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GIEMSA N-BANDING PATTERN IN TWO TETRAPLOID CYTOTYPES OF WILD BARLEY (*HORDEUM BULBOSUM* L.)

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Abstract

Distribution pattern of constitutive heterochromatin in 2 tetraploid cytotypes of *Hordeum bulbosum* was studied by Giemsa N-banding technique. Ideograms were developed for the description of individual N-bands.The N-banding patterns is characterized by having one or two centromeric or juxtacentromeric very small bands per chromosome. Further, single bands are present at one side of the nucleolar organizers. The N-banded karyotype of *H.bulbosum* (4x) supports the argument that it is an autopolyploid derivative of the diploid cytotype.

Introduction

Hordeum bulbosum L., is a wild perennial grass, native to the Mediterranean region and is considered the closest relative of cultivated barley, apart from subsp. *spontaneum* and shares the **I** genome with *H. vulgare*. It has been widely used in cereal breeding, because its chromosomes are normally eliminated in the young *H. vulgare x H. bulbosum* hybrid embryos during the first day of development, leaving one set (7) of barley chromosomes. The embryo develops into a haploid barley plant, which can be chromosome-doubled through application of colchicine. It gives rise to a completely homozygous line of barley, which may be used directly in a breeding programme. The chromosomal pairing during meiosis is often very high in the hybrids, but the fertility is extremely low (Xu & Snape, 1988). Some time during later meiotic phases or during the development of the pollen, the normal developmental process fails, leading to formation of sterile gametes, but this process is not understood in detail. Several characters in *H. bulbosum* are of putative interest for transfer into cultivated barley e.g., resistance to powdery mildew (Jones & Pickering, 1978). The wild relative of crop species is invaluable source of genes for novel characters. The optimal utilization and conservation of this natural material for plant breeding purposes depends upon the knowledge of the variation in the wild material and the relationships between the wild plants and their cultivated relatives (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 cytogenetic studies (Vahidy & Jahan, 1995). Several C-banding studies have been attempted by Linde-Laursen 1978, 1979, 1981; Linde-Laursen *et al*., 1980, 1986, 1989a, b) on the cytology and cytogenetics of cultivated barley and its wild relatives. Linde- Laursen *et al*., (1990) compared the Giemsa C-banded karyotypes of several populations of the two *H. bulbosum* cytotypes with previously reported C- banded karyotypes (Vosa, 1976; Noda, 1978; Papes & Bosiljevac, 1984; Thomas & Pickering, 1988; XU & Snape, 1988). The C-banding patterns of the diploid and tetraploid cytotypes of *H. bulbosum* were characterized by having one or two centromeric or juxtacentromeric

very small to larger bands per chromosome. Similar banding patterns were previously observed in diploid *H. bulbosum* by Vosa (1976); Noda (1978) and Thomas & Pickering, (1988) in diploid and tetraploid *H. bulbosum* by XU & Snape (1988) and in tetraploid *H. bulbosum* by Papes & Bosiljevac (1984). 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 at global, local or no concern. 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. The aim of the present study is to seek and exploit the N-banding pattern present in *Hordeum bulbosum* 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 N-banding pattern was studied in two accessions of *H. bulbosum*. Both were tetraploid with 2n=4x=28(HHHH) (Figs. 1& 2). Chromosomes were arranged in 7 groups of 4 homologous chromosomes. Group 1 showed a centromeric band on each arm and interstitial band on the short arm of both accessions (Table 1). A centromeric band on each arm and interstitial band only on the long arm were found in-group 2. The interstitial band occupied different positions. Centromeric band on each arm and two interstitial bands on the long arm of group 3 were common in each accession. An interstitial band on the short arm was found only in 'H127'. Centromeric and interstitial bands on each arm of group 4 chromosomes were common in both accessions. Darkly stained interstitial band proximal to the telomere on the long arm of one chromosome indicated banding pattern polymorphism among homologous chromosomes in 'H77'. Group 5 of 'H127' possessed a centromeric band on each arm while in 'H77' only two chromosomes of this group had this band. Centromeric and one or two interstitial bands respectively on the long and short arms of group 6 were found in 'H127', while of 'H77' had only a centromeric band in each arm. A terminal band on the satellite and a centromeric, interstitial (proximal to the centromere) and telomeric bands were found on each arm of group 7 chromosomes in 'H127'. In 'H77', the centromeric and interstitial bands were observed on each arm of all chromosomes. A terminal band on the satellites of only two chromosomes and an interstitial band (proximal to the telomere on the long arm of other two chromosomes indicated banding pattern polymorphism within homologous chromosomes in the latter accession (Table 1, Fig. 2).

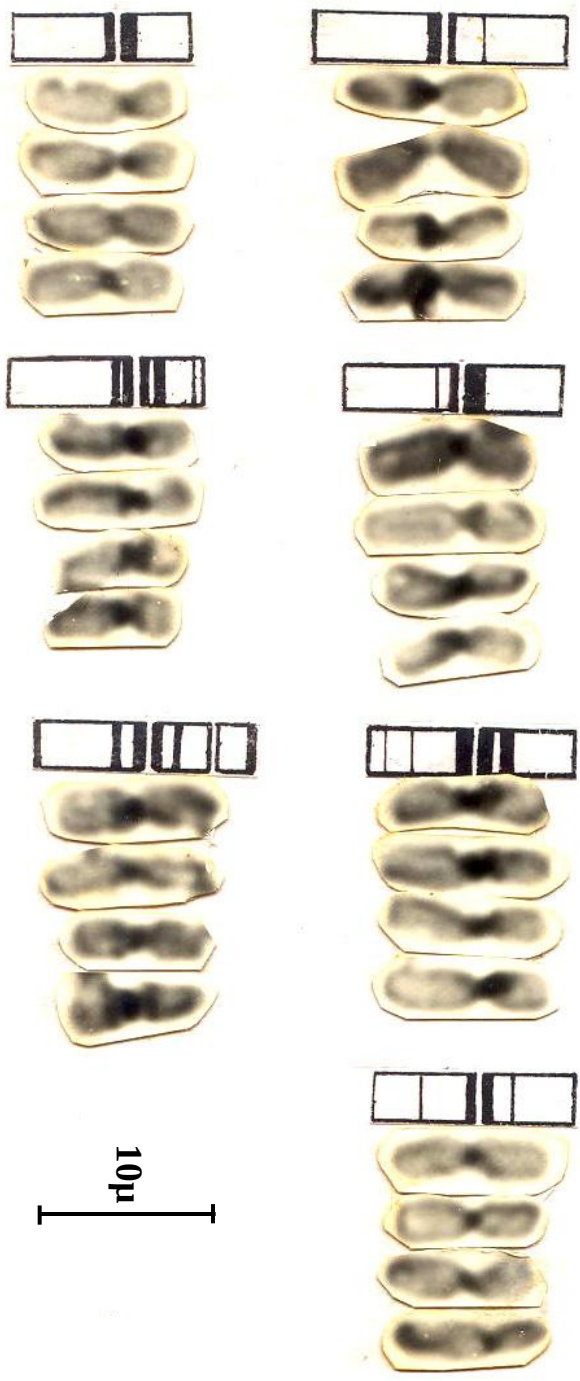

Fig. 1. Karyogram and ideogram of *H. bulbosum* (H127) through Giemsa N-banding technique.

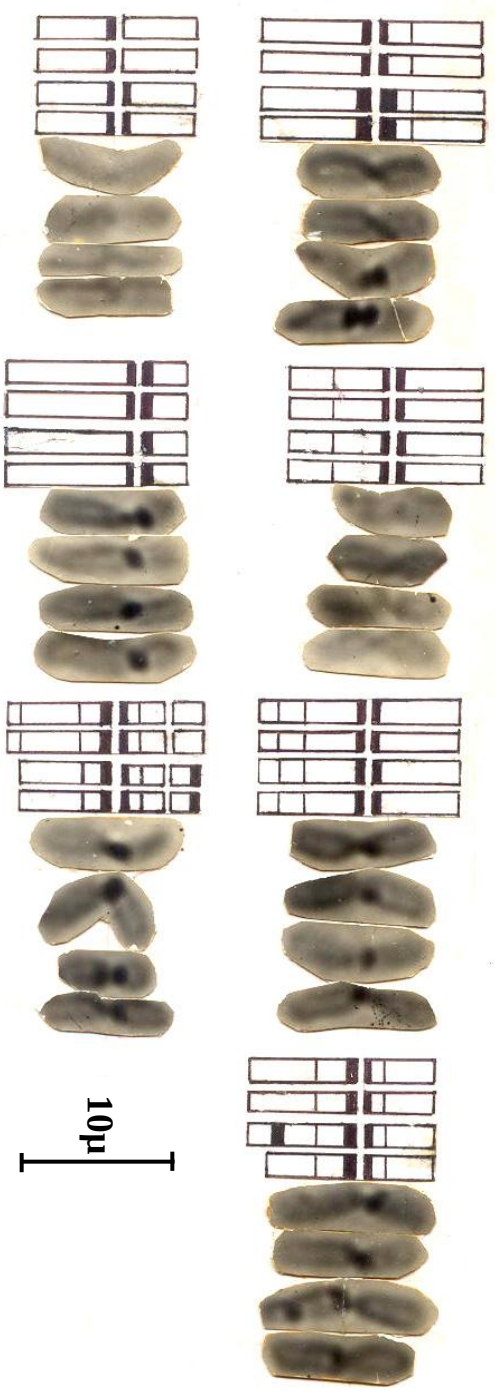

Fig. 2. Karyogram and ideogram of *H. bulbosum* (H77) through Giemsa N-banding technique.

Table 1. Giemsa N-banding pattern (considering constitutive heterochromatin) in Cytotypes `H127' and `H77'*Hordeum bulbosum* L.

	Band position	Homologous groups						
		1	2	3	4	5	6	7
		S/L	S/L	S/L	S/L	S/L	S/L	S/L
H127 (Fig. 1)	C	1/1	1/1	1/1	1/1	1/1	1/1	1/1
	IPC	1/0	0/1	1/0	1/0	0/0	1/1	1/1
	IMP	0/0	0/0	0/1	0/1	0/0	0/0	0/0
	IPT	0/0	0/0	0/1	0/0	0/0	1/0	0/0
	T	0/0	0/0	0/0	0/0	0/0	0/0	0/1
	SAT-L/M/T							0/0/1
H77 (Fig. 2)	C	1/1	1/1	1/1	1/1	1/1	1/1	1/1
	IPC	1/0	0/0	0/0	1/0	0/0	0/0	1/1
	IMP	0/0	0/1	0/1	0/1	0/0	0/0	0/0
	IPT	0/0	0/0	0/1	0/1	0/0	0/0	0/1
	T	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	SAT-L/M/T							0/0/1

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

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. The karyotype of *H. bulbosum* consisted of 24 metacentrics including four SAT-chromosomes and four submetacentric chromosomes showed an overall similarity with those described by Chin, (1941), Linde-Laursen *et al*., (1990) and Morrison (1959). Presence of four chromosomes with satellites was supported by observations of up to three NORs and four nucleoli in $AgNO_3$ stained cells at metaphase and interphase respectively (Linde-Laursen *et al*., 1990). *H. bulbosum* is generally considered an autopolyploid (Berg, 1936, Chin, 1941, Morrison, 1959, Xu & Snape, 1988) combining four homologous or near homologous genomes. Our observations support this conclusion. The presence of four chromosomes of each type should theoretically render the possible identification of 7 homologues groups (Vahidy & Jahan, 1998).

The N-banding patterns of the tetraploid cytotypes of *H. bulbosum* are characterized by having one or two centromeric or juxtacentromeric very small bands per chromosome. Further, single bands are present at one side of the nucleolar organizers. Similar banding patterns were previously observed in diploid *H. bulbosum* by Linde-Laursen *et al.,* 1990, Vosa, 1976, Noda, 1978; Thomas & Pickering (1988) in diploid and tetraploid *H. bulbosum* by Xu & Snape (1988) and in tetraploid *H. bulbosum* by Papes & Bosiljevac (1984). The similarity of the banding patterns at both ploidy levels supports the view that the tetraploid cytotype is an autopolyploid derivative of the diploid one (Berg, 1936; Chin, 1941; Lein, 1948; Papes & Bosiljevac, 1984; Bothmer *et al*., 1987a). Variation in the banding patterns even in between the homologous chromosomes was observed in 4 groups of 'H77' (Table 1).

The banding pattern polymorphism in homologous chromosomes of diploid and tetraploid cytotypes of *H. bulbosum* agreed well with their out breeding nature and is at a level comparable to that found in other outbreeding *Hordeum* taxa (Linde-Laursen *et al*., 1980, 1986, Linde-Laursen & Bothmer, 1984).

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