Pak.J.Bot., 27(2): 417-423, 1995.

INTERVARIETAL POLYMORPHISM OF CONSTITUTIVE HETEROCHROMATIN IN *HORDEUM VULGARE* L.

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Abstract

An intervarietal chromosome banding polymorphism in 8 botanical varieties of *Hordeum vulgare* viz., *convar. hexastichon* vars: *coeleste* and *ingrum*; *convar. distichon* vars: *inerme*, *pallidum* and *nutans*; *convar. vulgare* vars: *coeleste*, *horsfordianum* and *dwndarbeyi* was studied by Giemsa N-banding technique. Chromosomes were numbered according to the wheat homoeologue groups. The classification of the bands was in accordance to the generalized cytological nomenclature of cereal chromosomes (GCNCC). Majority of bands recognized in all varieties were centromeric or interstitial. Band polymorphism was observed on all chromosomes except 1H, which exhibited typical banding pattern in all varieties.

Introduction

Karyotype is one of the criteria for elucidating the phylogenetic relationships of related species. *Hordeum vulgare* has been used as a model crop for genetic and cytogenetic studies for many years. Chromosome identification mostly relied on the relative length and arm ratio of chromosomes, however these characteristics may be some what ambiguous and lead to erroneous classification. Barley chromosome 2H, 3H,4H and 7H are not distinguished easily by conventional karyotype techniques because these chromosomes are almost equal in size and possess about the same centromeric position. The banding techniques have been used successfully in many plant species to demonstrate hitherto undetected variation in the chromosomes both within and between individuals of the same taxon and among taxa. Giemsa banding technique is considered as a qualitative tool to identify individual chromosomes, while conventional staining methods are used as a quantitative approach to establish the standard karyotype (Singh & Tsuchiya, 1981). Previously the karyotype of barley was considered uniform, but the introduction of Giemsa C and N-banding techniques has revealed an unrecognized variation in the chromosomes (Linde-Laursen, 1978, 1982). Linde-Laursen (1975) demonstrated that C-banding method can be used to identify each of the barley chromosomes and this was confirmed by Noda & Kasha (1978) and Vosa (1976). Chromosome banding techniques thus 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. This could be extremely useful in plants such as barley, in which location of many gene loci are yet to be determined.

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Table 1. Investigated varieties of barley, *Hordeum vulgare* with their germplasm source.

Botanical varieties	Germplasm source	Accession Number
Convar. hexastichon L. var. *coeleste* Viborg ex Kunth	Botanischer Garten der universitat Main, Frankfurt. Germany	H42
var. *ingrum*	-do-	H45
Convar. distichon (L.) Alef. var. *inerme*	-do-	H43
var.*pallidum* Ser.	Botanischer Garten der universitat Bayreuth, Germany.	H59
var. *nutans* (Rode) Alef.	-do-	H175
Convar. vulgare var. *coeleste* L.	-do-	H163
var. *horsfordianum* Wittm.	-do-	H165
var. *dwndarbeyi* Zhuk.	-do-	H164

The fact that chromosome bands are inherited as Mendelian units and exhibit polymorphisms led Linde-Laursen *et al.*, (1982) to propose the use of C-bands in barley as chromosome markers for pedigree analysis and as descriptors for varieties. In a previous study Vahidy *et al.*, (1993) found heterochromatin polymorphism in the interstitial regions of all except chromosomes 5 and 7 (1H and 5H respectively on wheat homoeology) of barley complement, and concluded that the barley varieties and cultivars may be identified on the basis of their N-banding pattern.

The present investigation was undertaken with a view to add additional information about polymorphism between barley varieties inferred from the composition of bands indicated by Giemsa N-banding technique.

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 have been described by 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 patterns and to establish karyograms. Chromosomes are numbered according to the wheat homoeologue groups. The classification of the bands was in accordance to the generalized cytological nomenclature of cereal chromosomes (GCNCC).

Results and Discussion

Karyotype analysis of species is useful in detecting the probable relationships, origin and nature of chromosome variation. Karyotype differences have been reported among varieties of barley even before the discovery of banding techniques. Chromosomal structural differences provided a genetic basis for the varietal diversity in barley. Sharma (1956) observed high number of satellites and stated that structural interchanges are directly responsible for the different karyotypes among the different cultivated strains which differ from each other only in minor morphological characters.

Barley chromosomes numbered 1-7 here, are labeled as 7H, 2H, 3H, 4H, 1H, 6H and 5H respectively for their homoeology to wheat chromosomes (Huen *et al.*, 1991) . The N-band polymorphism was observed for all chromosomes except chromosome 1H among the 8 barley varieties. By comparing the Giemsa N-banding pattern among varieties of the same *convar.*, it was observed that chromosome 3H, 4H and 5H were polymorphic in vars. *coeleste* (H163), *dwndarbeyi* (H164) and *horsfordianum* (H165), as the band 3HL2.1 was absent in var. *dwndarbeyi* (Table 2). The bands 4HL4.1 and 5HL2.3 were absent in var. *horsfordianum* while these were present in other varieties (Table 2) belonging to the convar. *vulgare*. Varieties *coeleste* (H42) and *ingrum* (H45) belong to *convar hexastichon*, differed by the absence of band 6HL1.5 in var. *coeleste*. Similarly absence of a band 3HL2.1 in var. *inerme* (H43; Table 2) differentiated the banding pattern of vars. *inerme* and *pallidum* (H59) which belong to the convar. *distichon*. Similar small intervarietal differences in banding pattern were observed by Linde-Laursen (1978) and Noda & Kasha (1978) in C-banded preparations of barley cultivars. Linde-Laursen (1981) stated that the different banding patterns in the chromosomes of *H. vulgare* suggest that in this species there may be at least three different kinds of constitutive heterochromatin; that revealed by both C- and N-banding, that consistently revealed by C-banding only and that consistently revealed by N-banding only. The bands 3HL3.3 and 5HL2.3 observed in the present study (Fig.1) and earlier by Singh & Tsuchiya (1982) were reported neither by Fukui & Kakeda (1990) nor by

Table 2. Polymorphic dark N-bands in barley varieties. The + and - indicate presence and absence of dark bands, respectively.

	Accession numbers*							
Dark N-band	H42	H45	H43	H59	H175	H163	H165	H164
2HL3.3	-	-	+	+	-	-	-	-
3HL2.1	+	+	-	+	+	+	+	-
3HL3.3	-	-	-	-	+	+	-	+
4HL4.1	+	+	+	+	-	+	-	+
5HL2.3	-	-	-	-	+	+	-	+
6HL1.5	-	+	+	+	-	-	-	-
7HS2.3	-	-	+	+	+	+	+	+

*For details please see Table 1.

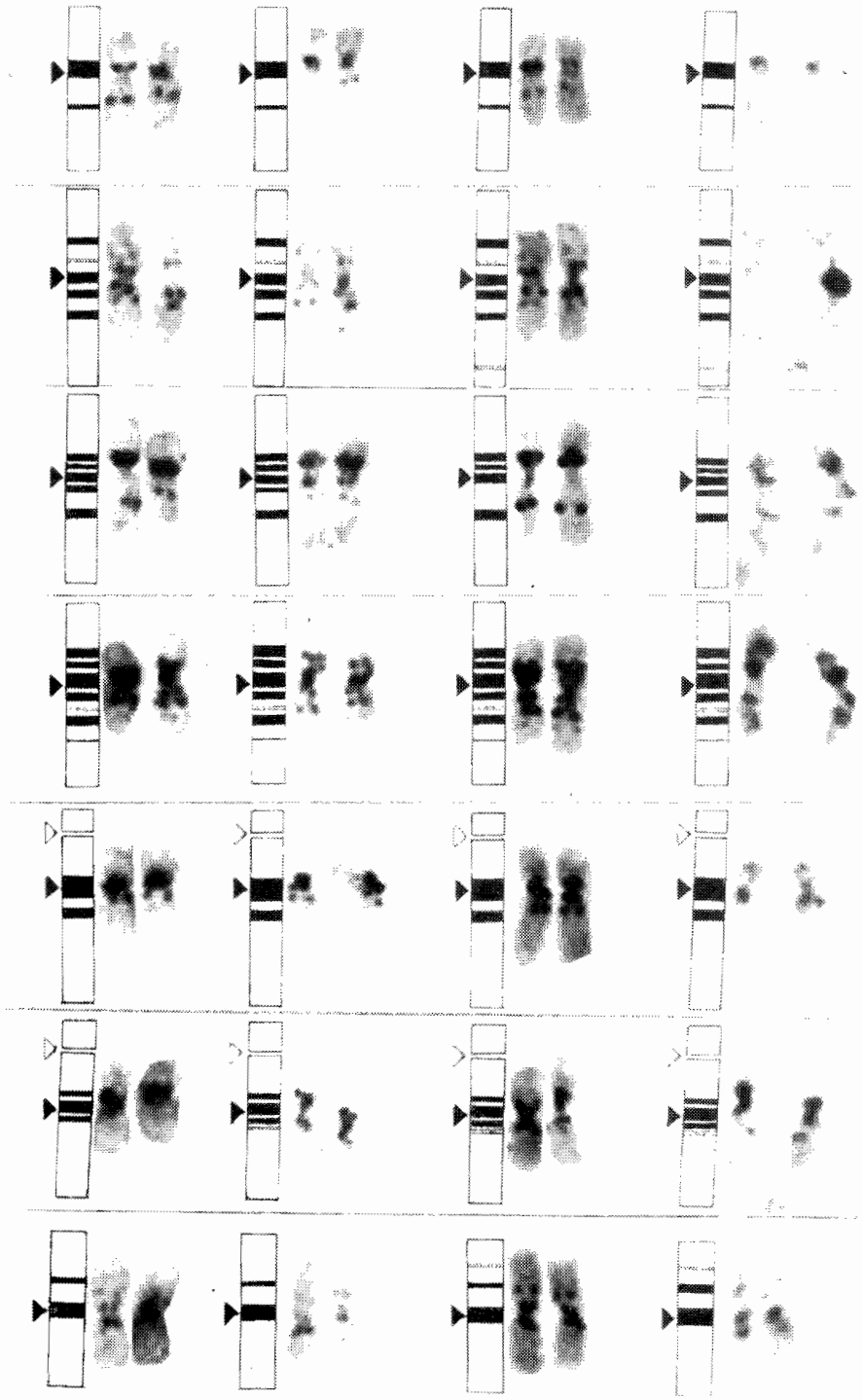
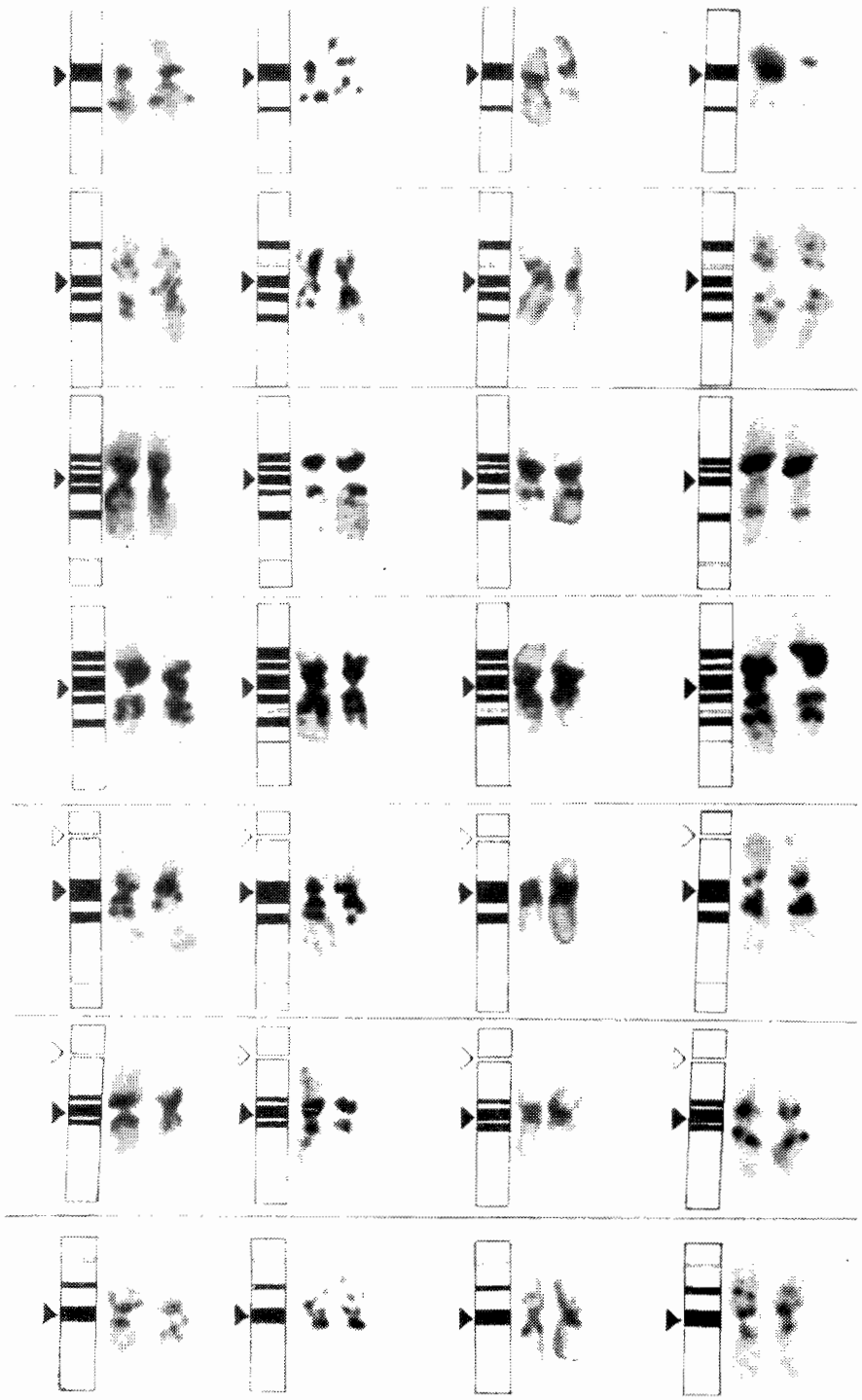


Fig. 1. N-banding polymorphism in 8 varieties of barley, *Hordeum vulgare*. Chromosomes arranged from left to right represent taxa: *convar. hexastichon* vars: *coeleste*, *ingrum*; *convar. distichon* vars: *inerme*, *pallidum*, nutans; *convar. vulgare* vars: *coeleste*, *horsfordianum*, *dwndarbeyi*. The chromosomes (1H-7H)

(Contd.)



according to wheat homoeology are arranged in rows from top to bottom.

Empty triangle and solid triangle indicating nucleolar organizing region and centromere respectively, closed and hatched bands indicate stable and unstable bands respectively.

Vahidy *et al.*, (1993). The later band was however, reported by Islam (1980). The bands 6HS2.3 and 5HS1.3 which were reported by Fukui & Kakeda (1990) as unstable bands were neither observed in the present study (Fig.1) nor in any variety investigated by us earlier (Vahidy *et al.*, 1993). The missing bands may represent deletions, differential susceptibility of the heterochromatic bands to the N- banding procedure or different staining affinity (Seal & Bennett, 1982).

Completely stable karyotypes in sexually reproducing species are unlikely to occur. Some evidence of a possible relationship between banding pattern and geographical origin can be obtained by considering the banded karyotypes. Little significant variation on a geographic scale has been reported in several *Hordeum* taxa by Linde-Laursen *et al.*,(1980). According to Linde-Laursen (1978) banding pattern polymorphism seems to indicate that in this species constitutive heterochromatin visualized as bands has no immediate impact on the fitness of the plant. Old barley varieties have shown more intervarietal C-band polymorphism than modern varieties. Linde-Laursen (1981) observed centromeric bands that were produced by N-banding but not by C-banding and pointed that there are wide differences among various barley lines in the size of particular bands and the number of bands on the same chromosome. On the other hand Singh & Tsuchiya (1982) stated that intervarietal heterochromatin polymorphism is absent in barley. Eventually, based on the fact that more bands can be found at prometaphase to early metaphase than at midmetaphase, they drew the conclusion that the variation in banding pattern observed in barley might be ascribed only to the mitotic stage examined and the technical procedure used. Kakeda *et al.*, (1991) investigated chromosome banding patterns in four barley cultivars by using C- and N- banding techniques. They demonstrated that N-banding positive and C-banding negative (N^+ C^-) heterochromatin was present at all centromeric sites and proved that intervarietal banding pattern polymorphism results from the intrinsicative characteristics of chromosomes.

The results indicated that the band polymorphism was found on all except chromosome 1H. In our earlier studies (Vahidy *et al.*, 1993) the band polymorphism was found in the interstitial regions of all except chromosomes 5 (1H) and 7 (5H). Each variety showed its distinctive banding pattern in most of the chromosomes. It can therefore, be concluded that the Giemsa N-banding technique is valuable for the analysis of polymorphic differences in the distribution of heterochromatic regions of different varieties of barley. Sources of error be minimized if comparisons are restricted to studies conducted within a working group, as variations due to different techniques employed in banding studies be reduced.

Acknowledgement

We are grateful to the germplasm sources mentioned in Table 1 for the supply of seed material. Financial aid to A.A.V. from the university of Karachi and research assistantship to B.J. from INFAQ Foundation are hereby acknowledged.

References

Fukui, K. and K. Kakeda. 1990. Quantitative karyotyping of barley chromosomes by image analysis methods. *Genome*, 33: 450-458.

Huen, M., A.E. Kennedy, J.A. Anderson, N.L.V. Lapitan, M.D. Sorrells and S.D. Tanksley. 1991. Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). *Genome*, 34: 437-447.

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

Kakeda, K., K. Fukui and H. Yamagata. 1991. Heterochromatic differentiation in barley chromosomes revealed by C- and N-banding techniques. *Theor. Appl. Genet.*, 81: 144-150.

Linde-Laursen, I.1975. Giemsa C- banding of the chromosomes of Emir' barley. *Hereditas*, 81: 285-289.

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

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, R.P. Ellis, A.M. Hayter and R.N.H. Whitehouse. Edinburgh University Press, Edinburgh, p. 786-795.

Linde-Laursen, I. 1982. Supplementary list of Giemsa C-banded karyotypes of 2-and 6-rowed barley. *Barley Genet. Newsl.*, 12: 63-65.

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., H. Doll and G. Nielsen. 1982. Giemsa C- banding patterns and some biochemical markers in a pedigree of European Barley. *Z. Pflanzenzuchtg.*, 88: 191-219.

Noda, K. and K. J. Kasha. 1978. A proposed barley karyotype revision based on the C-band chromosome identification. *Crop. Sci.*, 18: 925-930.

Seal, A.G. and M.D. Bennett. 1982. Preferential C-banding of wheat and rye chromosomes. *Theor. Appl. Genet.*, 63: 227-233.

Sharma, A. K. 1956. Chromosome studies in some Indian barley I.*Proc. National Inst. Sci. India.* B., 22: 246-254.

Singh, R. J. and T. Tsuchiya. 1981. Identification and designation of barley chromosomes by Giemsa banding technique: a reconsideration. *Z. Pflanzenzuchtg.*, 86: 336-340.

Singh, R. J. and T. Tsuchiya. 1982. Identification and designation of telocentric chromosomes in barley by means of Giemsa N-banding technique. *Theor. Appl. Genet.*, 64: 13-24.

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

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

(Received for Publication 4 April, 1995)