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GIEMSA N-BANDING PATTERN IN TETRAPLOID TAXA OF HORDEUM MARINUM HUDS

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Abstract

The N-band patterns of heterochromatin distribution in tetraploid taxa of *Hordeum marinum* chromosomes was studied to identify general patterns or preferential sites for heterochromatin. An ideogram was developed for each studied taxa of *Hordeum marinum* for the description of individual N-bands. Giemsa N-banding patterns of tetraploid taxa of *H. marinum* and ssp. *marinum* were characterized by having 3-4 bands per chromosome on an average, distributed at centromeric, telomeric and intercalary positions.Chromosomes1, 3, 4, 7, 9, 13 and 14 of tetraploid taxa showed minor polymorphism in their banding pattern. Negligible variation in banding pattern among tetraploid taxa, tend to suggest that N-heterochromatin has played a minor role in bringing about the species differentiation in *H. marinum*.

Introduction

Hordeum marinum is a species of barley commonly known as sea barley grass, considered an important early indicator of emerging soil salinity. Native to coastal western and southern Europe. Naturalized in Victoria, New South Wales, Queensland, South Australia and Western Australia. *H. marinum* (XX) is tolerant to salinity (Mano & Takeda, 1998; Garthwaite *et al*., 2005), and can be hybridized with wheat (Jiang and Liu, 1987). *H. marinum* displays a capacity to control concentrations of Na^+ (and also Cl^-) in leaves, even when exposed to very high concentrations of NaCl in the root-zone (Garthwaite *et al*., 2005). *H. marinum*–bread wheat amphiploid was produced by A.K.M.R. Islam (Colmer *et al*., 2005). In addition to salt tolerance, *H. marinum* also showed better root aeration and higher water logging tolerance, as compared with several other species in the Triticeae, including wheat (McDonald *et al*., 2001; Garthwaite *et al*., 2003). Salt and water logging tolerance in the amphiploid was reported to be intermediate to that of its parents (Colmer *et al*., 2005). 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 (Jahan & Vahidy, 2007). Cytological analysis become an essential screening tool in breeding programs, determining the hybrid fate in the cultivar development process.The C-banded karyotype of *H. marinum* subsp. *gussoneanum*, 4x, and studies using *In situ* hybridization (Linde-Laursen *et al.*, 1992) support the idea that it is an autopolyploid derivative of diploid *H. marinum.* The annual species *H. marinum* shows, despite morphological similarities to some of the other *Hordeum* species, no chromosomal homologies to the other species and appears to have a separate genome, preliminarily called "**X**" (Bothmer *et al.*, 1986, 1987). This genome is common to the two diploid forms, subsp. *marinum* and subsp. *gussoneanum* (Bothmer *et al.*, 1989). The cytogenetic behavior of the tetraploid cytotype of *H. marinum* subsp. *gussoneanum* is debated. It forms almost exclusively bivalents at meiosis, which should indicate an alloploid nature. Data from, for example, isoenzyme patterns and the presence of only one satellited chromosome pair in the karyotype also agree with the hypothesis that two unrelated genomes are represented in the tetraploid (Jørgensen 1982, 1986, Linde-Laursen *et al.,* 1989). However, the hybrid between the di- and tetraploid forms of *H. marinum* has full meiotic pairing. It forms almost exclusively trivalents, which indicates a very high degree of homology among the chromosomes (Bothmer *et al.,* 1989). Contrary to the other evidence, this suggests an autoploid nature of *H. marinum,* 4x, combined with a very strong genetic regulation for bivalent pairing. Further support for an autoploid origin is gained from the demonstration of a chromosome pair, morphologically similar to the satellited chromosome pair, having an inactive nucleolus organizer region (Linde-Laursen *et al.,* 1992). One chromosome of each of the two pairs forms a bivalent in *H. marinum* subsp. *gussoneanum,* 4x x *Secale cereale*-hybrids with up to seven *Hordeum* ring bivalents. Thus, the tetraploid *H. marinum* has most probably originated as a hybrid between two biotypes of diploid *H. marinum.* Giemsa C-banded karyotypes of the annual, self compatible species *H. marinum* and *H. murinum* were reported by Linde-Laursen *et al.,* (1989). Both species showed mostly very small bands at centromeric and telomeric positions, at one or both sides of the nucleolar constrictions and at intercalary positions. The C-banding patterns of *H. marinum* ssp. *marinum* and ssp. *gussoneanum* showed some divergence in spite of the close relationship. The C-banded karyotypes of *H. murinum* ssp. *murinum* and ssp. *leporinum* were very similar, supporting conspecificity. Morphology of C-banded metaphase chromosomes, C-banding patterns and the numbers of homologous arms of *H. marinum* ssp. *marinum* and ssp. *gussoneanum* were studied by Lushnikova (1988). The study revealed that the two subspecies were distantly related. The genome of *H. marinum* ssp. *gussoneanum* appeared to comprise two closely related diploid genomes, one of them well corresponding to the ssp. *marinum* (2x) genome. The tetraploid cytotype suggested its alloploid origin. The aim of the present study is to seek and exploit the patterns of heterochromatin distribution in *Hordeum marinum* chromosomes in order to evaluate the use of the bands as markers in cytogenetic investigations.

Materials and Methods

The sources of the plant material used are presented in Table 1. 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

The N-banded karyotype of *H. marinum* (Fig. 1) and *H. marinum* ssp. *marinum* (Fig. 2) showed more or less similar banding patterns. Chromosome 1 of *H.marinum* had three bands, one centromeric and two interstitial in each arm, while of ssp. *marinum* possessed two bands in the short arm, a centromeric and an interstitial. Chromosome 2 of *H. marinum* showed two bands, the dark centromericin the long arm and a light distal band in the short arm while each arm of ssp. *marinum* had only a centromeric band (Table 1). Chromosome 3 of both taxa had two interstitial bands, one in each arm. A telomeric band on the short arm was present only in ssp. *marinum*. Short arms of chromosome 4 of both taxa and a long arm of ssp. *marinum* showed a light centromeric and dark telomeric band, while the long arm of *H.marinum* had three bands, light centromeric, interstitial and a dark distal band. Chromosome 5 with an interstitial band in the long arm showed similar banding pattern in both taxa. Chromosome 6 was also similar and possessed only centromeric band in each arm. Chromosome 7 of *H. marinum* had a dark interstitial band in the short arm and a light interstitial band in the long arm, while in ssp. *marinum* centromeric and interstitial bands were present only on the short arm. Chromosome 8 of both taxa showed a centromeric and two interstitial bands in the short arm and a centromeric and distal band in the long arm. Chromosome 9 of both showed an interstitial band in the short arms. Chromosome 10 was also similar with a centromeric band in each arm. Chromosome 11 had a centromeric band in each arm and an interstitial band in the median position of the long arm in both the taxa. Chromosome 12 possessed a centromeric in each and an interstitial band in the long arm. Chromosome 13 of *H. marinum* had two interstitial and one distal band in the short arm while ssp. *marinum* possessed centromeric band in both arms and an interstitial and a telomeric band in the short arm. Long arm of a chromosome 14 showed a centromeric and an interstitial band in the middle portion in both taxa and the short arm (excluding satellite) showed a centromeric and telomeric bands in both taxa. An interstitial band was also present in the short arm of *H. marinum* which differentiated the banding pattern from ssp. *marinum*.

Discussion

H. marinum is tolerant of both salinity and water logging, so *H. marinum*-wheat amphiploids could help address the challenging issue of the severe adverse interaction between water logging and salinity on cereal production.

Table 1. Giemsa N-banding pattern (considering constitutive heterochromatin) in *Hordeum marinum* Huds. and ssp. *Marinum*

***H. marinum* (Fig. 1)**	**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	1/1	0/1	0/0	1/1	0/0	1/1	0/0	1/1	0/0	1/1	1/1	1/1	0/0	1/1
IPC	0/0	0/0	1/1	0/1	0/1	0/0	1/1	1/0	0/0	0/0	0/0	0/1	2/0	1/0
IMP	1/2	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0	0/1	0/0	0/0	0/1
IPT	1/0	1/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	1/0	0/0
T	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0
SAT L/M/T														0/0/0

Ssp. *marinum* (Fig. 2)	**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	0/0	1/1	0/0	1/1	1/0	1/1	0/0	1/1	1/1	1/1	1/1	1/1
IPC	1/0	0/0	1/1	0/0	0/1	0/0	1/0	1/0	1/0	0/0	0/0	0/1	1/0	0/0
IMP	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/1
IPT	0/0	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
T	0/0	0/0	0/0	1/1	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	1/0
SAT L/M/T														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

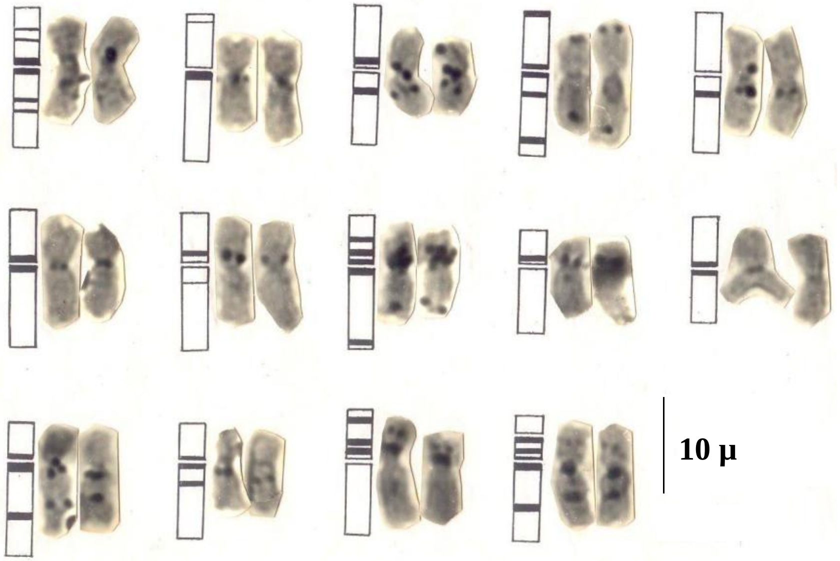

Fig. 1. Karyogram and ideogram of *H. marinum* (H132) through Giemsa N-banding technique.

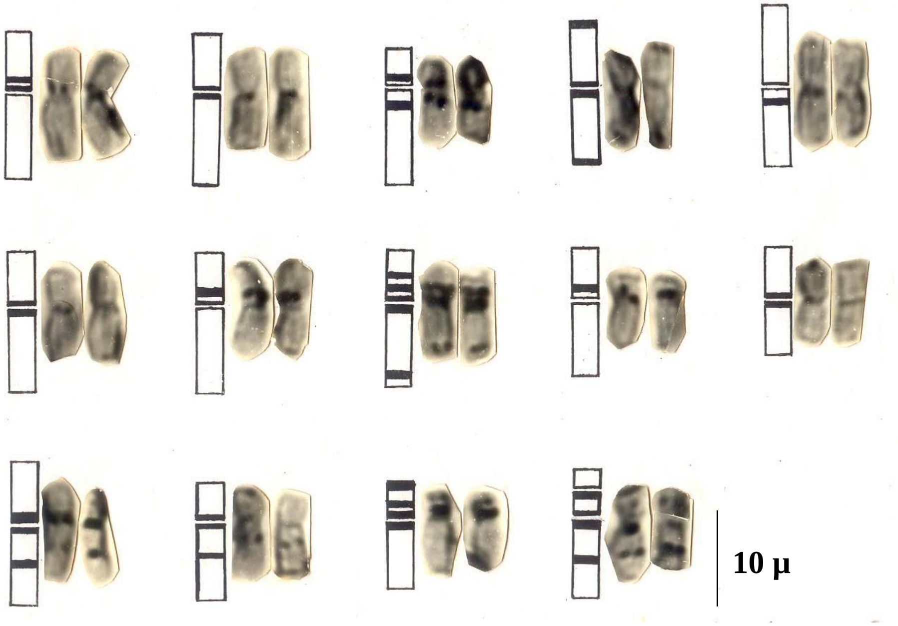

Fig. 2. Karyogram and ideogram of *H.marinum* ssp. *marinum* (H160) through Giemsa N-banding technique.

H. marinum and *H. murinum* were included in the section *Trichostachys* by Tzvelev (1976) but are now included in the sections *Stenostachys* and *Hordeum* respectively (Bothmer & Jacobsen, 1985). The differences in chromosome morphology and banding patterns support the conclusion that the taxa are not very closely related (Rajhathy & Morrison, 1962). The chromosome complement of diploid *H. marinum* consisted of five sub median and two median chromosome pairs, one of the former with satellites (Vahidy & Jahan, 1998). Previous investigators reported five metacentric, one submetacentric and one SAT- chromosome pairs (Morrison, 1959, Vosa, 1976, Lushnikova, 1988). Some discrepancies with respect to the number of SAT- chromosomes were also apparent. Chin (1941) and Covas (1949) reported two chromosome pairs with satellites in diploid as well as in tetraploid cytotypes. The karyotype of the tetraploid cytotype studied by Lushnikova (1988) corresponds to a doubling of the diploid one. However the investigation of her material and 9 other lines of tetraploid cytotype by Linde-Laursen *et al.,* (1989) revealed only one pair of chromosomes with satellites. Presence of one pair of SAT-chromosomes in both cytotypes is in agreement with that of Linde-Laursen *et al.,* 1989 and Morrison (1959). Giemsa N-banding patterns of the diploid and tetraploid cytotypes of *H. marinum* (Fig. 1) and ssp. *marinum* (Fig. 2) were characterized by having three-four bands per chromosome on an average, distributed at centromeric, telomeric and intercalary positions. Chromosomes 1, 3, 4, 7, 9, 13 and 14 of tetraploid taxa showed minor polymorphism in their banding pattern. Negligible variation in banding pattern among tetraploid taxa, tend to suggest that N-heterochromatin has played a minor role in bringing about the species differentiation in *H. marinum.* Centromeric C-bands on each chromosome of diploid *H. marinum* (Vosa, 1976) were also observed by N-banding except chromosomes 1 and 2. The tetraploid cytotype of *H. marinum* is of alloploid origin. Morrison (1959) on cytological grounds, considered that the taxon was an alloploid combining the genomes of the diploid cytotype of *H. marinum* ssp. *gussoneanum* and an unknown species. This point of view was supported by Jorgensen (1986), using electrophoretic evidence and exclusive bivalent formation in the tetraploid cytotype as observed by Bothmer *et al.,* (1986). The C-banded karyotype studied by Linde-Laursen *et al.,* (1989) and Vosa (1976) mostly favoured an alloploid origin. However, the observations of high levels of autosyndetic pairing in hybrids between the tetraploid *H. marinum* ssp. *gussoneanum* and S. *cereale* (Staat *et al.,* 1985), of a high pairing lever in polyhaploids (Pohler *et al.,* 1986) suggest that parental genomes of the tetraploid cytotype are closely related. Electrophoresis of easterases carried out by Mitra *et al.,* (1970) led these authors to suggest an autoploid origin. *In situ* hybridization has disclosed that a submetacentric pair carries rDNA segments at positions matching the positions of the NORs in the diploid cytotype, suggesting autoploidy (Linde-Laursen *et al.,* 1992).

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