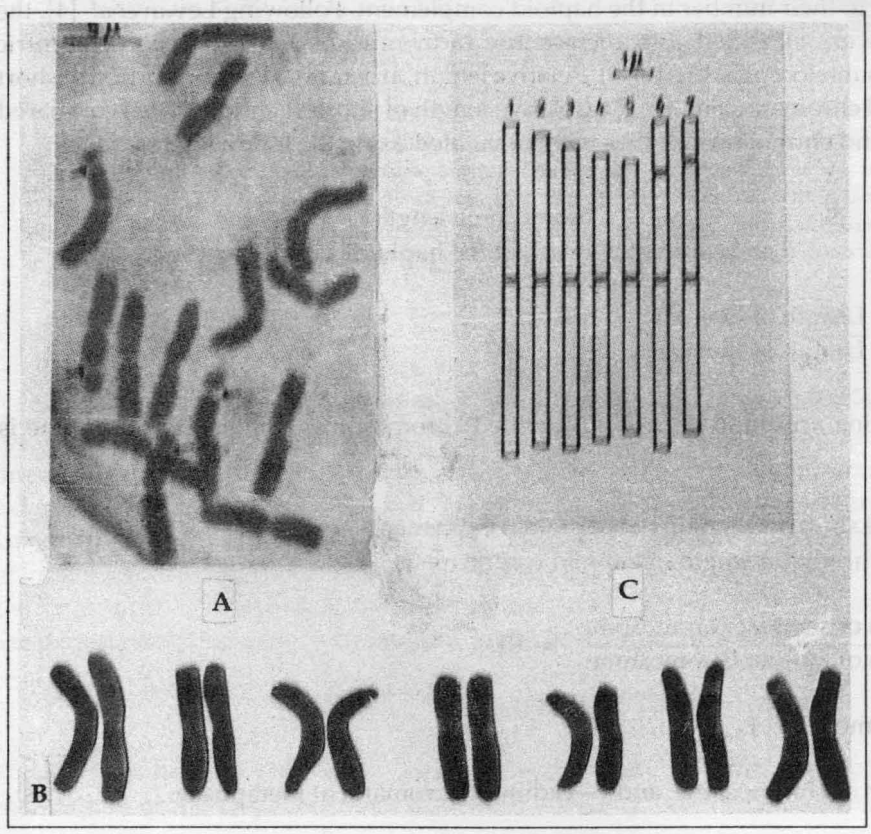

Fig. 1.
Somatic chromosomes of barley var. K-244: (a) somatic metaphase (arrows indicate the position of secondary constriction in the satellited chromosomes), (b) Karyotype, and (c) idiogram.

Table 1. Somatic chromosome morphology in thirteen varieties of barley

Variety		Values for different chromosomes							Total chromatin length (μm)	Total chromatin volume (μm^3)	S%	TF%
		1	2	3	4	5	6sat	7sat				
Vijay	AL	11.07 (16.46)[a]	10.40 (15.46)	9.31 (13.84)	9.18 (13.65)	8.06 (11.99)	7.23 + 1.62[b] (13.12)	8.72 + 1.60[b] (15.45)	67.23	30.15	72.81	40.41
	AR	1.11	1.19	1.36	1.23	1.20	1.75	2.04				
DL-243	AL	10.07 (16.31)	9.61 (15.37)	8.91 (14.43)	8.59 (13.91)	6.65 (10.77)	7.10 + 1.08 (13.25)	8.76 + 0.96 (15.74)	61.73	32.34	66.04	40.77
	AR	1.16	1.21	1.25	1.12	1.47	1.64	2.16				
K-272	AL	9.66 (16.58)	8.84 (15.17)	8.43 (14.47)	7.89 (13.54)	7.20 (12.36)	6.66 + 1.37 (13.68)	8.76 + 0.96 (14.18)	58.25	33.99	74.53	41.54
	AR	1.47	1.37	1.10	1.15	1.22	1.02	1.78				
DL-36	AL	9.81 (16.95)	9.11 (15.74)	8.84 (15.27)	8.69 (15.02)	7.03 (12.15)	6.59 (11.39)	6.76 + 1.09 (13.48)	57.87	32.98	67.18	44.74
	AR	1.34	1.08	1.55	1.00	1.17	1.33	1.05				
K-244	AL	8.87 (15.54)	8.35 (15.10)	8.10 (14.16)	7.63 (13.37)	7.25 (12.74)	7.28 + 1.34 (15.10)	7.15 + 1.10 (14.45)	57.07	34.23	81.96	40.55
	AR	1.11	1.12	1.23	1.27	1.29	1.59	1.33				
DL-270	AL	9.76 (17.51)	8.69 (15.59)	8.40 (15.07)	7.55 (13.54)	7.15 (12.82)	6.96 (12.48)	6.38 + 0.86 (12.99)	55.75	31.57	71.21	43.71
	AR	1.36	1.17	1.18	1.27	1.22	1.01	1.67				
Ratna	AL	8.68 (16.13)	8.37 (15.57)	7.68 (14.27)	7.31 (13.58)	6.56 (12.19)	6.08 + 1.40 (13.90)	6.55 + 1.19 (14.38)	53.82	25.97	75.58	41.01
	AR	1.28	1.06	1.10	1.22	1.24	1.47	2.42				

(Contd.)

Table 1 (contd.)

Variety		Values for different chromosomes							Total chromatin length (μm)	Total chromatin volume (μm^3)	S%	TF%
		1	2	3	4	5	6sat	7sat				
DL-89	AL	8.89	8.00	7.31	6.89	5.27	6.41 + 1.31	6.96 + 0.99	51.97	32.28	59.28	41.04
		(17.11)	(15.39)	(14.06)	(13.26)	(10.14)	(14.85)	(15.18)				
	AR	1.21	1.07	1.03	1.17	2.37	1.37	1.83				
K-192	AL	8.88	8.36	6.96	6.76	6.55	6.19	6.46 + 1.22	51.93	33.13	67.70	44.69
		(17.28)	(16.27)	(13.55)	(13.16)	(12.75)	(12.05)	(14.94)				
	AR	1.31	1.10	1.16	1.13	1.46	1.21	1.15				
DL-265	AL	8.39	7.41	7.11	6.55	6.02	6.29 + 1.32	6.76 + 0.96	50.76	46.80	71.75	41.15
		(16.53)	(14.59)	(14.01)	(12.90)	(11.86)	(14.99)	(15.11)				
	AR	1.24	1.18	1.19	1.08	1.10	1.42	2.02				
K-270	AL	9.11	7.99	6.78	6.05	5.43	5.54 + 1.63	6.70 + 1.36	50.29	22.27	59.60	42.06
		(18.11)	(15.83)	(13.48)	(12.03)	(10.80)	(13.66)	(16.03)				
	AR	1.20	1.13	1.22	1.29	1.61	1.07	1.05				
K-234	AL	8.06	7.73	7.21	5.94	5.94	7.74 + 1.10	6.39 + 0.81	48.92	25.28	73.70	39.57
		(16.47)	(15.80)	(14.74)	(12.14)	(12.14)	(16.03)	(12.67)				
	AR	1.27	1.10	1.12	1.13	1.20	2.54	2.99				
K-169	AL	7.85	7.18	5.77	5.61	5.47	6.02 + 1.33	5.95 + 0.84	46.02	22.09	69.68	41.50
		(17.06)	(15.60)	(12.54)	(12.19)	(11.89)	(15.97)	(14.75)				
	AR	1.23	1.07	1.27	1.20	1.40	1.09	2.34				

Note: AL—absolute length in microns; AR—arm ratio.

a) Figures in parentheses represent relative length in percentage; b) satellite lengths given separately.

of the oldest cultivated crops and has been subjected to selection and breeding over a long period of time. Cultivated barley is adapted to a very wide range of ecological and climatic conditions leading to the development of large number of varieties differing in physiological, ecological, yield, grain quality characteristics, etc. Differences in total chromatin lengths and volumes among these varieties suggest that structural alterations might have been associated with the evolution of barley.

In general, the chromosome complements carried two pairs of satellited chromosomes with a distinct terminal satellite on the short arm (Fig. 1, 2). This is in agreement with the standard karyotype of barley established by Tjio and Hagberg [1]. However, in three

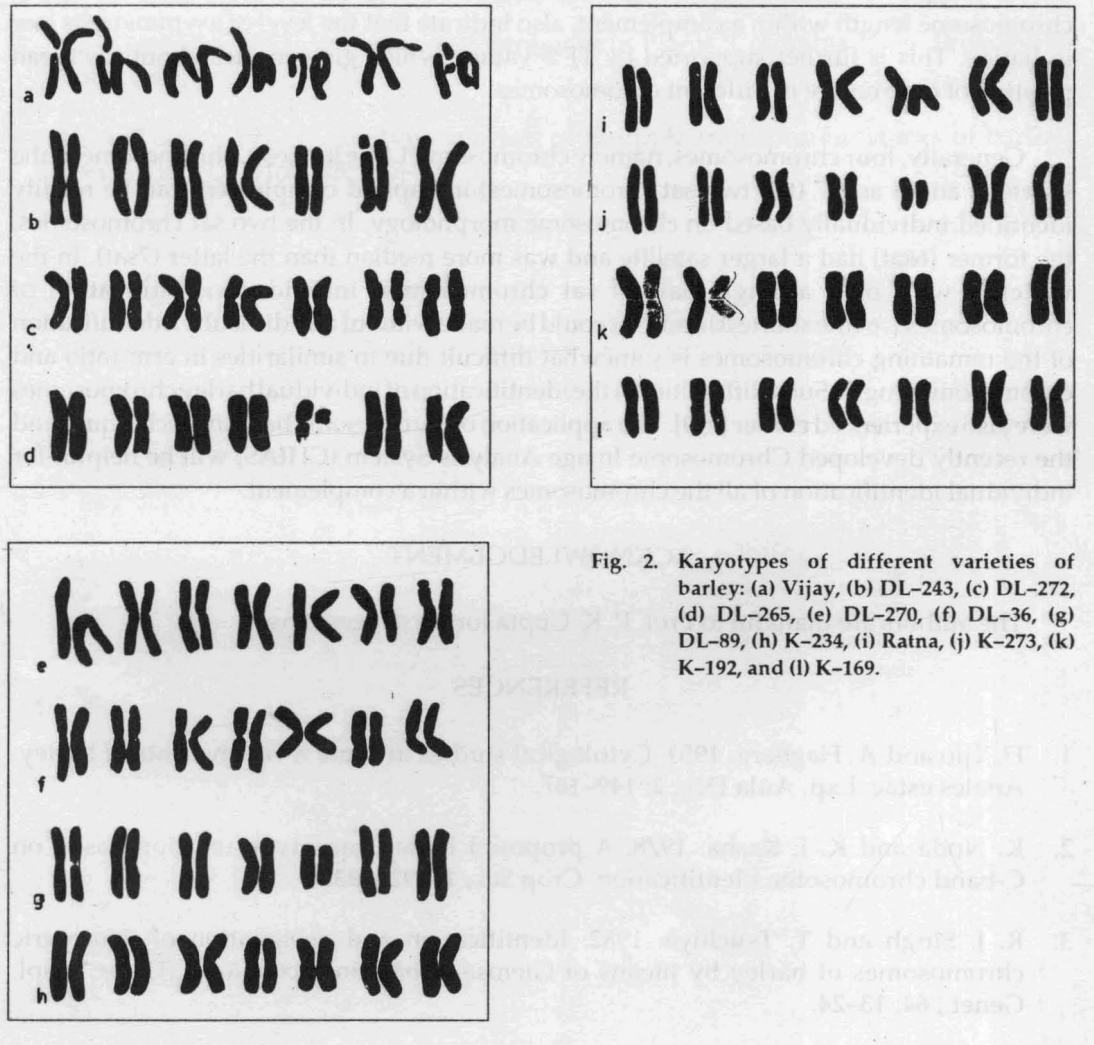

Fig. 2. Karyotypes of different varieties of barley: (a) Vijay, (b) DL–243, (c) DL–272, (d) DL–265, (e) DL–270, (f) DL–36, (g) DL–89, (h) K–234, (i) Ratna, (j) K–273, (k) K–192, and (l) K–169.

varieties included in the present study only one pair of sat chromosomes was found (Fig. 2). This may be attributed to the total loss of sat region in one pair due to translocation, hybridization or deletion. Linde-Laursen et al. [6, 7] also reported different numbers of sat chromosomes in several populations of a single *Hordeum* species and concluded that this character is less fixed than generally appreciated.

Symmetric karyotypes, mostly with metacentric chromosomes, were observed in all the varieties studied. Karyotype symmetry was examined using Stebbins [8] classification based on four degrees of asymmetry in centromere position and 3 degrees of asymmetry in chromosome size. All but DL-89 belonged to 1A category, indicating symmetric nature of the karyotypes. S% and relative length estimates, which give an idea about variation in chromosome length within a complement, also indicate that the level of asymmetry is low in barley. This is further supported by TF% values, which give an idea about the mean position of centromere in different chromosomes.

Generally, four chromosomes, namely chromosome 1 (the longest), chromosome 5 (the shortest) and 6 and 7 (the two sat chromosomes) in haploid complement can be readily identified individually based on chromosome morphology. In the two sat chromosomes, the former (6sat) had a larger satellite and was more median than the latter (7sat). In the varieties with only a single pair of sat chromosomes, individual identification of chromosomes 1, 6 (the shortest) and 7sat could be made without any difficulty. Identification of the remaining chromosomes is somewhat difficult due to similarities in arm ratio and chromosome length. Such difficulties in the identification of individual barley chromosomes were also experienced earlier [2, 9]. The application of chromosome banding techniques and the recently developed Chromosome Image Analysis System (CHIAS) will be helpful for individual identification of all the chromosomes within a complement.

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