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GIEMSA N- BANDING PATTERN IN *HORDEUM MURINUM*

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Abstract

Giemsa N-banding pattern in some tetraploid taxon of *Hordeum murinum* was studied. An ideogram was developed for each studied taxon of *Hordeum murinum* for the description of individual N-bands. N-banded karyotype of tetraploid taxa of *H. murinum* had 4-6 bands per chromosome, *subsp. murinum* showed 5 and *subsp. leporinum* 6 bands per chromosome on an average. Most of the bands were intercalary. The pattern showed high levels of band heteromorphism and banding pattern polymorphism but heteromorphisms were not observed between homologous chromosomes within these taxa.

Introduction

Hordeum murinum, wall barley - European annual grass is often found as a weed in waste ground especially along roadsides and hedgerows. The taxa in the *H. murinum* complex have during the past been ascribed to various taxonomic levels, Baum & Bailey (1984) reproposed at the specific level, but Lind-Laursen *et al.*, (1989) proposed to maintain them at sub specific level. The morphological difference between the taxa is definitely insufficient, can not be distinguished without karyological analysis. Cytological analysis becomes an essential screening tool in breeding programs, determining the hybrid fate in the cultivar development process. The karyotypes are symmetrical with chromosomes of approximately the same size and centromeres located at median or sub median positions. The length of the chromosomes at somatic metaphase are variously reported to be from about 5 to 12µm, seldom longer (the variation can partly be ascribed to differences in cytological technique). Some of the chromosomes, especially the satellite (SAT) chromosomes, are morphologically so characteristic that in some cases they can be used as markers for species identification (Rajhathy *et al.*, 1964). In *Hordeum*, the pattern of relationship is complicated due to the presence of several genomes (Bothmer *et al.*, 1987b). As deduced from studies of polyploid interspecific hybrids, the diploid form of *H. murinum* (subsp. *glaucum)* has yet another genome, preliminarily called "**Y**" (Bothmer *et al*., 1987b). There is no chromosomal homology among the polyploids of the *H. murinum* complex (subsp. *murinum*, 4x, and subsp. *leporinum*, 4x and 6x), and other *Hordeum* species (Rajhathy & Morrison 1962, Bothmer *et al.*, 1988). Like the tetraploid of *H. marinum*, the polyploids of *H. murinum* also behave as alloploids, i.e., exclusively forming bivalents. However, there are also some cytogenetic indications that *H. murinum*, 4 x and 6x, are also of an autoploid origin, but have very strong diploidizing mechanisms (Rajhathy and Morrison 1962, Bothmer *et al.*, 1988). 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 level). 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). The aim of the present study is to seek and exploit the N-banding pattern present in *Hordeum murinum* 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. Details of locality (origin), accession number is given in Table 1.

Results

***H. murinum* subsp. *murinum* L.:** The Giemsa N-banded karyograms of 'H61', 'H136' and 'H154' are shown in Figs. 1,2 and 3 respectively. All of them were tetraploid with 2n=4x=28 (Table 1). Chromosome 1 of 'H61' had a centromeric band in the long arm and two interstitial bands in the short arm while of other accessions had a centromeric band on both arms and an interstitial band in the long arm . An additional distal band on the long arm of H136 and interstitial band on the short arm of 'H154' were also observed. Chromosome 2 showed centromeric and interstitial bands on each arm of 'H136' and 'H154'. A darkly stained telomeric band on the long arm of 'H154' was also present. Short arm of chromosome 2 ('H61') had dark centromeric, interstitial and light distal bands, long arm had light centromeric and interstitial bands.

Chromosome 3 possessed centromeric and interstitial bands on each arm of 'H136' and 'H154', while short arm of chromosome 3 ('H61') showed a centromeric, three interstitial bands and long arm had centromeric and distal bands. Chromosome 4 of 'H136' showed heavily stained centromeric and an interstitial bands on the short and a centromeric and two interstitial bands on the long arms, while of 'H154' showed light centromeric and an interstitial bands on each arm (Table 1). Only a centromeric band was present on both arms of chromosome 4 in ('H61'). Chromosome 5 of all accessions possessed a centromeric band on each arm. An interstitial band on the short arm was also present in 'H136'. Centromeric, interstitial and distal bands on the long arm and two interstitial bands on the short arm were present on chromosome 6 of 'H61'. A centromeric, one and two interstitial bands were present on each arm of chromosome 6 of 'H154' and 'H136' respectively (Table 1). A telomeric band on the short arm of 'H136' was also observed. Chromosome 7 had interstitial and centromeric bands in the short and long arms respectively ('H61'). Centromeric and distal bands in the short arm and centromeric, interstitial and distal bands in the long arm of chromosome 7 of 'H136', while of 'H154' had a centromeric on each and an interstitial bands on the short arm. An interstitial band was present at a median portion of short arm and near the telomere on the long arm of chromosome 8 ('H61'). Short arm of this chromosome in 'H136' had a centromeric and two interstitial bands and the long arm a centromeric and an interstitial bands while in 'H154'centromeric bands on both arms were absent. Long arm of chromosome 9 had only a centromeric band, while short arm had centromeric, interstitial and distal bands ('H61'). While this chromosome of other accessions was heavily stained consisted of centromeric, one, two interstitial and telomeric bands on the short arm and a centromeric and interstitial bands on the long arm of 'H154' and 'H136' respectively. A telomeric band on the long arm of 'H136' was also observed. Heavily stained chromosome 10 showed centromeric and interstitial bands in both arms of all accessions. Dark telomeric and light distal bands were also observed respectively on the short and long arms of 'H136' only. N-banding pattern of chromosome 11 in all accessions was similar with centromeric, interstitial and telomeric bands on each arm. Chromosome 12 showed a centromeric and terminal bands on the short arm and satellite respectively and very light centromeric, interstitial and distal bands on the long arm of 'H136', whereas in 'H154' it had a centromeric band on each arm and an interstitial band on the short arm. Chromosome 12 of 'H61' possessed very light distal band in the short arm (excluding satellite) and a centromeric band in the long arm. A terminal band at the lower end of satellite, a centromeric and two interstitial bands in short arm and a centromeric and an interstitial bands on the long arm were present in chromosome 13 of 'H61'. Banding pattern of chromosome 13 showed variation with telomeric bands at each end of satellite and centromeric and telomeric band on the short arm. The long arm had a centromeric, two interstitial and a tetomeric bands in 'H136', while in'H154' a telomeric band on satellite and short arm (excluding satellite) and a centromeric and interstitial bands on each arm were observed (Table 1).

Chromosome 14 of 'H136' and ('H61') showed centromeric and interstitial bands on each arm. A telomeric band in 'H136' and distal band in 'H154' were also observed. A telomeric band on the lower and upper end of satellite was observed in 'H136' and ('H61') and a band only at the upper end of satellite was present in 'H154' (Table 1).

subsp. *leporinum* (Link) Arcang.: The N-banding pattern was studied in two accessions 'H157' (Fig. 4) and 'H84' (Fig. 5). Both were tetraploid with 2n=4x=28 (Table 1). Chromosome 1 had centromeric and interstitial bands on both arms of each accession and distal and telomeric bands respectively on the short and long arm of 'H157'. Chromosome 2 of 'H157' had a centromeric, two interstitial and a distal bands on the short and centromeric and an interstitial bands on the long arm, while of 'H84' had a centromeric and three interstitial bands on the long arm and an interstitial and distal bands on the short arm. Chromosome 3 of both accessions possessed centromeric and interstitial bands on each arm. A distal band on each arm of this chromosome was found only in 'H84'. Heavily stained chromosome 4 showed centromeric, interstitial and distal bands in each arm of both accessions (Table 1). Chromosome 5 showed centromeric and interstitial bands on each arm and a darkly stained distal band on the short arm of both accessions. Chromosome 6 of both accessions showed heavily stained centromeric and interstitial bands on the short arm and a centromeric, two interstitial and distal bands on the long arm. A distal band on the short arm was present only in 'H84'. Chromosome 7 of 'H157' showed a centromeric and interstitial bands on each arm, while of 'H84' had a centromeric and interstitial bands on the short arm and a distal and three interstitial bands on the long arm (Fig. 5). Chromosome 8 of both accessions had centromeric and interstitial bands on short and two interstitial bands on long arm. The interstitial bands in 'H157' were proximal to the cemromere, while in 'H84' proximal and distal to the centromere. A centromeric, two interstitial bands in the long arm and a centromeric, three interstitial and telomeric bands were present on the short arm of chromosome 9 of each accession (Table 1). Chromosome 10 showed a centromeric and two interstitial bands on each arm of 'H157' and a centromeric and three interstitial bands on each arm of 'H84'. The centromeric and interstitial bands on each arm and telomeric band on short arm of chromosome 11 were common in both accessions. A terminal band on the long arm of this chromosome was found only in 'H157'. Chromosome 12 showed a centromeric and one, two interstitial bands on the short arm of 'H154' and 'H84' respectively and centromeric, two interstitial and a distal bands on the long arm of each accession. Centromeric and interstitial bands on short and a centromeric and two interstitial bands on long arm of chromosome 13 were observed in 'H157', while in 'H84' the long arm had a centromeric, interstitial and distal bands and short arm possessed a centromeric and an interstitial bands. A thin band at terminal end of satellite in 'H157'and near the lower end of satellite in 'H84' were also found. Chromosome 14 of 'H157'possessed a centromeric, interstitial and distal bands on each arm and a light band on the terminal end of satellite, while of 'H84' showed a centromeric and interstitial bands on each arm and a distal band on the long arm only. A band on the lower end of satellite was found in each accession (Table 1).

Table 1. Giemsa N-banding pattern (considering constitutive heterochromatin) in *Hordeum murinum* L. taxa.

Species	Accession number	Locality	Band position	Homologous group													
				1 S/L	2 S/L	3 S/L	4 S/L	5 S/L	6 S/L	7 S/L	8 S/L	9 S/L	10 S/L	11 S/L	12 S/L	13 S/L	14 S/L
H.murinum Subsp. *murinum* (Fig. 1)	H61	Jardin Botanique de. Nantes, France	C	0/1	1/1	1/1	1/1	1/1	0/1	0/1	0/0	1/1	1/1	1/1	0/1	1/1	1/1
			IPC	1/0	1/1	2/0	0/0	0/0	1/1	1/0	0/0	1/0	1/3	1/2	0/0	1/1	1/2
			IMP	1/0	0/0	1/0	0/0	0/0	1/0	0/0	1/0	0/0	0/0	1/0	0/0	0/0	0/1
			IPT	0/0	1/0	0/1	0/0	0/0	0/1	0/0	0/1	1/0	0/0	0/0	1/0	1/0	0/0
			T	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
			SAT L/M/T												0/0/0	1/0/0	1/0/1
H.murinum Subsp. *murinum* (Fig. 2)	H 136	Okol Botanischer Garten, Bayreuth, Germany	C	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
			IPC	0/1	1/0	0/0	1/1	1/0	1/2	0/1	1/0	1/1	0/1	1/1	0/0	0/2	1/1
			IMP	0/0	0/1	1/0	0/0	0/0	1/0	0/0	1/0	1/0	1/0	0/1	0/1	0/0	0/0
			IPT	0/1	0/0	0/1	0/1	0/0	0/0	1/1	0/1	0/1	0/1	0/0	0/1	0/0	0/0
			T	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	1/1	1/0	1/0	1/0	1/1	0/1
			SAT L/M/T												0/0/1	1/0/1	1/0/1
H.murinum Subsp. *murinum* (Fig. 3)	H154	Okol Botanischer Garten, Bayreuth, Germany	C	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
			IPC	1/1	1/1	1/1	1/1	0/0	0/1	1/0	1/0	0/1	1/1	0/2	1/0	1/1	1/1
			IMP	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/0	1/0	0/1	1/0	0/0	0/0	0/0
			IPT	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/1
			T	0/0	0/1	0/0	0/0	0/0	1/1	0/1	0/1	1/0	0/0	1/0	0/0	1/0	0/0
			SAT L/M/T												0/0/0	0/0/1	0/0/1
H.murinum Subsp. *leporinum* (Fig. 4)	H157	Okol Botanischer Garten, Bayreuth, Germany	C	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/0	1/1	1/1	1/1	1/1	1/1	1/1
			IPC	1/0	1/1	1/1	1/1	1/1	1/2	1/1	1/2	1/2	1/2	0/0	1/2	1/1	1/0
			IMP	0/1	1/0	0/1	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/1	0/0	0/1	0/0
			IPT	1/0	1/0	0/0	1/1	1/0	0/1	0/0	0/0	1/0	1/0	0/0	0/1	0/0	1/1
			T	0/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/1	0/0	0/0	0/0
			SAT L/M/T												0/0/0	0/0/1	1/0/1
H.murinum Subsp. *leporinum* (Fig. 5)	H84	Plant Breeding station Clermont, Ferrand, INRA	C	1/1	0/1	1/1	1/1	1/1	1/1	1/0	1/0	1/1	1/1	1/1	1/1	1/1	1/1
			IPC	1/0	1/1	1/1	1/1	1/1	1/2	1/2	1/1	2/2	1/2	0/0	1/2	1/0	1/1
			IMP	0/1	0/2	0/0	0/0	0/0	0/0	0/1	0/0	1/0	0/0	1/1	1/0	0/1	0/0
			IPT	0/0	1/0	1/1	1/1	1/0	1/1	0/1	0/1	0/0	2/1	0/0	0/1	0/1	0/1
			T	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/0	0/0	0/0	0/0
			SAT L/M/T												0/0/0	1/0/0	1/0/0

S= Short arm, L= Long arm, 0, 1, 2, 3= 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 terminal positions of satellites

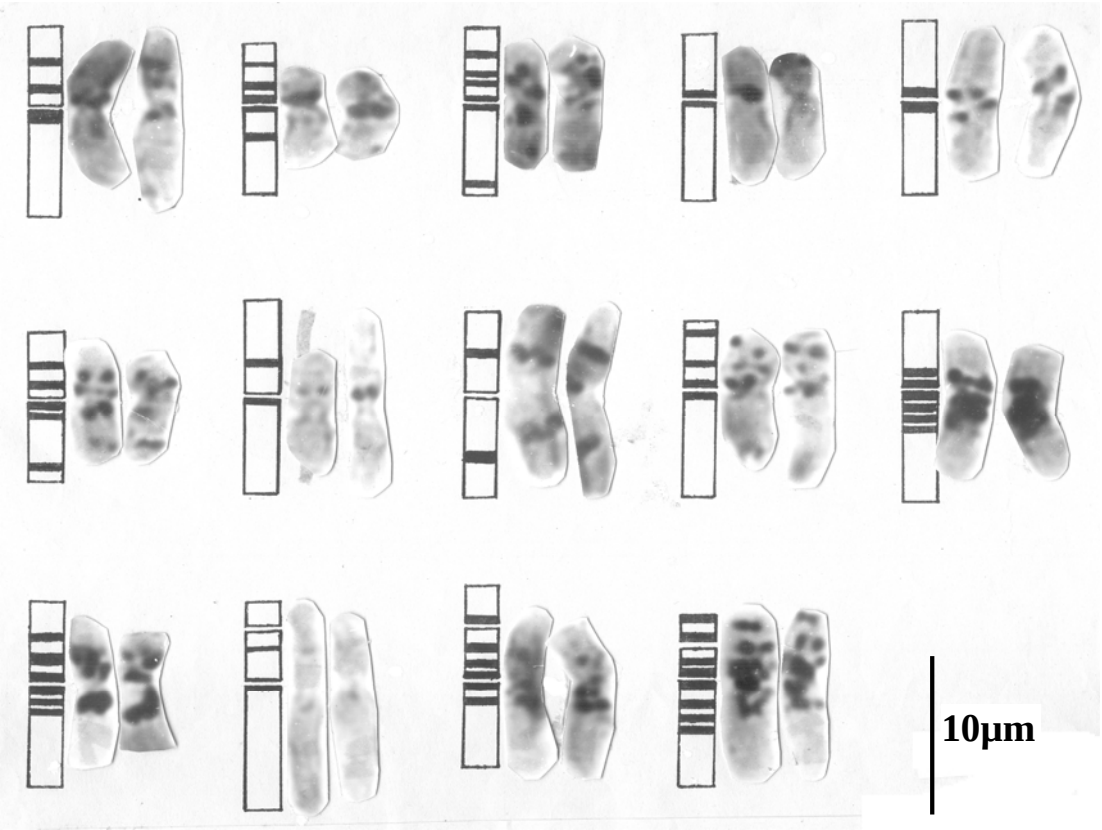

Fig. 1. Karyogram and ideogram of *H.murinum* sub sp. *murinum* (H61) through Giemsa N-banding technique (10µm).

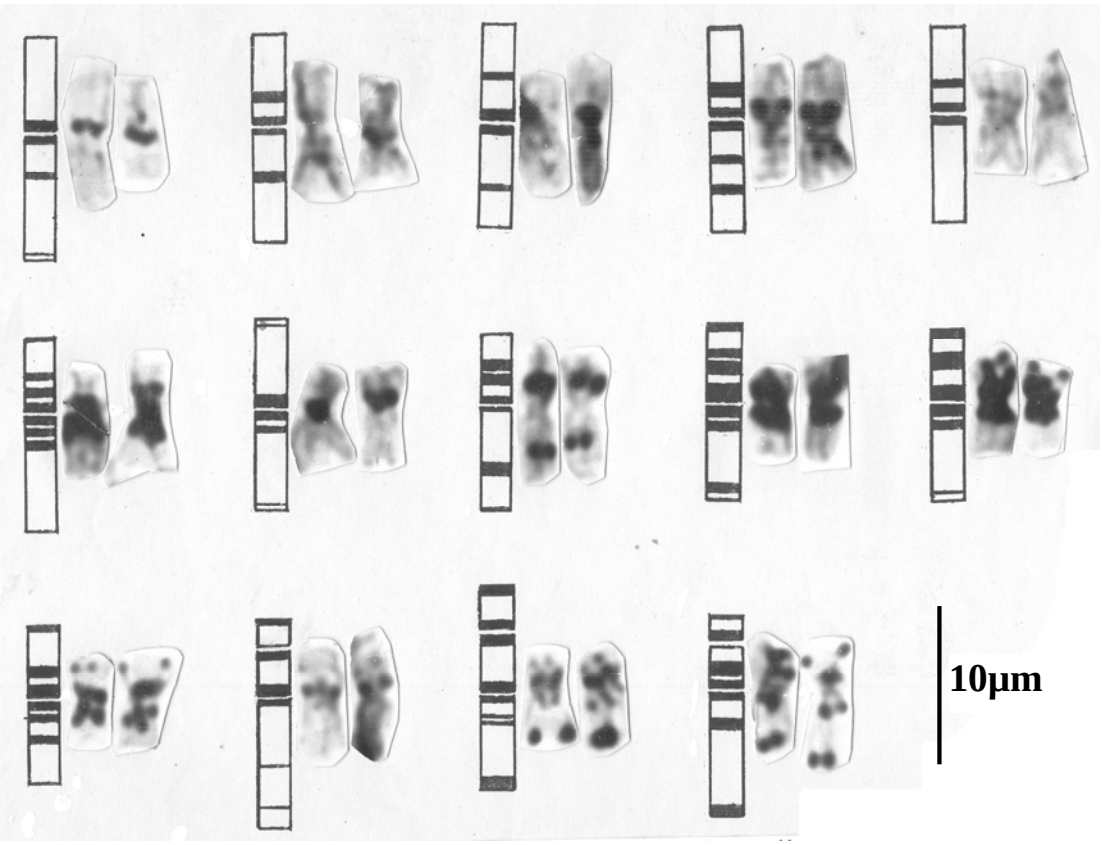

Fig. 2. Karyogram and ideogram of *H.murinum* sub sp. *murinum* (H136) through Giemsa N-banding technique (10µm).

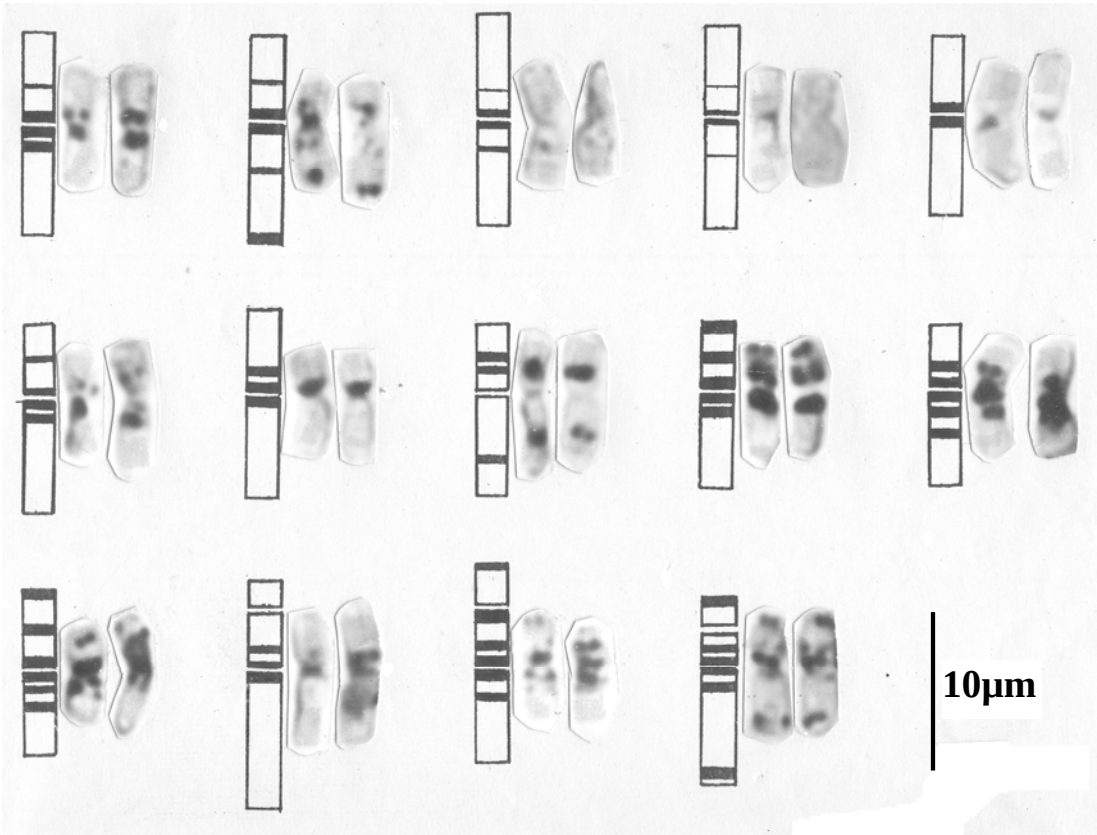

Fig. 3. Karyogram and ideogram of *H.murinum* sub sp. *murinum* (H154) through Giemsa N-banding technique (10µm).

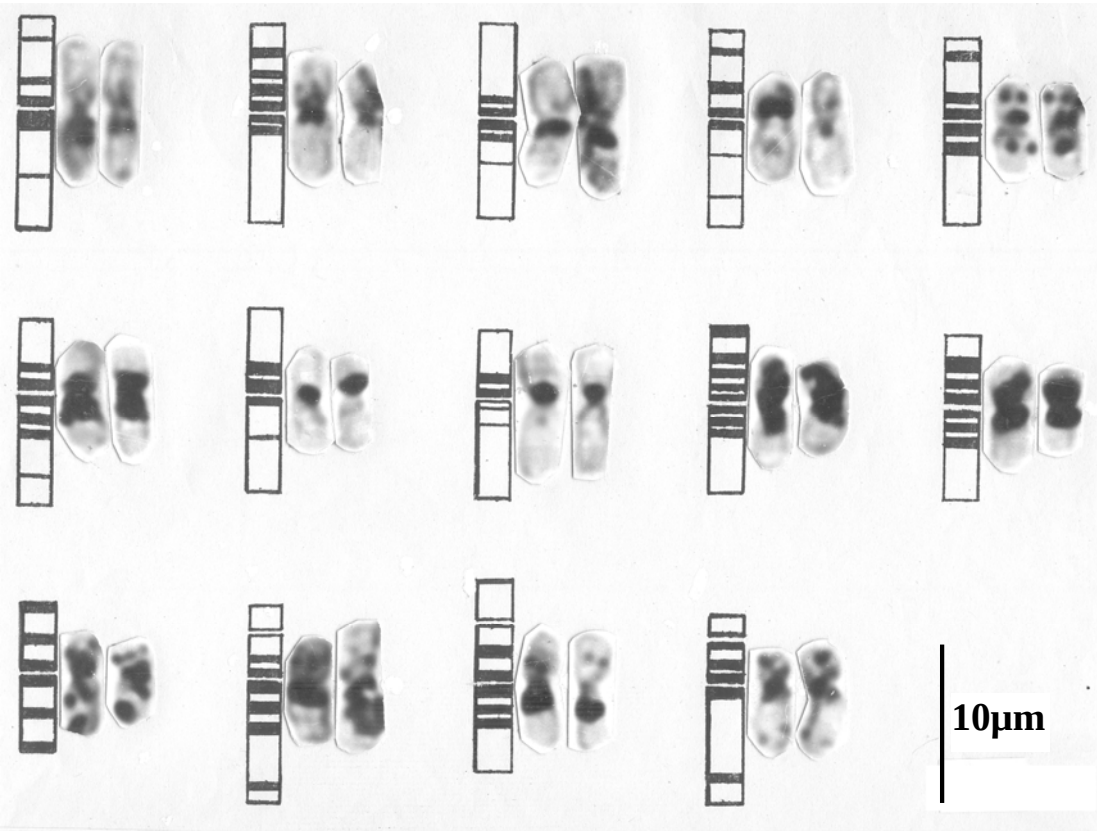

Fig. 4. Karyogram and ideogram of *H.murinum* sub sp. lepo*rinum* (H157) through Giemsa N-banding technique (10µm).

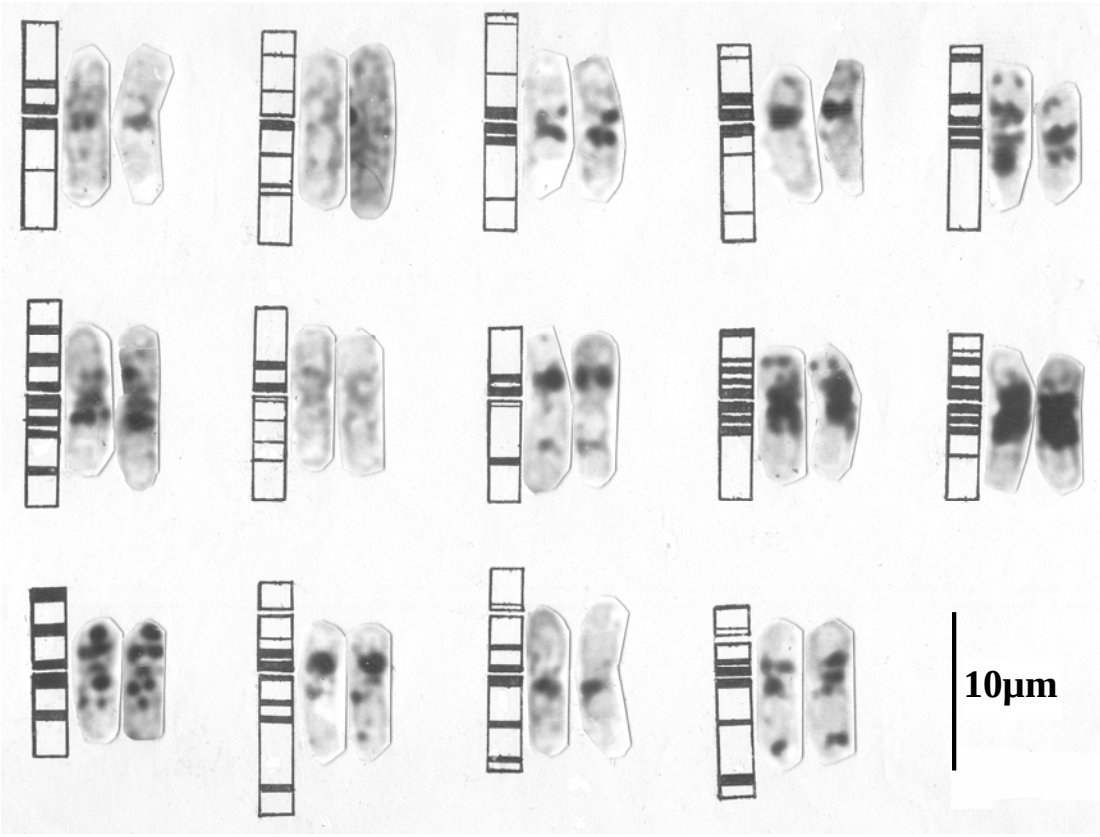

Fig. 5. Karyogram and ideogram of *H.murinum* sub sp. *leporinum* (H84) through Giemsa N-banding technique (10µm).

Discussion

Discovery and incorporation of genes from wild species provide means to sustain crop improvement, particularly when levels of resistance in the cultigens are low and virulent strains of pests and pathogens overcome the host plant resistance. Due to the possibility for wide hybridization, wild species of *Triticeae* are potentially important genetic resources in plant breeding (Jahan & Vahidy, 2008). 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). The taxa in the *H. murinum* complex have during the past been ascribed to various taxonomic levels, Baum & Bailey (1984) reproposed at the specific level, but Lind-Laursen *et al.*, (1989) proposed to maintain them at sub species. The morphological difference between the taxa is definitely insufficient for distinguishing between them without karyological analysis. There are three pairs of SAT-chromosomes in tetraploid taxa of *H. murinum* which are not always visible in every cell but in good preparations are quite clear (Vahidy & Jahan, 1998). The satellites of one pair were larger than those of the two other pairs, which appeared similar. The number of SAT-chromosomes matched the presence of six nucleoli in interphase as reported by Linde-Laursen *et al.*, (1989). N-banded karyotype of diploid (Jahan & Vahidy, 2007) and tetraploid taxa of *H. murinum* had 4-6 bands per chromosome, *subsp. glaucum* (Jahan & Vahidy, 2007) and *murinum* showed 5 and *subsp. leporinum* had 6 bands per chromosome on an average**.** Most of the bands were intercalary (Table 1). The patterns showed high levels of band heteromorphism and banding pattern polymorphism but heteromorphisms were not observed between homologous chromosomes within these taxa (Figs. 1, 2, 3, 4 and 5). Each and every chromosome except those arranged at 3 and 11 in subsp. *murinum* and 4,8 and 9 positions in ssp. *leporinum* showed banding pattern polymorphism among accessions. Variations in the banding patterns of above taxa were found in the occurrence of the intercalary and telomeric bands, while the centromeric heterochromatin in most of the chromosomes seems to be similar. Linde- Laursen *et al.*, (1989) reported 5-13 conspicuous bands per chromosome in ssp. *glaucum*, 6-10 inconspicuous C-bands in ssp. *murinum* and 5-8 in ssp. *leporinum.* Vosa (1976) reported telomeric C-band on each arm of chromosomes 1, 2, 4 and 6, while N- banding showed this band respectively on the long and short arms of chromosome 1 and 6. The alloploidy of the polypolid cytotypes of *H. murinum* was proposed from karyological results (Richards & Booth, 1976), from an electrophoretic examination of seed proteins (Booth & Richards, 1978) and from crosses (Rajhathy & Morrison, 1962). Chromosomes 1-7 of diploid *H. murinum* (Jahan & Vahidy, 2007) showed more or less similar bands respectively on chromosomes 10,5,6,9,1,13 and 14 of a tetraploid cytotype. Banding patterns of *H. murinum* taxa indicated that the chromosome complements of the polyploids comprised the genome of the related diploid as well as one or two

"unidentified" genomes. This agrees with an alloploid origin of polyploids. According to Linde-Laursen *et al.*, (1989) the diploid genome found in *H. murinum* ssp. *glaucum* is refound in *H. murinum* ssp. *murinum* and ssp. *leporinum*, while a second genome in the tetraploid cytotype cannot be refound in any known *Hordeum* species. Bothmer *et al.*, (1987a, 1988) have suggested that the high pairing level found in some interspecific hybrids of *H. murinum* may be autosyndetic and the parental genomes of polyploids of *H. murinum* are closely related.

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