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GIEMSA N- BANDING PATTERN IN SOME WILD DIPLOID SPECIES OF *HORDEUM*

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Abstract

Distribution pattern of constitutive heterochromatin in some wild diploid *Hordeum* species was studied by Giemsa N-banding technique. It was possible to discriminate between the species by the number and morphology of the nucleolar chromosomes and the presence or absence of certain characteristic N-bands on chromosomes. An ideogram was developed for each studied species/taxa of *Hordeum* for the description of individual N-bands. The banding patterns, especially the finer intercalary and distal bands were more easily observed and reliably scored in late prophase and early metaphases when the chromosomes are at the right stage of contraction. N-banding procedure indicated that a clearly detectable mass of constitutive heterochromatin was located at the centromeric and interstitial regions of most chromosomes; however a wide variation in the intensities of bands, number of bands and their position in the chromosomes were observed. Among a mean number of bands 0.63 centromeric, 0.17 telomeric, 0.50 intercalary and 0.14 were found on satellites of diploid *Hordeum* species.

Introduction

Hordeum is a genus of 25-30 species widely distributed in temperate regions of the world (Jahan *et al*., 1992). The genetic diversity in cultivated and wild forms in the genus *Hordeum* is extremely rich and interesting. Morrison (1959) reported the detailed chromosome morphology of 16 *Hordeum* species. Prior to that time, cytological especially the karyomorphological studies remained limited mostly to the cultivated barley, and enough attempts were not made to study critically the wild occurring closely related species. On the basis of similarities and dissimilarities of chromosome morphology Rajhathy& Morrison (1959, 1961,1962) analysed relationships between various *Hordeum* species and their hybrids. Rajhathy *et al*., (1964) suggested that the chromosomes of *Hordeum* species, particularly the satellites, were distinctive and could be used as "markers" that would greatly assist in determining species relationships, genome homologies and the structure of polyploidy in this complex genus. Chromosome banding is a lengthwise variation in staining properties along a chromosome. This variation in staining properties is normally independent of any immediately obvious structural variation. Karyotype analysis on Giemsa banded chromosomes may provide useful information on chromosome relationships than conventional staining techniques. However the usefulness of this method of genome analysis will depend on the bandedness of chromosomes i.e., the number of bands and uniqueness of their distribution on each chromosome, heterochromatin DNA in C- and N-bands can be stained usually with Giemsa. Islam (1980) claimed that the N-banding technique is superior to C-banding but Singh &Tsuchiya (1982) did not find any difference in the quality of banding patterns between them. They advocate the use of their improved

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acetocarmine N-banding technique because it does not produce distorted chromosome morphology and allows a more precise demarcation of chromosomal constrictions and ends by photomicrography after squashing, than can be had after banding. The determination of the centromere position is essential in karyotype analysis (Singh & Tsuchiya, 1982). The N-banding method was originally thought to be specific for the chromosomal material of nucleolus organizer regions (Funaki *et al*., l975), but was later considered a variant of C-banding (Bostock & Sumner 1978). As a complementary technique to C-banding and because of comparatively simple handling, N-banding would be very useful for the rapid identification of chromosomes (Schlegel *et al.,* 1986). 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. Several C-banding studies have been attempted by Linde-Laursen (1978a, b, 1979, 1981); Linde-Laursen *et al*., (1980, 1986, 1989a, b) on the cytology and cytogenetics of cultivated barley and its wild relatives. 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 was to seek and exploit the N-banding pattern present in *Hordeum* species 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

H. bogdanii Wil.

The long arms of chromosome 1 to 4 of *H. bogdanii* did not show any band. Short arm of first three chromosomes possessed a centromeric and interstitial band, while of chromosome 4 had one telomeric and two interstitial bands (Table 1). Chromosome-5 showed strong centromeric band on each arm. The short arm also had an interstitial band proximal to the centromere. Short arm of chromosome 6 had a centromeric, interstitial and a terminal bands and the long arm had two interstitial bands proximal to the telomere. A terminal band at both ends of satellite and a centromeric and a telomeric bands (excluding satellite) were present on the short arm of chromosome 7. The long arm had four weak interstitial bands, two proximal to the centromere, one in the median position and one proximal to the telomere (Table 1).

Table 1. Giemsa N-banding pattern (considering constitutive heterochromatin) in some wild diploid *Hordeum* species.

Taxon	Band position	Chromosome number						
		1	**2**	**3**	**4**	**5**	**6**	**7**
		S/L	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**	**S/L**
H. bogdanii Wil.								
	C	1/0	1/0	1/0	0/1	1/1	1/0	1/0
	ICP	1/0	0/0	1/0	0/0	1/0	0/0	0/2
	IMP	0/0	0/0	0/0	1/0	0/0	1/0	0/1
	IPT	0/0	1/0	0/0	1/0	0/0	1/2	0/1
	T	0/0	0/0	0/0	1/0	0/0	1/0	1/0
	SAT							
	L/M/T						1/0/1	-
H. chilense Roem & Schult. (Fig. 1)								
	C	0/0	0/0	1/1	1/1	1/1	1/1	1/1
	ICP	1/2	1/2	1/2	1/1	1/3	0/1	0/0
	IMP	1/1	0/0	1/1	1/0	0/1	1/1	0/2
	IPT	2/1	0/1	1/0	1/1	0/0	0/1	0/1
	T	0/0	0/0	0/1	0/0	0/1	1/0	0/0
	SAT							
	L/M/T						1/0/1	0/0/1
H. marinum Huds. (Fig. 2)								
	C	0/0	0/0	0/1	0/0	1/0	1/0	0/1
	ICP	1/2	2/0	0/1	1/1	0/1	1/0	0/0
	IMP	0/1	0/1	1/0	0/0	1/0	0/0	0/1
	IPT	0/0	0/0	1/1	1/1	1/0	0/0	0/0
	T	0/0	0/0	0/0	0/0	0/0	1/0	1/0
	SAT							
	L/M/T						-	0/0/0
H.murinum L. (Fig. 3)								
	C	1/1	1/1	0/0	1/1	0/1	1/1	1/1
	ICP	1/3	0/1	1/1	0/0	1/0	0/0	1/1
	IMP	1/1	0/0	0/0	1/0	1/0	1/0	0/0
	IPT	0/0	0/0	1/2	1/0	0/1	0/1	0/0
	T	0/0	0/0	0/0	0/0	0/0	0/0	1/0
	SAT							
	L/M/T						0/1/0	1/0/1
H.murinum L. ssp. *glaucum* (Steud.) Tzvelev. (Fig. 4)								
	C	1/1	1/0	1/1	1/1	1/0	1/1	1/1
	ICP	1/0	0/1	1/0	1/0	1/0	1/1	0/1
	IMP	0/1	0/1	0/0	1/0	0/0	0/0	0/0
	IPT	0/0	1/0	0/1	1/1	0/1	1/0	0/0
	T	0/1	0/0	1/0	0/0	0/0	0/0	1/0
	SAT							
	L/M/T						0/0/1	1/0/0

S= Short arm, L= Long arm, 0,1,2,3= Number of dark bands, C= Centromeric bands, IPC= Interstitial bands proximal to centromere, IMP= Interstitial bands at median position, IPT= Interstitial bands proximal to telomere, T= Telomeric bands, SAT-LMT= Dark bands at lower, median and a terminal positions of satellites.

***H. chilense* Roem & Schult.**

Giemsa N-banded karyogram revealed that the short and long arms of chromosome 1 had four equally spaced interstitial bands along the length of the chromosome. The short arm of chromosome 2 had a thick interstitial and the long arm three interstitial bands, chromosome 3 possessed a centromeric and three interstitial bands on each arm (Table 1). A telomeric band on the long arm was also present. Chromosome 4 showed strong centromeric bands on each arm and 2/3 interstitial bands on the long/short arm. Chromosome 5 was the smallest in the complement, short arm had a centromeric and an interstitial band while the long arm had a centromeric, four interstitial and one terminal bands. In addition to the centromeric band each arm of chromosome 6 possessed two-three interstitial bands. There was a terminal band on each end of the satellite. A terminal band on the satellite and a centromeric band were present on the short arm of chromosome 7. The long arm had a centromeric and three interstitial bands at different locations (Fig. 1).

***H. marinum* Huds.**

The basic N banded karyotype of *H. marinum* had two-three very small to large bands per chromosome. Chromosome 1 showed one to three interstitial bands respectively in the short and long arms, most of them being proximal to the centromere. Chromosome 3 possessed two interstitial bands in the short arm; the dark one was proximal to the centromere and the lighter one in the median position. The long arm had 3 bands, a centromeric, an interstitial which was proximal to the centromere and a distal band. Chromosome 4 showed very light bands, two in each arm, one of which was interstitial and the other was distal. Bands of chromosome 5 were also very light, one centromeric and two interstitial in the short and one interstitial in the long arm (Fig. 2). Chromosome 6 showed three heavy bands in the short arm, at centromere, intercalary and telomere. The SAT- chromosome 7 possessed two bands in the long arm. Of these one was centromeric and the other distal (Table 1).

***H. murinum* L.**

The Giemsa N-banding pattern of the diploid cytotype (Fig. 3) showed that chromosome 1 had three heavy bands, a centromeric and two interstitial in the short arm and a centromeric and four interstitial bands in the long arm (Table 1). In addition to a light interstitial band in the long arm chromosome 2 possessed a dark centromeric band in each arm. Chromosome 3 had a dark interstitial band proximal to the centromere in each arm, one distal in the short arm and two distal bands in the long arm. Chromosome 4 had a centromeric band in each arm and an interstitial in the middle and a distal bands in the short arm. Long arm of chromosome 5 had a dark centromeric and light distal bands, while the short arm an interstitial and distal bands. Chromosome 6 was satellited and showed a centromeric band in each arm and an interstitial and a dark distal bands on the short and long arm respectively. A faint band was also present at the basal portion of satellite. Chromosome 7 was also satellited with three heavy bands in the short arm, at centromeric, interstitial and telomeric regions. Long arm had centromeric and interstitial bands only while the satellite possessed two bands, one at each end (Table 1).

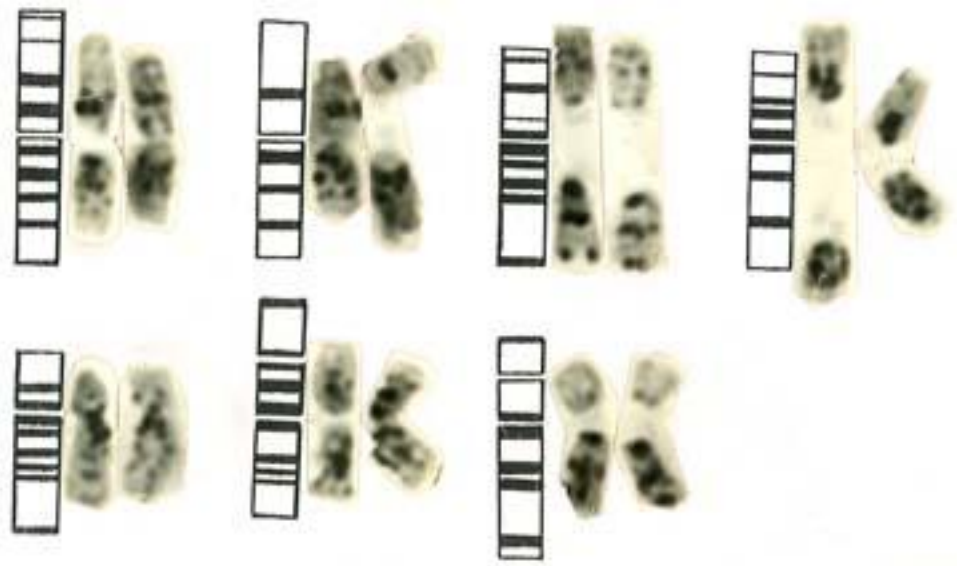

Fig. 1. Karyogram and ideogram developed from the cell of *H. chilense* through Giemsa N-banding technique.

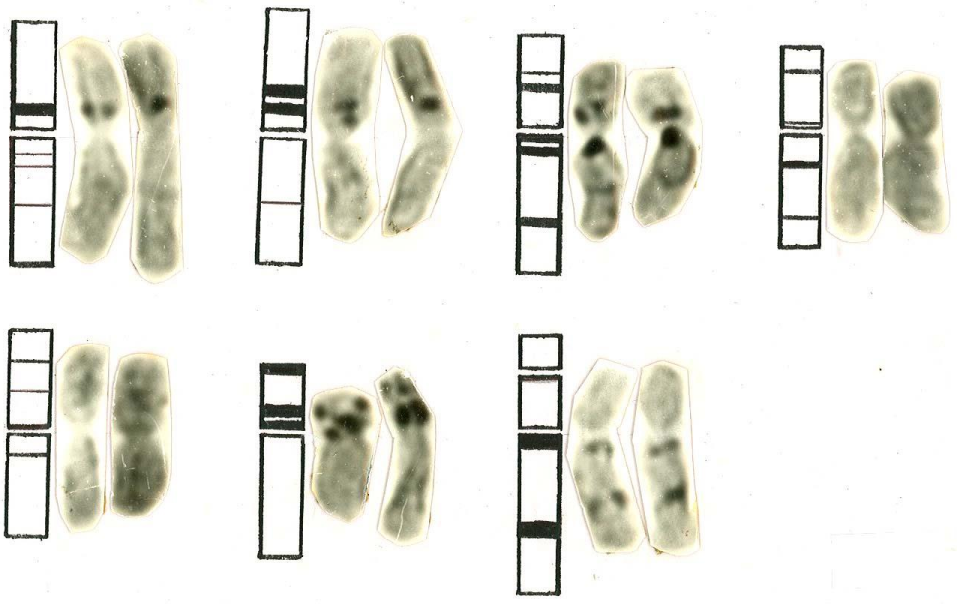

Fig. 2. Karyogram and ideogram developed from the cell of *H. marinum* (2x) through Giemsa N-banding technique.

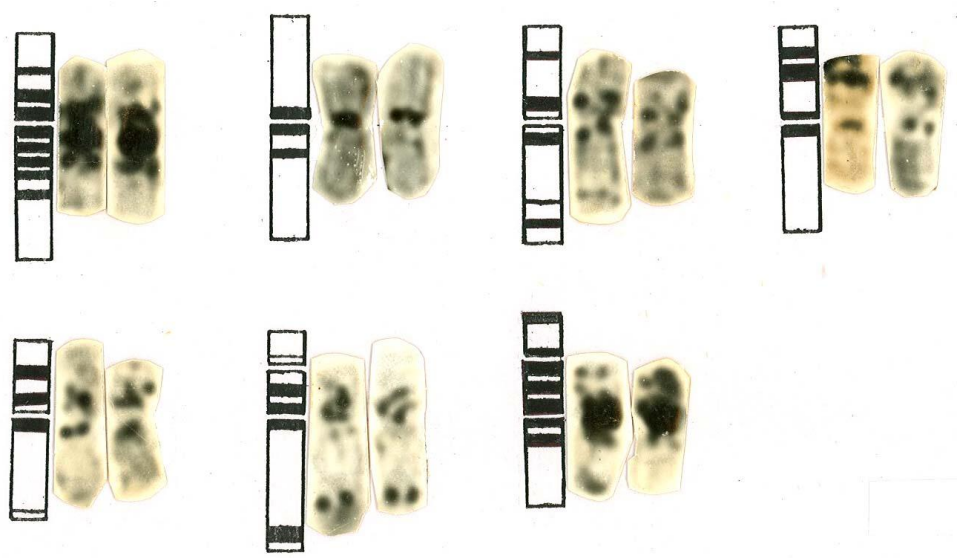

Fig. 3. Karyogram and ideogram developed from the cell of *H. murinum* (2x) through Giemsa N-banding technique.

Fig. 4. Karyogram and ideogram developed from the cell of *H. murinum* ssp. *glaucum* (2x) through Giemsa N-banding technique.

Table 2. Mean number of dark N-bands at different regions of chromosomes in wild diploid *Hordeum* species.

Taxon	Centromere	Intercalary	Telomere	Satellite
H. bogdanii Wil.	0.5	0.31	0.21	0.14
H.chilense Roem & Schult	0.71	0.83	0.21	0.21
H. marinum Huds.	0.29	0.47	0.14	0.00
H. murinum L.	0.79	0.5	0.71	0.21
H. murinum L. ssp. *glaucum* (Steud.) Tzvelev.	0.86	0.4	0.21	0.14
Total	**0.63**	**0.5**	**0.17**	**0.14**

H. murinum L. ssp. *glaucum* (Steud.) Tzvele

Chromosome 1 had a dark centromeric and interstitial band on both arms. Chromosome 2 showed dark centromeric and light distal bands on the short arm. Two interstitial bands one proximal to the centromere and the other in the middle portion of long arm were present (Table 1). Short arm of chromosome 3 had a centromeric, interstitial and telomeric band while the long arm had centromeric and distal bands. Chromosome 4 was heavily stained possessed a centromeric, two interstitial and distal bands in short arm and a centromeric and distal bands in the long arm. Chromosome 5 was the smallest in the complement possessed a centromeric and interstitial bands in the short arm and a distal band in the long arm. Chromosome 6 showed a terminal band on the satellite, a centromeric and two interstitial bands in short arm and a centromeric and an interstitial band in the long arm (Table 1, Fig. 4).

Discussion

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 a karyotype analysis of some diploid species of *Hordeum.* N-banded karyotype of *H. bogdanii* was characterized by 0.5 centromeric, 0.31 intercalary localized distally, 0.21 telomeric mostly on short arms and 0.14 bands on both sides of the nucleolar constriction (Table 2). Linde-Laursen *et al*., (1980) reported that C-banded karyotype of five plants were basically same with few centromeric, intercalary and most telomeric bands. Telomeric N-bands were present only on the short arms of chromosomes 4, 6 and 7, while C-bands (Linde-Laursen *et al*., 1980) on this position were found on each chromosome of *H.bogdanii*. *H. chilense* 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 tolerant quality of grain or nutritional value of cereals. It showed 7 bands per chromosome on an average in their N-banding pattern (Fig. 1 and Table 1), which can be used in conjunction with length and arm ratios to identify the individual chromosomes. Fernandez & Jouve (1984) also reported 7 bands per chromosome, whereas those of Armstrong *et al*., (1987), Zerneke (1987) and Linde-Laursen *et al*., (1989a) reported 13 bands per chromosome. Bands were smaller in size than those depicted by Fernandez & Jouve (1984) and Zerneke (1987). Armstrong *et al*., (1987) and Linde-Laursen *et al*., (1989a) also reported smaller bands. The metacentric SAT-chromosome pair of *H. chilense* has longer satellites than the submetacentric one. 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 differences suggest

chromosome polymorphism. The correspondence between the N- and C- banded karyotypes of *H. chilense* as observed presently and those previously reported indicated that telomeric N-bands were observed only on the Long arms of chromosomes 1, 3, 4 and short arm of chromosome 6, while C-bands were found on the telomeres of most chromosomes (Linde-Laursen *et al.*, 1989a). N-banding pattern of *H. marinum* (Fig. 2) was characterized by having 3-4 bands per chromosome on an average, distributed at centromeric, telomeric and intercalary positions. Centromeric C-bands on each chromosome of diploid *H. marinum* (Vosa, 1976) was also observed by N-banding except chromosome 1 and 2. N-banded karyotype of *H. murinum* had 4-6 bands per chromosome, ssp. *glaucum* showed 5 bands per chromosome on an average. Most of the bands were intercalary (Table 2). Linde-Laursen *et al*., (1989b) reported 5-13 conspicuous bands per chromosome in sp. *glaucum*. Vosa (1976) reported telomeric C-band on each arm of chromosomes 1, 2, 4 and 6, while N-banding shows this band respectively on the long and short arms of chromosome 1 and 6. In diploid *H. murinum*, telomeric N- band was found only on the short arm of chromosome 7, while C-banding (Vosa, 1976) revealed band at this position on the short arm of chromosome 3, long arms of chromosomes 4,7 and each arm of 1, 2 and 5 chromosomes. Polymorphism in banding patterns in *Hordeum* species/taxa revealed that there was considerable band polymorphism and particularly all the plants studied were unique in their banding patterns. The smaller intercalary and distal bands are the most variable in occurrence while the bands adjacent to the centromere vary in size. Banding pattern similarity together with duplication both within and between species, seem to preclude their use as outright specific markers.

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References

Armstrong, K.C., I.L. Craig and C. Merritt. 1987. *Hordeum chilence* (2n=14) Computer-assisted Giemsa Karyotypes. *Genome*, 29: 683-688.

Bostock, C.J. and A.T. Sumner. 1978. *The eukaryotic chromosome*. pp. 225 North Holland, Amsterdam.

Fernandez, J.A. and N. Jouve. 1984. Giemsa C-banding of the chromosomes of *Hordeum chilense* and its amphidiploid x *Triticum turgidum* Conv. *durum*. *Z. Pflanaenzuchtg*, 93: 212-221.

Funaki, K., S. Matsue and M. Sasaki. 1975. Location and nucleolar organizers in animal and plant chromosomes by means of an improved N-banding technique. *Chromosoma*, 49: 357-370.

Islam, A.K.M.R. 1980. Identification of wheat barley addition lines with N-banding of chromosomes. *Chromosoma*, 76: 365-373.

Jahan, B., A.A. Vahidy and S.I. Ali. 1992. Chiasma frequency and chromosome associations in sixteen taxa of *Hordeum* species. *Cytologica*, 57: 51-57.

Kubalakova, M., M. Valarik, J. Bartos, J. Vrana, J. Cihalikova, M. Molnar-Lang and J. Dolezel. 2003. Analysis and sorting of rye (*Secale cereale* L.) chromosomes using flow cytometry.*Genome,* 46(5): 893-905.

Linde-Laursen, I. 1978a. Giemsa C-banding of barley chromosome. I. Banding pattern polymorphism. *Hereditas*, 88: 55-64.

Linde-Laursen, I. 1978b. Giemsa C-banding of barley chromosome. II. Banding patterns of trisomics and telotrisomics. *Hereditas*, 89: 37-41.

Linde-Laursen, I. 1979. Giemsa C-banding of barley chromosomes. III.Segregation and linkage of C-bands on chromosomes 3, 6 and 7. *Hereditas*, 91: 73-77.

Linde-Laursen, I. 1981.Giemsa banding patterns of the chromosomes of the cultivated and wild barleys. In: *Barley Genet. IV Proceedings of the Fourth international barley Genetics symposium, Edinburgh*, 22-29 July, 1981, (Eds.): M.J.C. Asher, P.P. Ellis, A.M. Hayter and R.N.H. Whitehouse, pp. 786-795, Edinburgh University press, Edinburgh.

Linde-Laursen, I., R.V. Bothmer and N. Jacobsen. 1980. Giemsa C-banding in Asiatic taxa of *Hordeum* section *Stenostachys* with notes on chromosome morphology. *Hereditas,* 93: 235-254.

Linde-Laursen, I., R.V. Bothmer and N. Jacobsen. 1986. Giemsa C-banded karyotypes of *Hordeum* taxa from North America. *Can. J. Genet. Cytol*., 28: 42-62.

Linde-Laursen, I., R.V. Bothmer and N. Jacobsen. 1989a. Giemsa C-banded karyotypes of South American *Hordeum* (Poaceae) I. 14 diploid taxa. *Hereditas*, 110: 289-305.

Linde-Laursen, I., R.V. Bothmer and N. Jacobsen. 1989b. Giemsa C-banded karyotypes of *H. marinum* and *H.murinum*. *Genome,* 32: 629-639.

Morrison, J.W. 1959.Cytogenetic studies in the genus *Hordeum.* I. Chromosome morphology. *Can. J. Bot*., 37: 527-538.

Rajhathy, T. and J.W. Morrison. 1959. Cytogenetic studies in the genus *Hordeum,* IV. Hybrids of *H. Jubatum, H.brachyantherum, H. vulgare* and a hexaploid *Hordeum* species. *Can. J. Genet and Cytol*., 1: 124-132.

Rajhathy, T. and J.W. Morrison. 1961. Cytogenetic studies in the genus *Hordeum* V. *H. jubatum* and the new world species. *Can. J. Genet. Cytol*., 3: 378-390.

Rajhathy, T. and J.W. Morrison. 1962. Cytogenetic studies in the genus *Hordeum* VI- The *murinum*-complex. *Can. J. Genet. Cytol*., 4: 240-247.

Rajhathy, T., J .W. Morrison and S. Symko. 1964. Interspecific and intergeneric hybrids in *Hordeum*. *Barley Genetics*, I: 195-212.

Schlegel, R., R. Kynast and J.C. Schmidt. 1986. Alien Chromosome transfer from wheat into rye. In: *Genetics manipulation in plant breeding*, (Eds): W. Horn, C.J. Jensen, W. Odenbach, Oleds Schieder, pp. 129-136, De Gruyler, Berlin.

Singh, R. J. and T. Tsuchiya. 1982. An improved Giemsa N-banding technique for the identification of barley chromosomes. *J. Hered*., 73: 227-229.

Vahidy, A. A., Q. Jahan and B. Jahan. 1993. Giemsa N-banding polymorphism in six botanical varieties and six cultivars of barley, *Hordeum vulgare* L. *Cytologia*, 58: 273-279.

Vahidy, A.A. and B. Jahan. 1995. Intervarietal polymorphism of constitutive heterochromatin in *Hordeum vulgare* L. *Pak. J. Bot*., 27(2): 417-423.

Vinogradov, A.E. 2003**.** Selfish DNA is maladaptive: evidence from the plant Red List. *Trends in Genetics*, 19: 609-614.

Vosa, C.G. 1976. Chromosome banding patterns in cultivated and wild barley. (*Hordeum* spp.). *Heredity*, 37: 395-403.

Zerneke, F. 1987. Leishman-C-banding der chromosomen des diploiden *Hordeum chilense* und des amphidiploiden *Hordeum chilense X 6x-Triticale*- Bastards. Arch. *Zuchtungsforsch*, 17:129-137.

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