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N- BANDING PATTERNS OF HETEROCHROMATIN DISTRIBUTION IN *HORDEUM JUBATUM* CHROMOSOMES

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Abstract

The N-band patterns of heterochromatin distribution in *Hordeum jubatum* chromosomes was studied to identify general patterns or preferential sites for heterochromatin. Mean number of Giemsa N-bands indicated that they are located mostly on the intercalary and centromeric regions and rarely on the terminal ends. Heterochromatic N-bands located at terminal and distal positions respectively on the satellites of first two pairs and the third pair had two bands in the middle and terminal portion of the satellite.

Introduction

Hordeum jubatum (Foxtail barley) is a perennials plant species in the grass family Poaceae. It occurs wild mainly in northern North America and adjacent northeastern Siberia. However, as it escaped often from gardens it can be found world-wide in areas with temperate to warm climates and is considered a weed in many countries. The species is a polyploid and originated *via* hybridization of an East Asian *Hordeum* species with a close but extinct relative of Californian *H. brachyantherum*. *H. jubatum* is the most compatible partner to be used in interspecific crosses not only with cultivated barley but also with wild *Hordeum* species (Bothmer *et al.*,1983; Bothmer & Jacobsen, 1985). The C-banded metaphase chromosomes of diploid and tetraploid cytotypes of *H. jubatum* were investigated by Lushnikova (1988). A comparative karyological analysis of the two cytotypes showed that the genome of hexaploid *H. jubatum* involved I genome of tetraploid *H. jubatum* and a diploid genome of unknown origin. It may have presumably originated from *H.brachyantherum* (2x) or *H. marinum* (2x). Kubalakova *et al*., (2003) developed procedures for chromosome analysis and sorting using flow cytometry (flow cytogenetics) for rye (*Secale cereale* L.) chromosomes. Vinogradov (2003) identified 3036 diploid species from the Plant DNA C-values database and compared each one against the United Nations Environmental Programme World Conservation Monitoring Centre (UNEP-WCMC) species database to determine its conservation status. He noted a striking relationship between genome size and conservation status; species with large genomes appeared to be at greater risk of extinction than those with smaller genomes. To analyze the phylogenetic relationships among diploid and polyploid taxa of the genus the nuclear rDNA internal transcribed spacer region (ITS) was analyzed for 91 accessions, representing all *Hordeum* species by Blattner (2004).The ITS data indicate times of independent evolution of paralogous rDNA clusters on different chromosomes intermitted by sweeps of homogenization among these clusters and bi-directional homogenization of the clusters in diploids (Blattner, 2004). rDNA–RFLP analysis detected rDNA polymorphisms more sensitively and corroborated the estimation of ancestry based on the FISH pattern. RFLP analysis showed that I genome polyploid species of *Hordeum* generally retain variants of 18S–25S rDNA repeat sequences contributed by their putative ancestral species, although quantitative changes in their copy numbers after polyploidization were apparent in some species (Taketa *et al*.,2005). An

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increasing interest in the use of wild relatives of crop species has led to considerable studies of such materials in order to obtain a crop with improved disease and pest resistance and with increase protein content. Due to the possibility for wide hybridization, wild species of *Triticeae* are potentially important genetic resources in plant breeding (Jahan & Vahidy, 2008). The aim of the present study is to seek and exploit the N-banding pattern present in *Hordeum jubatum* in order to evaluate the use of the bands as markers in cytogenetic investigations.

Materials and Methods

Chromosome preparations followed the Giemsa N-banding technique after squashing meristematic cells from root tips. Detailed methods have been described earlier (Vahidy *et al.*, 1993). At least five cells were screened and the cells with good spreads and bands were photomicrographed and used for analyzing banding pattern and to establish karyograms.

Results

The karyogram of a tetraploid cytotype of *H. jubatum* is shown in Fig. 1. In addition to a centromeric band the short arm of chromosome 1 had three equally spaced interstitial bands along the length of the chromosome. The long arm had only a light band near the centromere. Chromosome 2 possessed a centromeric, interstitial at a median position and a terminal band on each arm. Each arm of chromosome 3 had a centromeric, two interstitial and a terminal bands. Among the interstitial bands on short arm one was strong proximal to the centromere and the other weak one proximal to the telomere. Interstitial bands on the long arm were in the medium position and distal to the centromere. Each arm of chromosome 4 showed a centromeric and two interstitial bands (Table 1). Centromeric, interstitial and terminal bands were present on each arm of chromosome 5, interstitial band on the short arm proximal to the telomere while on long arm was proximal to the centromere. A light interstitial band proximal to the telomere was also found on the long arm of this chromosome. Short arm of chromosome 6 had a cntromeric, interstitial and a terminal bands, while the long arm showed a centromeric and an interstitial band at a median position. Chromosome 7 showed only interstitial band proximal to the centromere on each arm. An interstitial band at a median position was also found on the long arm . A heavy centromeric and interstitial bands were present on the short arm of chromosome 8. Its long arm had a thick and thin interstitial bands proximal to the centromere and telomere respectively. Both arms of chromosome 9 showed two heavily stained interstitial bands. Bands on the short arm were proximal to the centromere and at a median position, while among the bands on the long arm one was proximal to the centromere and the other proximal to the telomere. Short arm of chromosome 10 possessed a centromeric and a telomeric bands while the long arm had four interstitial bands at different locations. Chromosome 11 showed a centromeric, interstitial (proximal to the centromere) and a terminal band on the short arm. The long arm had a centromeric and three interstitial bands. Short arm of chromosome 12 had a thin centromeric and a thick telomeric bands (excluding satellite). A terminal band on the satellite was also found. The long arm showed a thin centromeric and two thick interstitial bands, proximal to the centromere and the telomere. Satellite of chromosome 13 possessed a band at a median position, short arm had a terminal and long arm a centromeric and three interstitial bands at different locations. Chromosome14 showed bands at a terminal and median positions of satellite, a centromeric and telomeric bands (excluding satellite) on the short arm and a centromeric and two interstitial bands on the long arm.

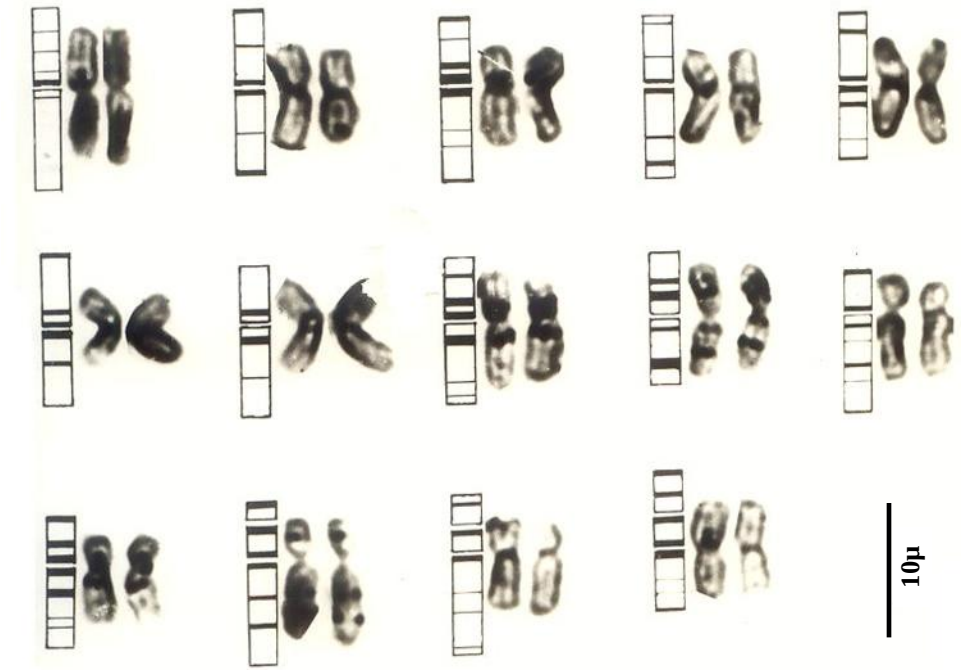

Fig. 1. Karyogram and ideogram developed from the cell of *H. jubatum* through Giemsa N-banding technique.

Table 1. Giemsa N-banding pattern (considering constitutive heterochromatin) in tetraploid *Hordeum jubatum*.

H. jubatum	Homologous groups													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Band position	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**
C	1/0	1/1	1/1	1/1	1/1	1/1	0/0	1/0	0/0	1/0	1/1	1/1	0/1	1/1
IPC	1/1	0/0	1/0	0/0	0/1	1/0	1/1	1/1	1/1	0/2	1/1	0/1	0/1	0/0
IMP	1/0	1/1	0/1	1/1	0/0	0/1	0/1	0/0	1/0	0/1	0/1	0/0	0/1	0/1
IPT	1/0	0/0	1/1	1/1	1/1	0/0	0/0	1/2	0/1	0/1	0/1	0/1	0/1	0/1
T	0/0	1/1	1/1	0/0	1/1	1/0	0/0	0/0	0/0	1/0	1/0	1/0	1/0	1/0
SAT L/M/T												0/0/1	0/1/0	0/1/1

S= Short arm, L= Long arm, 0, 1= Number of dark bands, C= Centromeric, IPC= Interstitial proximal to centromere, IMP= Interstitial at median position, IPT= Interstitial proximal to telomere, T=Telomeric, SAT-LMT= Dark bands at lower, median and a terminal positions of satellites.

Discussion

Hordeum is a genus of 25-30 species widely distributed in temperate regions of the world.The genetic diversity in cultivated and wild forms in the genus *Hordeum* is extremely rich and interesting (Jahan & Vahidy, 2007). Chromosome banding techniques provide an important tool in analyzing karyotypes and detecting chromosome polymorphism. Band polymorphism affords the possibility of using bands simultaneously with genetic markers in cytogenetgic studies (Vahidy & Jahan, 1995). The present study deals with the N-band patterns of heterochromatin distribution in *Hordeum jubatum* chromosomes. There are two pairs of SAT-chromosomes in diploid (Vahidy & Jahan, 1998) and three in tetraploid *H. jubatum* (Fig.1), whereas Chin (1941) and Covas (1949)

reported two pairs of chromosomes with satellites in latter cytotype. The observation of three pairs of SAT- chromosomes per genome was supported by the observation of six nucleoli in tetraploid cytotypes (Linde-Laursen *et al.*, 1986). Mean number of Giemsa N-bands indicated that they are located mostly on the intercalary and centromeric regions and rarely on the terminal ends. Heterochromatic N-bands located at terminal and distal positions respectively on the satellites of first two pairs and the third pair had two bands in the middle and terminal portion of the satellite. Centromeric bands on long arms of chromosomes 1,7, 10 and short arms of 9 and 13 observed by N-banding technique were not revealed by C-banding (Linde-Laursen *et al.*, 1986). C-banded karyotypes of tetraploid *H. jubatum* and *H. brachyantherum* are similar to that of *H. roshevitzii*. Linde-Laursen *et al.*,1980 interpreted a close relationship between these taxa as suggested by Jorgensen (1986) based on meiotic and electrophoretic analysis respectively. *H. jubatum* is the most compatible partner to be used in interspecific crosses not only with cultivated barley but also with wild *Hordeum* species (Bothmer *et al*.,1983; Bothmer & Jacobsen,1985). Studies on the genome analysis of *H. jubatum* revealed it to be a segmental allotetraploid (2n =4x =28), with a genome formula of HI HI H2 H2 (Rajhathy *et al*., 1964). The two genomes of *H. jubatum* are believed to be contributed by *H. brachyantherum* ssp. *californicum* and *H. cordobense.* There has been controversy in interpreting the nature of the polyploidy found in *H. jubatum* (Bothmer *et al*., 1987; 1988) but a very strong genetic regulation of homoeologous meiotic pairing (a diplodizing mechanism) is known to exist (Wagenaar, 1960; Gupta & Fedak, 1985; Bothmer *et al*., 1988). A comparative karyological analysis of the tetra- and hexaploid cytotypes by Lushnikova (1988) showed that the genome of hexaploid *H. jubatum* involves a genome of tetraploid *H. jubatum* and a diploid genome of unknown origin. It may have originated from *H. brachyantherum* (2x) or *H. marinum* (2x).

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