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PATTERNS OF HETEROCHROMATIN DISTRIBUTION IN *HORDEUM DEPRESSUM* (SCHRIBN. & SMITH) RYD., CHROMOSOMES

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Abstract

The N-band pattern of heterochromatin distribution in tetraploid *Hordeum depressum* chromosomes was studied to identify general patterns or preferential sites for heterochromatin. An ideogram was developed for the description of individual N-bands. The karyotype of *H. depressum* had 9 metacentrics, 3 submetacentrics and 2 SAT chromosome pairs with small spherical and elongated satellites. Giemsa N-banding patterns of *H. depressum* showed 6 bands per chromosome on an average. Mean number of bands indicated that it possessed 0.32 centromeric, 0.5 intercalary and 0.18 telomeric bands.

Introduction

Hordeum depressum is a species of barley commonly known as low barley and dwarf barley. It is native to the western United States from Idaho to California, where it can be found in moist habitats such as vernal pools. This is a small annual grass forming petite patches of thin, hairy leaves and erect stems to half a meter in maximum height. The green or reddish green inflorescence is 2 to 6 centimeters long and about half a centimeter wide. Like other barleys the spikelets come in triplets. There is a large fertile central spikelet about a centimeter long and two smaller, often sterile spikelets on pedicels, each 3 to 5 millimeters long. The North American annual tetraploid *H. depressum,* has previously been considered a pure alloploid with the H genome in combination with an unrelated genome (Bothmer *et al.*, 1988). It may, however, be a segmental alloploid or even an autoploid, but with a very strong diploidizing mechanism, similar to the one found in *H. marinum* and *H. murinum.* This is confirmed by results from *Secale* sp. x *H. depressum* hybrids that show a very high pairing between the two *H. depressum* genomes (Petersen 1991). The quantitative measurement of individual chromosomes using digital methods is an efficient method for species comparison. Merritt & Craig (1984) used an interactive chromosome analysis system for the genetic study of barley varieties. *H. chilense* and *H. depressum* grow in different areas of the world, but their chromosome morphology indicated that they may be closely related genetically and thus have good potential to cross. *H. depressum* arose as a consequence of hybridization between *H. brachyantherum* ssp. *californicum* and *H. intercedens*, probably with *H. brachyantherum* ssp. *californicum* as the female parent (Salomon & Bothmer; 1998). Taketa *et al*., (2005) studied the ancestry of American polyploid *Hordeum* species with the I genome inferred from 5S and 18S–25S rDNA and reported that *H. depressum* had three pairs of 5S rDNA sites and four pairs of 18S–25S rDNA

sites on four pairs of chromosomes. Genome size variation in plants is thought to be correlated with cytological, physiological, or ecological characters. However, conclusions drawn in several studies were often contradictory. To analyze nuclear genome size evolution in a phylogenetic framework, DNA contents of 134 accessions, representing all but one species of the barley genus *Hordeum* L., were measured by flow cytometry.The group of tetraploid species combining two H genomes revealed genome sizes between 15.52 pg (*H. depressum*) and 18.57 pg (*H. brachyantherum* subsp. *brachyantherum*) (Jakob *et al.*,2004). The aim of the present study was to seek and exploit the patterns of heterochromatin distribution in *Hordeum depressum* chromosomes 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 were 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

H. depressum (Schribn. & Smith) Ryd.

The karyogram is shown in Fig. 1. In addition to the centromeric band the short arm of chromosome 1 possessed weak interstitial band at a median position. The long arm had a centromeric and four interstitial bands, two of them were proximal while the others distal to the centromere. The short arm of chromosome 2 had a centromeric, two interstitial and a terminal bands and the long arm had two distal and one terminal bands (Table 1). Each arm of chromosome 3 had a centromeric, interstitial and telomeric bands. In chromosome 4 short arm showed a centromeric, two interstitial and a telomeric bands. The long arm had only a centromeric band. Each arm of chromosome 5 showed a centromeric and interstitial band distal to the centromere. In addition to the centromeric bands each arm of chromosome 6 possessed telomeric bands. Chromosome 7 showed a centromeric and two interstitial bands on each arm and a telomeric band on the long arm only. Chromosome 8 showed centromeric bands on each arm. A terminal on the long and interstitial bands on the short arm was also found. In addition to the centromeric bands, each arm of chromosome 9 had three equally spaced interstitial bands along the length of the chromosome. A terminal band on the long arm was also present. Chromosome 10 had a centromeric and two interstitial bands on each arm. Chromosome11 had a centromeric and one, two interstitial bands respectively on the short and long arms. A centromeric band was present only on the short arm of chromosome 12, while the long arm possessed two interstitial (proximal and distal to the centromere) and a terminal band. Only a weak terminal band was present on the short arm of chromosome 13. The long arm had a centromeric and three interstitial bands. Centromeric and telomeric bands were common in each arm of chromosome 14. In addition to this an interstitial band on the long arm was also found.

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

***H. depressum* (Fig. 1)**

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

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

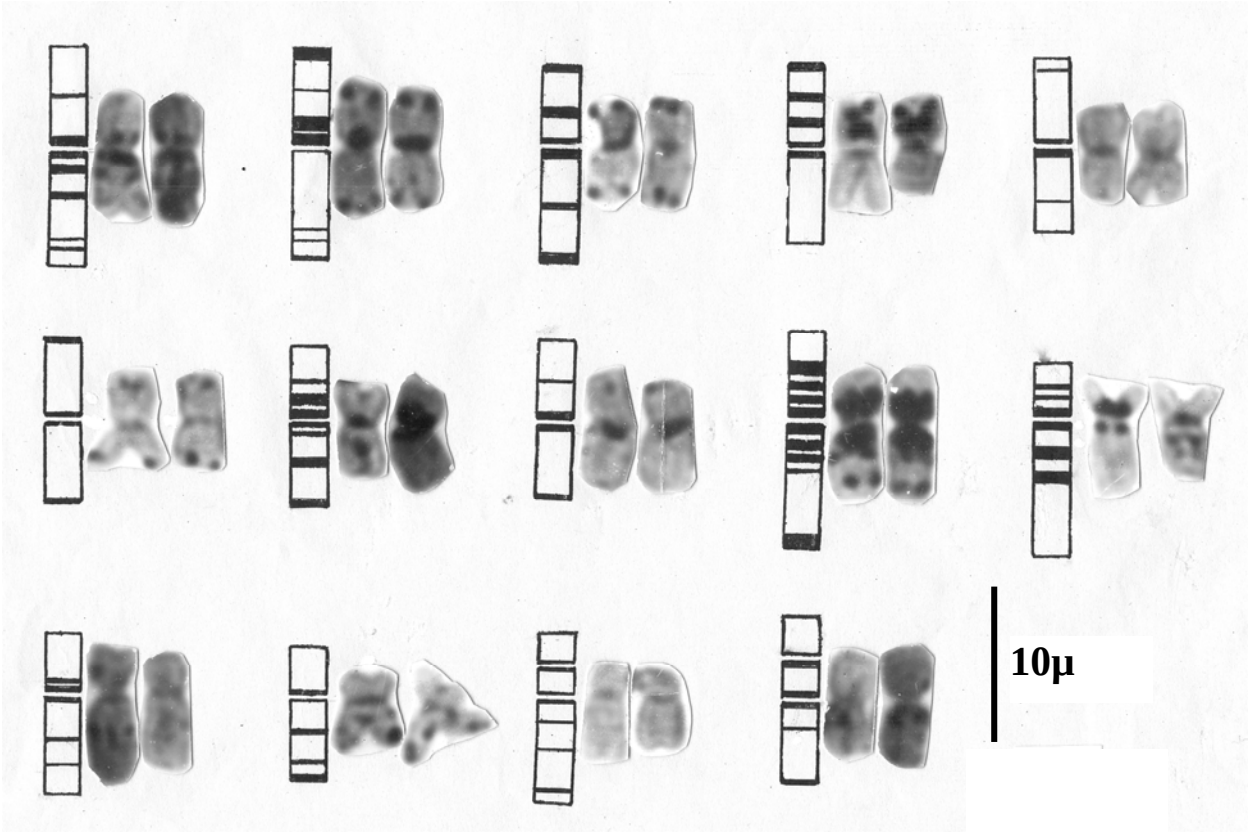

Fig. 1. Karyogram and ideogram developed from the cell of *H. depressum* through Giemsa N-banding technique.

Discussion

An 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. 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 karyotype of *H. depressum* had 9 metacentrics, 3 submetacentrics and 2 SAT chromosome pairs with small spherical and elongated satellites (Vahidy & Jahan, 1998). Covas (1949) also reported one pair with small spherical and the other with elongated satellites. The chromosomes of *H. depressum* showed 6 bands per chromosome on an average in their N-banding pattern (Fig. 1). Mean number of bands indicated that it possessed 0.32 centromeric, 0.5 intercalary and 0.18 telomeric bands (Table 1). Centromeric bands on the short arms of chromosomes 5, 6, 7 and 10 and long arms of chromosomes 3, 6-9 and 11 were observed only by N-banding technique, while bands on the telomeric region of 4, 5, 7, 9 and 10 chromosomes were observed only by C-banding. 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*., 1986). The diploid *H. depressum* is generally considered closely related to *H. brachyantherum* (Covas, 1949, Rajhathy & Morrison, 1959). A similar genome is present in tetraploid *H. depressum* but the other genome in this cytotype is different from that present in tetraploid *H. brachyantherum* (Linde-Laursen *et al.*, 1986). A possible donor may be *H. intercedens* (Linde-Laursen *et al.,* 1986). The suggestion is supported by the sympartic distribution of the two species (Bothmer & Jacobsen, 1985) and the observation of up to 7 closed bivalents and a mean of 12.5 chiasmata in MI cells of *H. depressum* (4x) x *H. intercedens* hybrid (Bothmer *et al.,* 1987). They also observed some pairing in diploid *H. marinum* x *H. depressum* hybrid and assumed that besides the 'H' genome it contains a modified *H. marinum* genome. However, in the intergeneric combinations between *H. depressum* and *Secale cerele* the level of autosyndetic pairing between the *Hordeum* genomes was found to be very high. The pronounced pairing ability between the two genomes of *H. depressum* are probably comparable to that between the genomes of the polyploid cytotypes of *H. marinum* and *H. murinum* and may possibly be correlated to the annual and predominantly inbreeding habit shared by these three species (Petersen, 1991).

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