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KARYOLOGICAL STUDIES IN SOME SPECIES OF *HORDEUM* L.

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Abstract

Karyological studies in *Hordeum* species viz., *H. bogdanii* (2x), *H. brachyantherum* ssp. *californicum* (2x), *H. brevisubulatum* (4x), *H. brevisubulatum* ssp. *nevskianum* (2x), *H. brevisubulatum* ssp. *turkestanicum* (4x), *H. bulbosum* (4x), *H. chilense* (2x), *H. comosum* (2x), *H. depressum* (4x), *H. jubatum* (2x), *H. marinum* (2x), *H. murimum* ssp. *leporinum* (4x), *H. lechleri* (6x), *H. roshevitzii* (2x), *H. secalinum* (4x), *H. stenostachys* (2x) and *H. vulgare* ssp. *agriocrithon* (2x) were made using aceto-orcein staining technique. Detailed morphological observations of chromosomes included total length, arm ratio and presence of satellites. The cytological technique employed gave good spread of chromosomes, an important prerequisite for karyotype analysis.

Introduction

Hordeum species of the family Poaceae, sub family Festucoideae and tribe Hordeae (Hitchcock, 1951), which grow in temperate regions of the world vary widely as to the extent of distribution or range of adaptability as reflected in their genetic constitution, morphology and physiology. Karyosystematics aims to reconstruct chromosomal evolution and diversification to elucidate the adaptiveness of karyotypes and to study their role in specification and their inherent organizational constraints upon evolutionary change. In taxonomy, karyotype features are helpful in reaching a conclusion alongwith macromorphological characteristics. The first report of chromosome number (2n=14) for *H. vulgare* was made by Kihara (1924). Tjio & Hagberg (1951) established the standard chromosome karyotype of barley by measuring arm ratios, relative lengths and secondary constrictions. Cytologic and cytogenetic studies of species and of interspecific and inter generic hybrids have been presented by Morrison (1959), Morrison & Rajhathy (1959), Rajhathy & Morrison (1959, 1961) and Wagenaar (1960). Chin (1941) studied the cytology of some of the wild species. An exhaustive cytotaxonomic study of the species occurring in Canada and United States has been published by Bowden (1962). In the present study karyotypes of different *Hordeum* taxa were studied. The criteria of discrimination between the species was the number and morphology of the nucleolar chromosomes.

Materials and Methods

The germplasms used in the present study are listed in Table 1. Root tips were harvested and preserved as described by Vahidy *et al.*, (1993). Preserved root tips were transferred in 2% aceto-orcein and stored for one week in a refrigerator at 4°C. Soften-

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Table 1. Karyological parameters of root tip mitotic chromosomes in 16 diploid species of *Hordeum*. Readings in each column represent total length of chromosome in micrometer, relative length and long/short arm ratio respectively, readings represent means of five cells.

Sr. No.	Taxon	Ploidy Level	Accession Number	Chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	*H. bogdanii*	2x	H107	10.60	10.10	9.80	9.20	9.35	*8.50	*9.60	---	---	---	---	---	---	---
	Wil (Fig.1A)			15.78	15.04	14.59	13.70	13.92	12.65	14.29	---	---	---	---	---	---	---
				1.35	1.06	1.22	1.19	1.22	1.43	2.00	---	---	---	---	---	---	---
2.	*H. brachyantherum*	2x	H323	10.10	9.90	8.80	8.20	8.20	*9.30	*9.10	---	---	---	---	---	---	---
	Nevski s.1. (Fig.1B)			15.88	15.56	13.84	12.89	12.89	14.62	14.31	---	---	---	---	---	---	--
	ssp. californicum,			1.29	1.20	1.38	1.05	1.83	2.00	0.98	---	---	---	---	---	---	--
	Covas & Stebbins																
3.	*H. brevisubulatum*	4X	H221	5.00	4.60	4.60	4.75	4.70	4.00	3.50	4.00	4.00	4.50	4.50	4.50	*3.75	*3.50
	(Trinius) Link (Fig.3B)			1.50	1.30	1.30	1.37	1.23	1.0	1.33	1.00	1.00	2.00	1.57	1.00	2.75	1.33
4.	*H. brevisubulatum*	2X	H120	12.40	12.00	10.00	10.00	9.60	*10.80	*11.80	---	---	---	---	---	---	---
	(Trinius) Link (Fig.1C)			16.18	15.66	13.05	13.05	12.53	14.09	15.40	---	---	---	---	---	---	---
	ssp. nevskiamum Bowden			1.20	1.66	1.77	1.22	1.50	1.25	1.68	---	---	---	---	---	---	---
5.	ssp. *turkestanicum*	4X	H222	9.20	9.20	8.60	8.00	7.80	7.20	7.20	7.20	7.00	8.70	9.60	9.40	*7.20	*6.20
	(Nevski) Tzvel. (Fig.3C)			1.55	1.30	1.04	1.22	1.05	1.00	1.00	1.40	0.94	1.72	1.68	1.14	1.57	2.10
6.	*H. bulbosum* L.	4X	H78	9.00	8.40	7.80	7.90	7.40	8.30	*7.65	---	---	---	---	---	---	---
	(Fig.3A)			1.25	1.21	1.43	1.25	1.05	1.80	1.04	---	---	---	---	---	---	---
7.	*H. chilense*	2X	H114	8.00	7.60	7.00	7.20	5.60	*7.20	*7.60	---	---	---	---	---	---	---
	Roem. & Schult (Fig.1D)			15.93	15.14	13.94	14.34	11.15	14.34	15.14	---	---	---	---	---	---	---
				1.10	1.23	1.05	1.00	1.54	1.00	1.71	---	---	---	---	---	---	---
8.	*H. comosum*	2X	H115	9.00	7.60	7.60	8.80	6.80	*8.00	*7.60	---	---	---	---	---	---	---
	Presl. (Fig.1E)			16.25	13.72	13.72	15.88	12.27	14.44	13.72	---	---	---	---	---	---	---
				1.25	1.11	1.37	1.44	1.83	1.00	2.16	---	---	---	---	---	---	---

Table 1 (Cont'd)

Sr. No.	Taxon	Ploidy Level	Accession Number	Chromosomes 1	2	3	4	5	6	7	8	9	10	11	12	13	14
9.	*H. depressum*	4X	H83	7.87	8.00	7.50	6.50	6.00	6.00	5.00	7.00	5.75	6.75	6.00	4.85	*5.00	*6.00
	(Schribn. & Smith) Ryd.			1.52	1.00	1.50	1.16	1.00	1.00	1.00	1.33	1.30	1.70	2.00	1.77	1.50	1.00
10.	*H. jubatum* L.	2X	H102	10.60	10.10	9.60	9.40	8.30	9.20	*11.50	---	---	---	---	---	---	---
	(Fig.2A)			15.43	14.71	13.97	13.68	12.08	13.39	16.73	---	---	---	---	---	---	---
				1.25	1.35	1.18	1.76	1.18	1.62	0.98	---	---	---	---	---	---	---
11.	*H. marinum* Huds.	2x	H105	10.90	11.60	10.40	9.80	9.70	9.10	*11.90	---	---	---	---	---	---	---
	(Fig.2B)			15.35	16.34	14.65	13.80	13.66	12.82	16.76	---	---	---	---	---	---	---
				1.59	1.00	1.60	1.72	1.11	2.13	2.05	---	---	---	---	---	---	---
12.	*H. murimum* L.	4x	H84	7.00	8.00	7.00	7.00	7.00	6.50	6.50	6.50	5.50	5.00	4.00	*6.50	*5.50	*7.00
	ssp. *leporimum* (Fig.4B)			1.33	1.66	1.00	1.33	1.33	1.60	1.16	1.60	1.75	1.50	1.00	1.60	1.08	2.50
	(Link) Arcang. {*H. leporimum* (as given by source}																
13.	*H. roshevitzii* Bowden	2z	H322	9.20	8.80	8.80	8.00	7.60	8.40	*8.40	---	---	---	---	---	---	---
	(Fig.2C)			15.54	14.86	14.86	13.51	12.83	14.18	14.18	---	---	---	---	---	---	---
				1.30	1.30	1.00	1.11	1.38	1.62	1.33	---	---	---	---	---	---	---
14.	*H. secalimum*	4x	H230	9.60	8.60	8.80	7.20	7.90	7.40	8.00	7.27	7.00	6.20	8.00	7.20	7.10	*6.40
	Schreber (Fig.4C)			1.40	1.60	1.00	1.00	1.39	1.05	1.50	1.00	1.18	1.06	1.75	1.57	2.23	1.28
15.	*H. stenostachys* Godr.	2x	H125	9.80	9.60	9.00	8.20	7.60	*10.20	*10.80	---	---	---	---	---	---	---
	(Fig.2D)			15.03	14.72	13.80	12.57	11.65	15.64	16.56	---	---	---	---	---	---	---
				1.33	1.00	1.14	1.27	1.70	1.31	1.70	---	---	---	---	---	---	---
16.	*H. vulgare* L.	2x	H47	7.00	9.25	8.75	8.25	*9.00	*7.5	8.37	---	---	---	---	---	---	---
	ssp. *agriocrithon* (Fig.2E)			12.04	15.91	15.05	14.19	15.48	12.90	14.40	---	---	---	---	---	---	---
	{*H. agriocrithon* Aberg			1.50	1.18	1.25	1.35	1.57	1.14	1.09	---	---	---	---	---	---	---
	(as given by source)}																

*Satellited Chromosomes

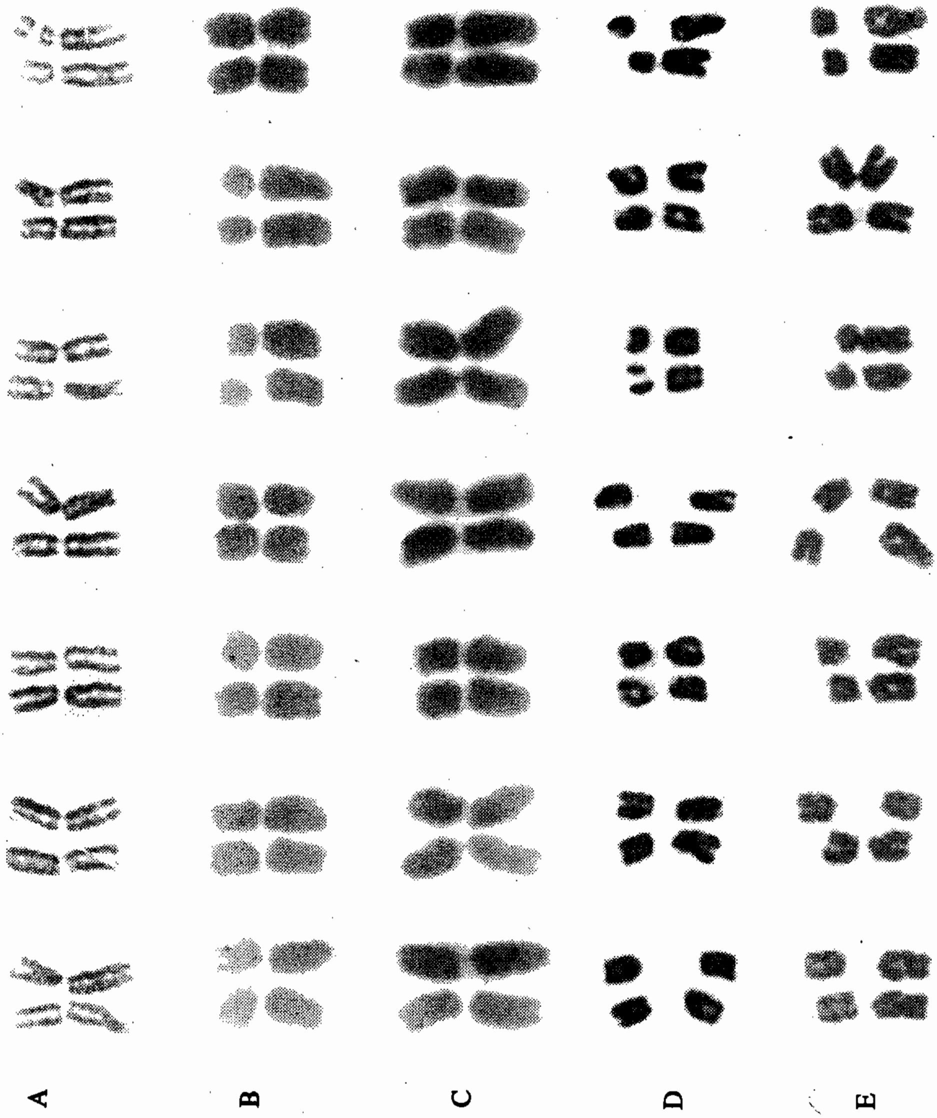

Fig.1. Karyograms constructed from the somatic chromosomes of diploid *Hordeum* species through aceto-orcein staining; A: *H. bogdanii*, B: *H. brachyantherum* ssp. *californicum*, C: *H. brevisubulatum* ssp. *nevskianum*, D: *H. chilense*, E: *H. comosum*.

ing of the tissue was carried out by heating the root tips in 45% acetic acid, avoiding the boiling. The meristematic region of the root tips was squashed in a drop of 45% acetic acid on a slide. A clean cover slip was then applied and the slide gently heated and pressed with the thumb to spread the chromosomes. Slides were made permanent by removing the cover slip in liquid nitrogen, rapidly dehydrating in absolute alcohol and mounting in Euparal or Canada balsam. At least 5 cells were screened and the cells with good spreads were photomicrographed and used to establish karyograms.

Results and Discussion

Karyotype analysis showed that different species and varieties of the genus *Hordeum* are di-, tetra- or hexaploid with a basic number of x = 7. The chromosome complement of *H. bogdanii* (Table 1) consisted of 6 metacentric and 1 submetacentric chromosome pairs with satellites (Fig.1A). The chromosome length ranged from 8.5-10.6μ (Table 1). Similar results were described by Linde- Laursen *et al.*, (1992), while of *H. brachyantherum* ssp. *californicum* (Table 1) had 5 metacentric, 1 submetacentric and 1 SAT-chromosome pairs (Fig.1B). The chromosome length ranged between 8.2-10.1μ (Table 1). The satellites of both species were about the same length as the adjoining short arms. Diamond shaped centromeres as reported by Singh & Tsuchiya (1982) in barley were also observed in some chromosomes of *H. bogdanii* (Fig.1A). In *H. brachyantherum* ssp. *Californicum*, Morrrison (1959) observed 1 pair of chromosomes with long satellites, 1 of long metacentrics and 1 of long submetacentrics. The other pairs were metacentrics or submetacentrics. The existence of accessions with 1 SAT-chromosome pair was previously reported by Chin (1941), whereas Covas (1949) observed 2 SAT-chromosome pairs. Linde-Laursen *et al.*, (1986a) reported 4 metacentrics, 2 submetacentrics and 1 SAT-chromosome pairs. Later they reported 2 pairs with satellites and stated that it may be found with 1 or 2 SAT-chromosome pairs (Linde-Laursen *et al.*, 1992). *H. bogdanii* and *H. brachyantherum* ssp. *californicum* have more or less similar karyotype pattern (Fig.1A and B) and similar genome lengths (Table 1). Both have 1 pair of chromosomes with large satellites on the short arms. The results are in agreement with that of Hsiao *et al.*, (1986). The close genomic relationship between *H. bogdanii* and *H. brachyantherum* ssp. *californicum* (= *Critesion californicum* Covas & Stebbins) was also supported by chromosome pairing data, indicating that they shared a similar 'H' genome of *Elymus canadensis* (Dewey, 1971).

H. chilense (Table 1) is highly polymorphic, mainly inbreeding, wild perennial barley native to central Chile and western Argentina, can be used to transfer genes of disease resistances, ecological adaptability, salt tolerance or nutritional value of grain. The metacentric SAT-chromosome pair of *H. chilense* has longer satellites than the submetacentric one (Fig.1D). The results are in agreement with that of Armstrong *et al.*, (1987) and Zerneke (1987), while Fernandez & Jouve (1984) reported satellites of similar size. The difference suggest chromosome polymorphism. The karyotype of *H. comosum* (Fig.1E) was similar to the South American diploid species as reported by Linde-Laursen *et al.*, (1992). *H. jubatum* (Fig.2A) showed 5 pairs of metacentrics and 2 of submetacentrics, each with 1 SAT-chromosome pair. The chromosome length ranged from 8.3-11.5 μ (Table 2), whereas Chin (1941) and Covas (1949) reported 2 pairs of chromosomes with satellites in tetraploid cytotype. The observation of 3 pairs of SAT-chromosomes per genome was supported by the observation of 6 nucleoli in tetraploid cytotypes (Linde-Laursen *et al.*, 1986a).

The chromosome complement of *H. marinum* (Table 1) consisted of 5 submedian and 2 median chromosome pairs, 1 of the former with satellites (Fig.2B). Previous investigators reported 5 metacentrics, 1 submetacetric and 1 SAT-chromosome pairs (Morrison, 1959, Vosa, 1976; Coucoli & Symeonidis, 1980; Lushnikova, 1988). Some discrepancies with respect to the number of SAT-chromosomes were also apparent. Chin (1941) and Covas (1949) reported 2 chromosome pairs with satellites in diploid as well as in tetraploid cytotypes. The karyotype of the tetraploid cytotype studied by Lushnikova (1988) corresponds to a doubling of the diploid one. However, the investigation of her material and 9 other lines of tetraploid cytotype revealed only 1 pair of chromosomes with satellites. Presence of 1 pair of SAT-chromosomes in both cytotypes is in agreement with that of Linde-Laursen *et al.*, (1989b) and Morrison (1959).

The chromosome complement of *H. roshevitzii* (Table 1) consisted of 5 metacentric and 2 submetacentric chromosome pairs, 1 of the latter with satellites. The satellites were of about half the length of the adjoining short arms (Fig.2C). The chromosome length ranged between 7.6-9.2 μ (Table 1). The diploid *H. roshevitzii* is considered to be closely related to the South American diploids, based on high levels of ring bivalents in interspecific hybrid with *H. chilense* and *H. euclaston* (Bothmer *et al.*, 1986), similarities of isoenzyme banding patterns (Jorgensen, 1986) and C-banding patterns (Linde-Laursen *et al.*, 1989a). Relative to the common South American diploid karyotype, that of *H. roshevitzii* has an additional metacentric chromosome pair and lacks submetacentric SAT-chromosome pair (Linde-Laursen *et al.*, 1980), however, satellites of rather similar size were observed in the metacentric SAT- chromosome pair of *H. chilense* population studied by Fernandez & Jouve (1984). The karyotypic differences indicate that the statement of a close relationship among South American diploids and *H. roshevitzii* must be based on consideration of more than one character.

The karyotype of *H. stenostachys* (Table 1) consisted of 5 metacentric and 2 submetacentric chromosome pairs 1 of each type with satellites, 1 metacentric pair with long satellites and 1 submetacentric pair with satellites about the length of the supporting short arms (Fig.2D). Similar karyotypes of *H. stenostachys* were previously described by Linde-Laursen *et al.*, (1989a), Morrison (1959) and Hunziker *et al.*, (1973). Similarities in banding patterns and chromosome morphology rendered it possible to identify homologues both within and between populations (Linde-Laursen *et al.*, 1989a). *H. vulgare* ssp. *agriocrithon* (Fig.2E) showed 5 pairs of metacentrics and 2 of submetacentric, each with one SAT-chromosome pair. The chromosome length ranged from 7-9.25 μ (Table 1).

The karyotype of *H. bulbosum* (Table 1) consisted of 24 metacentrics including 4 SAT-chromosomes and 4 submetacentric chromosomes (Fig.3A), showed an overall similarity with those described by Chin (1941), Linde-Laursen *et al.*, (1990b) and Morrison (1959). Presence of 4 chromosomes with satellites was supported by observations of up to 3 NORs and 4 nucleoli in $AgNO_3$ stained cells at metaphase and interphase respectively (Linde-Laursen *et al.*, (1990b). *H. bulbosum* (4x) is generally considered an autopolyploid (Berg, 1936; Chin, 1941; Morrison, 1959; Xu & Snape, 1988)

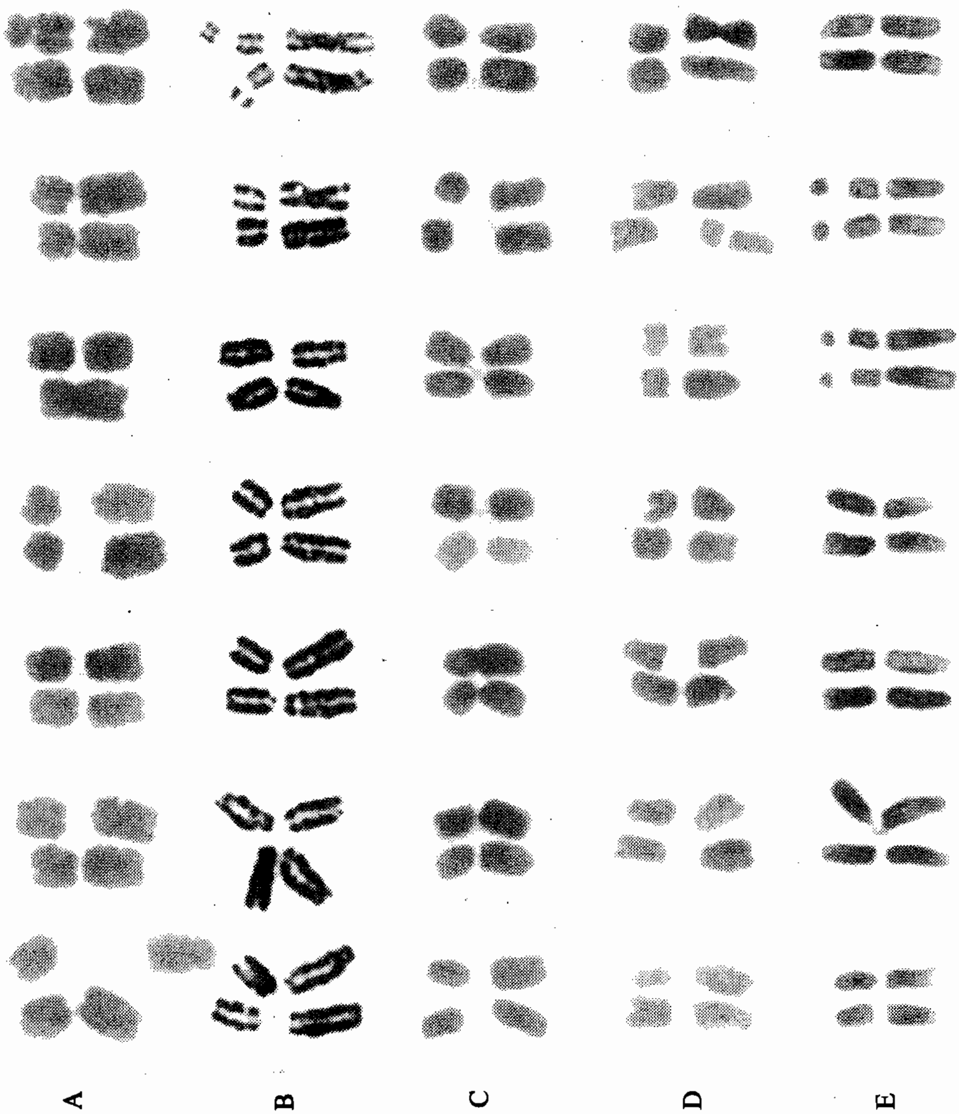

Fig.2. Karyograms constructed from the somatic chromosomes of diploid *Hordeum* species through aceto-orcein staining; A: *H. jubatum*. B: *H. marinum*, C: *H. roshevitzii*, D: *H. stenostachys*, E: *H. vulgare* ssp. *agriocrithon*.

combining 4 homologous or near homologous genomes. Our observations support this conclusion. The presence of 4 chromosomes of each type theoretically means the presence of 7 homologous groups (Table 1; Fig.3A).

In the present study only 4 chromosomes with satellites were observed in diploid *H. brevisubulatum* ssp. *nevskianum* (Table 1; Fig.1C) as well as in tetraploid *H. brevisubulatum* and its spp. *turkstanicum* (Table 1; Figs.3B and C). Observation of 6 nucleoli, 4 large, 1 small and micronucleolus in tetraploid ssp. *turkestanicum* by Linde-Laursen & Bothmer (1984), indicated the existence of 6 chromosomes with nucleolar forming capacity. The 4 large nucleoli obviously corresponded to the 4 commonly observed SAT-chromosomes, the small one to the SAT-chromosome observed rarely, and the micronucleolus to a chromosome with very low nucleolar forming capacity, which did not result in the formation of an observable secondary constriction. The observation of 5 chromosomes with satellites in the tetraploid ssp. *turkestanicum* by Linde-Laursen & Bothmer (1984) indicates a polymorphism for this character. Linde-Laursen *et al.*, (1980) also reported a wide variation in C-banding patterns among *H. brevisubulatum* taxa.

The karyotype of *H. depressum* (Table 1) had 9 metacentrics, 3 submetacentrics and 2 SAT-chromosome pairs with small spherical and elongated satellites (Fig.4A). The chromosome length ranged between 4.85-8 μ (Table 1). Covas (1949) also reported one pair with small spherical and the other with elongated satellites. It has usually been considered an allotetraploid possessing 'H' genome and another unidentified genome (possibly a modified 'H' genome) between which only a low level of pairing occurs (Bothmer *et al.*, 1988). The chromosomes with satellites resembled those present in diploid *H. brachyantherum* (Linde-Laursen *et al.*, 1986a).

H. murinum ssp. *leporinum* (Table 1) had 8 metacentric including 1 satellited and 6 submetacentric, including 2 satellited chromosome pairs (Fig.4B). The chromosome length ranged between 4-8 μ (Table 1). The 3 pairs of SAT-chromosomes in this taxon are not always visible in every cell but in good preparations are quite clear (Fig.4B). The satellites of 1 pair were larger than those of the 2 other pairs, which appeared similar. The number of SAT-chromosomes matched the presence of 6 nucleoli in interphase as reported by Linde-Laursen *et al.*, (1989b).

H. secalinum (Table 1) comprised of 10 metacentric, including 1 satellited and 4 submetacentric chromosome pairs (Fig.4C). The chromosome length ranged between 6.2-9.6 μ (Table 1). The presence of 1 chromosome pair with satellites in *H. secalinum* is in agreement with the results of Linde-Laursen *et al.*, 1986b; Cauderon & Cauderon, 1956; Rajhathy *et al.*, 1964. Vosa (1976) reported 2 SAT-chromosome pairs. The presence of 1 pair of SAT-chromosomes matched observation of $AgNO_3$-stained interphase with a maximum of 2 nucleoli (Linde-Laursen *et al.*, 1986b). Observations of similarity in chromosome morphology and C-banding patterns of *H. secalimum* and *H. capense* (Linde-Laursen *et al.*, 1986b), high paring in PMCs of a *H. secalinum* X *H. capense* hybrid compared to other tetraploid cross combinations comprising one of the two species (Bothmer *et al.*, 1988), similarities in growth habit (Bothmer & Jacobsen, 1979) and isoenzyme patterns (Jorgensen, 1986) indicated a close relationship between the two species.

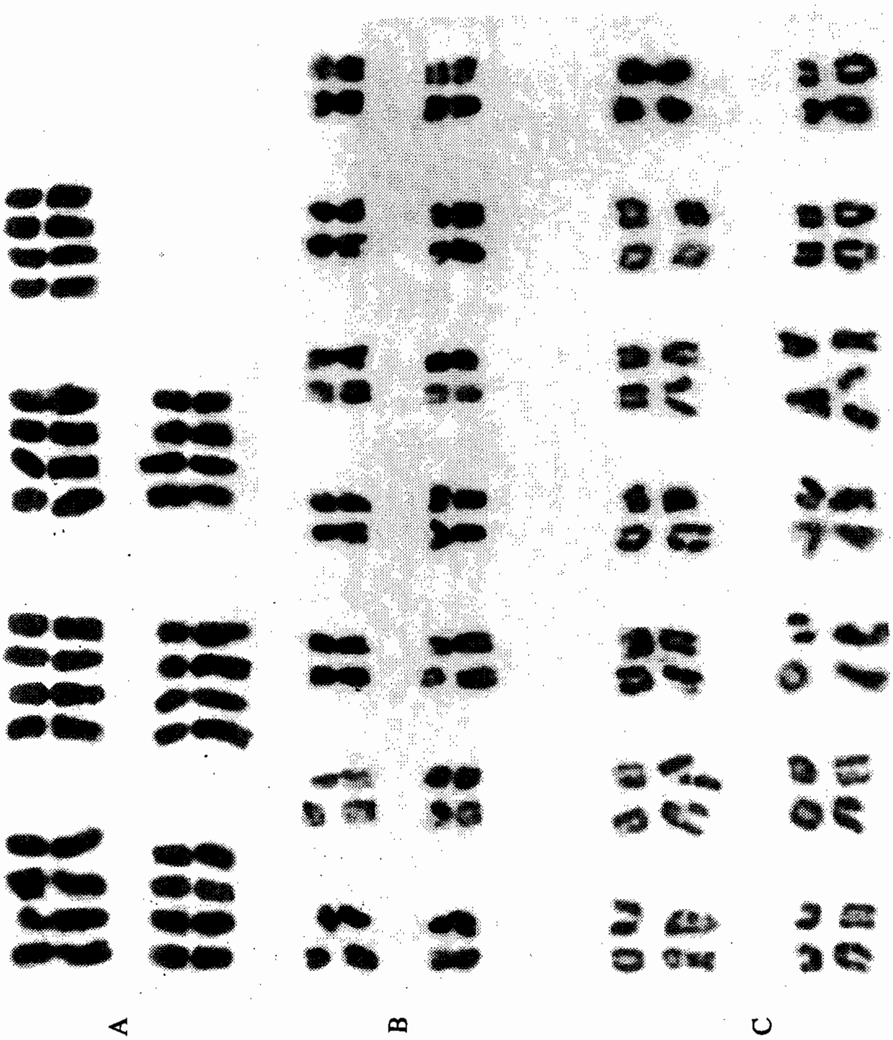

Fig.3. Karyograms constructed from the somatic chromosomes of tetraploid *Hordeum* species through aceto-orcein staining; A: *H. bulbosum*, B: *H. brevisubulatum*, C: *H. brevisubulatum* ssp. *turkestanicum*.

Table 2. Karyological parameters of root tip mitotic chromosomes in hexaploid *H. lechleri*, readings represent means of five cells

Chromosome No.	Total Length µm	Arm ratio L/S	Centromeric Index
1	7.00	1.33	42.85
2	7.00	1.33	42.85
3	7.00	1.33	42.85
4	7.00	1.33	42.85
5	6.50	2.25	30.76
6	6.00	2.00	33.33
7	5.00	1.50	40.00
8	5.00	1.50	40.00
9	6.00	1.00	50.00
10	5.00	1.50	40.00
11	5.00	1.50	40.00
12	5.00	1.50	40.00
13	4.00	1.00	50.00
14	6.50	1.16	46.15
15	4.50	2.00	50.00
16	6.00	2.00	50.00
17	*5.50	1.75	57.14
18	*6.00	2.00	50.00
19	*6.00	2.00	40.00
20	*6.00	1.00	50.00
21	*6.00	1.00	50.00

*Satellited Chromosomes

The karyotype of *H. lechleri* (Table 2) had 13 metacentrics, 3 submetacentrics and 5 SAT-chromosome pairs (Fig.5). Of the latter, the 2 similar metacentric chromosome pairs had long satellites, 1 metacentric and 2 submetacentric ones had short satellites. The chromosome length ranged between 4-7 µ (Table 2). The results are in agreement with that of Linde-Laursen *et al.*, (1990a) and contrary to the karyotype reported by Rajhathy & Morrison (1961) and Rajhathy & Symko (1974), who found a maximum of only 4 SAT-chromosomes. Presence of 5 SAT-chromosomes per genome, agrees with the observations of 5 SAT-chromosomes and 5 nucleoli in 4 polyhaploids derivatives of *H. lechleri* (Linde-Laursen & Bothmer, 1986). The normal presence of 5 chromosomes with nucleolus forming capacity is further inferred from the maximum number of nucleoli in 29 aneuploid *H. lechleri* X H. *vulgare* hybrids (Linde-Laursen & Bothmer, 1986). The species is considered to have an alloploid origin (Rajhathy & Morrison, 1961, Reddy & Subrahmanyam, 1985). The basic karyotype of *H. lechleri* was similar to the basic one of *H. procerum* except for the replacement of the metacentric chromosome pair with small satellites of *H. procerum* by a metacentric pair with long satellites

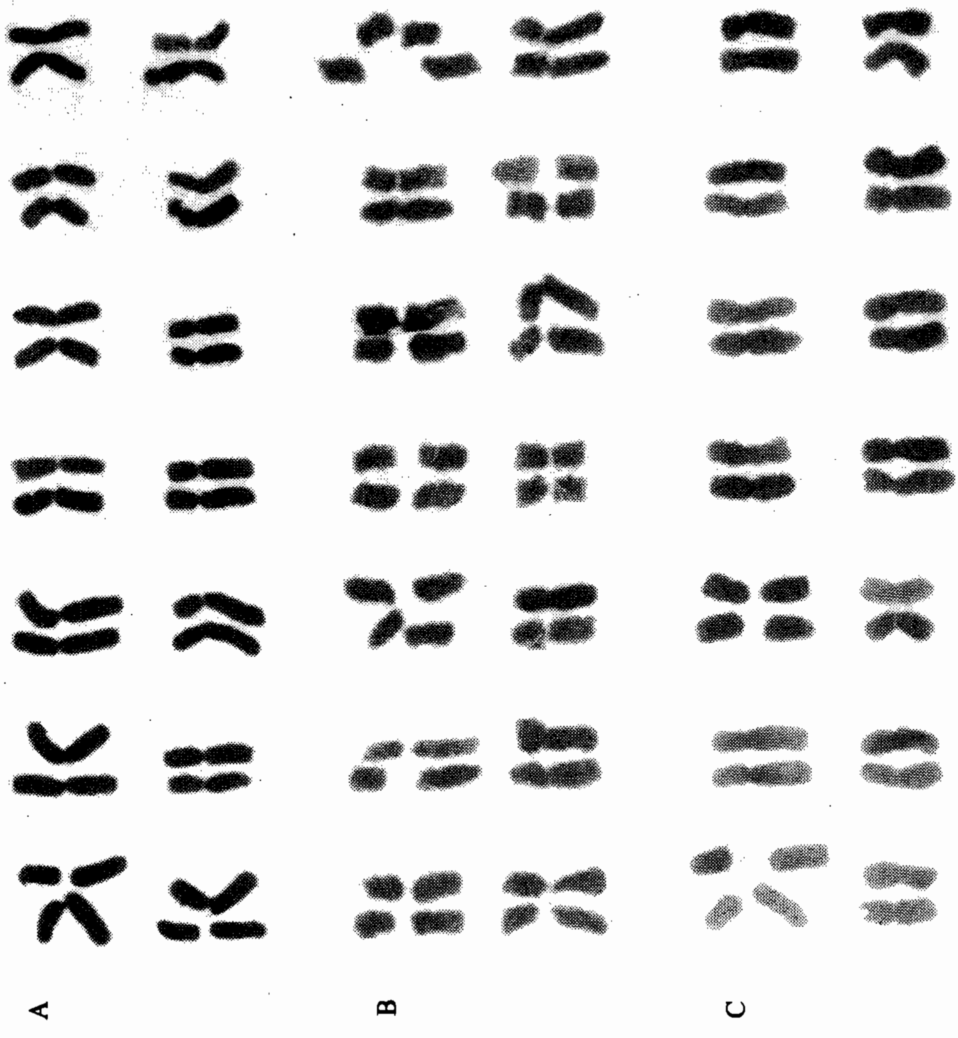

Fig.4. Karyograms constructed from the somatic chromosomes of tetraploid *Hordeum* species through aceto-orcein staining; A: *H. depressum*, B: *H. murinum* ssp. *leporinum*, C: *H. secalinum*.

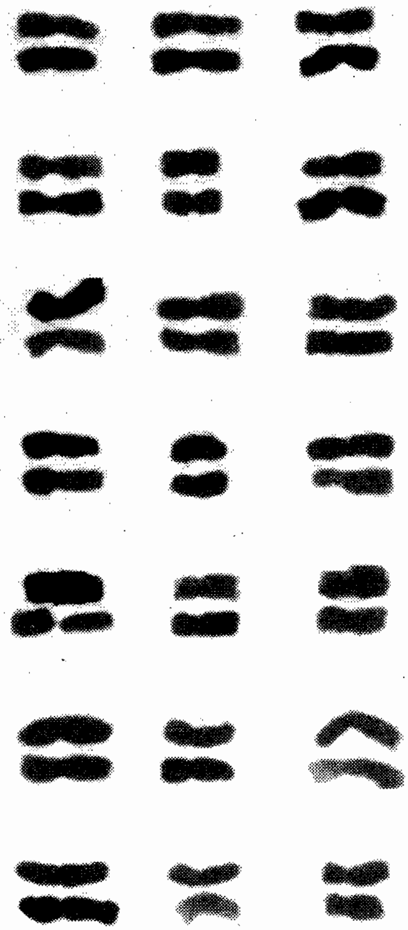

Fig.5. Karyogram constructed from the somatic chromosomes of *H. lechleri* through aceto-orcein staining.

(Linde-Laursen *et al.*, 1990a). The similarity of the latter pair with the pair normally found in diploid American *Hordeum* species (Hunziker *et al.*, 1973; Linde-Laursen *et al.*, 1986a; 1989a) supports the belief that *H. lechleri*, in addition to the two genomes, which are similar to those of those of *H. jubatum, H. brachyantherum* (4x) and *H. guatemalense* (Rajhathy & Morrison, 1961; Reddy & Subrahmanyam, 1985; Linde-Laursen *et al.*, 1990a), has a third genome derived from a South American diploid *Hordeum* species. Jorgensen (1986) proposed that the species was either *H. flexuosum* or *H. pubiflorum*. However the similarity of the karyotypes of all diploid South American Hordeum species except *H. cordobense* and *H. muticum* renders it impossible to suggest any single species as the genome donor on this basis (Linde-Laursen *et al.*, 1989a).

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