Asian Journal of Plant Sciences 2 (2): 224-227, 2003
ISSN 1682-3974

Chromosomal Variation in Two Species of *Hordeum*

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Abstract: The present study was conducted for karyomorphological investigation in 15 lines of two species of *Hordeum*. All the lines showed a diploid chromosome number of 14. Differences were observed regarding chromosome length, total chromatin length (TCL), type etc. among the 15 entries. The longest (11.06 μm) chromosomes were observed in BTON-10 while the shortest (4.23 μm) in BEL-4 and BEL-36 of *Hordeum vulgare*. Extra large chromosomes were found in BEL-4 (1 pair) and BTON-10 (5 pairs) of *H. vulgare*. Large type chromosomes were found in BHV-105, BTON-10 and conquest of *H. vulgare*. Medium type and relatively short type chromosomes were absent in BEL-36, BHV-1 and BTON-10 of *H. vulgare*, respectively. More metacentric chromosomes (7 pairs) were found in BHV-1 of *H. vulgare*. Maximum chromatin length (70.21 μm) was also found in BTON-10 and minimum (40.96 μm) in BEL-36 of *H. vulgare*. Maximum (47.76%) and minimum (39.88%) total fractional percentage (TF %) were found in BHV-1 and API-19 of *H. vulgare*, respectively. Each line has a specific chromosomal morphology which might be responsible for the variations of *Hordeum* species.

Key words: Chromosome, variation, morphology, metaphase, *Hordeum* sp.

Introduction

Barley plant is remarkably stable genetically, although numerous hereditary changes have occurred. Of the seven linkage groups in barley studies with chromosome translocation indicate that groups III and IV should be considered as one (Kramer *et al*., 1954). However, the presence or absence of a linkage disequilibrium affecting the genes controlling a quantitative character will determine the best strategy for producing desirable recombinant lines. Furthermore, the presence of linkage will bias cross prediction methods since estimates of additive gene variance or normally obtained from rank I variances (Caligari *et al*., 1985). In fact, almost any genetic changes sought by plant breeders may be induced as new mutations (Nybom, 1954). Many barley varieties have been induced artificially and most of the induced mutations are deleterious recessives. Barley lines with genes for resistance to disease have been studied by Jensen *et al*. (1980) and Per Koster *et al*. (1986). Meiosis in haploid barley and relationship between *H. vulgare* and *H. bulbosum* have been studied by Pickering (1983) and reported the occurrence of chromosome duplication within the haploid set and the basic chromosome number as seven. Although at a lower frequency, but a relative proportion of barley germplasms are induced spontaneously and along with that morphological changes both qualitatively and quantitatively are being caused. Thus, a keen morphological study was essential to detect the genetic diversity among the barley germplasm for potential breeding programme.

Materials and Methods

The experiment was conducted in the laboratory of the Department of Botany of Rajshahi University, Rajshahi, Bangladesh during the year 1999-2000. Fifteen lines of *Hordeum* species were collected from CIMMYT, Mexico and Bangladesh Agricultural Research Institute (BARI), Bangladesh (Table 1). Fresh and dry seeds of 15

Table 1: Sources of different varieties/lines of two species of *Hordeum*

Species	Varieties/lines	Sources
Hordeum nudum	-	CIMMYT, Mexico
Hordeum vulgare	API-19	„
„	BEL-4	„
„	BEL-34	„
„	BEL-36	„
„	BEL-72	„
„	BHV-1	BARI, Gazipur Bangladesh
„	BHV-91	„
„	BHV-95	„
„	BHV-105	„
„	BTON-10	CIMMYT, Mexico
„	BTYN-8	„
„	BTYN-37	„
„	Centinella	„
„	Conquest	„

CIMMYT = International Centre for Maize and Wheat Improvement
BARI = Bangladesh Agricultural Research Institute

lines/species of the *Hordeum* were allowed to germinate in petridishes lined with moistened Whatman filter paper at room temperature (20-22^{0}C) in the laboratory of Botany of Rajshahi University. When the roots grew upto 1-1.5 cm in length, the root tips were treated with saturated solution of paradichloro-benzene at 10^{0}C for 3 and 1/2 h. Then they were fixed in 1:3 acetoalcohol at room temperature and after 48 h of fixation the root tips were preserved in 70% ethanol. Root tips were stained using haematoxylin as stain following the schedule of Haque *et al*. (1976) with certain modifications. After removing from 70% ethanol the root tips were washed with distilled water for 6-7 min and were then hydrolyzed in 50% HCl for 25 min to dissolve the middle lamella of cells and again washed with distilled water for 5 min. Those were modanted by 2% aqueous solution of iron alum for 10 min and washed with distilled water for 20 min. Then the roots were stained with 0.5% haematoxylin for 30 min and again washed with distilled water for 5 min.

Meristematic zone of the root tip was cut with a razor blade and squashed in a drop of 0.5% of acetocarmine. Then it was warmed slight and the cells were covered with a cover glass. The slide was again warmed over an alcohol flame and a gentle pressure was applied by thumb over the cover glass keeping the slide duly wrapped in blotting paper to achieve the desired degree of spreading the cells and therefore, also of the chromosomes. Slides were then observed under microscope.

Measurement of chromosomes was taken from five metaphase plates of each line with the help of a divider and a millimeter scale. Then the values for chromosome size were converted into μm on the basis of enlargement of chromosome size in photomicrographs. Based on chromosome length, position of the centromere and their ratios, karyotypic formulae were derived for all the entries. It revealed chromosome complement to be made up off extra large (XL), large (L), medium (M), relatively short (S_1) and short (S_2) type of chromosomes with metacentric (m), submetacentric (sm) and sub-telocentric (st) centromeric position. For analysis, chromosomes were classified according to the position of the centromere and their arm (short arm/long arm) ratios. The chromosome having the arm length ratio less than 0.50 were placed in subterminal (st), the arm length ratios between 0.51 and 0.75 into the submedian (sm) and those having the arm length ratio above 0.75 into the median group (m) as suggested by Kutarekar and Wanjari (1983). The TF percent was calculated using the following formula of Huziwara (1962):

$$TF\ \% = \frac{\text{Total sum of short arm}}{\text{Total sum of chromosome length}} \times 100$$

Results and Discussion

It was observed that for karyotype analysis root tips collected between 20-22^{0}C showed maximum number of dividing cells. In order to carry out the karyotypic analysis of 15 entries *Hordeum* sp. root tips were treated with saturated solution of paradichloro-benzene (PDB) for 3 and 1/2 h, at 10^{0}C and it gave good results. Meyer (1947) developed a technique using saturated solution of PDB to facilitate chromosome study for root tip cells. In this study haematoxylin method (Haque *et al*., 1976) was used with certain modifications and it gave good results. All the 15 entries showed 2n= 14 chromosomes and hence, *Hordeum* sp. may be considered as homogeneous monobasic one. Such a similarity in chromosome number of these lines indicated their evolutionary trend from a common ancestor which has formed an uniform congregation.

The morphology of chromosomes was found to vary from line to line (Table 2). Differences were observed regarding chromosome length, total chromatin length (TCL), centromeric position, type, TF % etc. Photomicrographs were taken of only well spread and properly contracted metaphase chromosomes (Fig. 1). Among 15 entries longest (11.06 μm) chromosome was observed in BTON-10, shortest (4.23 μm) in BEL-4 and BEL-36 of *H. vulgare*. Maximum total chromatin length (70.21 μm) was found in BTON-10 and minimum (40.96 μm) in BEL-36 of *H. vulgare*. The widest range of arm ratio (0.33 to 1.00) was observed in BHV-91 and the smallest range (0.92 to 1.00) in BEL-4, BHV-95 and BHV-105 of *H. vulgare*. The differences in the total chromatin length might be considered as one of the most important factors in the evolutionary trend (Stebbins, 1950). On the basis of this factor BEL-36 of *H. vulgare* having minimum length may be considered as advanced and BTON-10 of *H. vulgare* with maximum chromatin length as primitive. Such a reduction in chromatin length might be due to the erosion of chromatid segments during the course of evolution.

On the other hand, taxa of symmetrical karyotype are thought to be primitive than those with asymmetrical karyotype (Stebbins, 1958; Singh and Moss, 1982). On the basis of this criterion the lines API-19 and BHV-105 of *H. vulgare* and *H. nudum* may be considered as the advanced genotype due to highest number (2 pairs) of subterminal (st) chromosomes. Such a situation may arise due to deletion, translocation or pericentric inversion (Singh and Moss, 1982). It also might be due to the deficiency in one arm of the chromosomes causing the shift of centromeric positions. This shifting of the centromeric position may not be in a similar fashion in all the chromosomes of the same line and this lead to asymmetric condition of the karyotypes. This karyotype

Table 2: TCL, total fractional percentage (TF%) and KF of the somatic chromosomes of fifteen lines of *Hordeum*

Lines/Varieties	Range of chromosome length (µm)	Total chromatin length (TCL) (µm)	TF %	Karyotype formula (KF)				
				XL 9.50 µm and above	L 8.50-9.49 µm	M 7.50-8.49 µm	S_1 6.50-7.49 µm	S_2 6.49 µm and less
Hordeum nudum	5.85-7.80	45.50	40.00	-	-	M^{sm}	$S_1^m+2S_1^{sm}+S_1^{st}$	$S_2^m+S_2^{st}$
Hordeum vulgare								
API-19	5.85-8.13	49.73	39.88	-	-	$2M^m+M^{sm}$	$S_1^{sm}+S_1^{st}$	$S_2^m+S_2^{st}$
BEL-4	4.23-10.40	51.36	44.94	XL^m	-	$2M^m$	$2S_1^m+S_1^{sm}$	S_2^m
BEL-34	6.83-8.13	53.64	41.82	-	-	$M^m+3M^{sm}+M^{st}$	$2S_1^m$	-
BEL-36	4.23-6.83	40.96	42.85	-	-	-	$2S_1^m+S_1^{st}$	$3S_2^m+S_2^{sm}$
BEL-72	5.53-7.80	47.14	43.45	-	-	Ms^m	$4S_1^m$	$2S_2^{sm}$
BHV-1	5.20-7.15	43.55	47.76	-	-	-	$4S_1^m$	$3S_2^m$
BHV-91	5.20-8.13	45.19	41.71	-	-	M^m+M^{sm}	$S_1^m+S_1^{sm}$	$S_2^m+S_2^{sm}+S_2^{st}$
BHV-95	4.55-8.13	48.76	45.32	-	-	$2M^m+M^{sm}$	$2S_1^m$	$S_2^m+S_2^{sm}$
BHV-105	5.85-9.10	52.01	42.49	-	L^{sm}	$2M^m+M^{st}$	$2S_1^m$	S_2^{st}
BTON-10	8.45-11.06	70.21	40.28	$3XL^m+XL^{sm}+XL^{st}$	L^{sm}	M^{sm}	-	-
BTYN-8	5.20-8.45	49.40	44.74	-	-	$2M^m+M^{sm}$	$S_1^m+S_1^{sm}$	$2S_2^m$
BTYN-37	4.55-7.80	44.88	44.92	-	-	M^m	$2S_1^m+S_1^{sm}$	$S_2^m+2S_2^{sm}$
Centinella	5.20-8.46	44.54	43.06	-	-	M^{sm}	$2S_1^m+S_1^{sm}$	$S_2^m+S_2^{sm}+S_2^{st}$
Conquest	6.50-9.10	53.63	42.42	-	L^{sm}	M^m+2M^{sm}	$2S_1^m+S_1^{sm}$	-

XL= extra large, L= Large, M= medium, S_1= relatively short and S_2= short

Fig. 1: Metaphase chromosomes of two species of *Hordeum*

evolution brought about by repatterning of chromosomes structure might be considered as one of the prime factors for evolution within the same species and thus the formation of different lines with the same chromosome numbers (Sinha and Kumar, 1979).

Chromosomes with subterminal constriction were not found in the lines BEL-4, BEL-72, BHV-1, BHV-95, BTYN-8, BTYN-37 and Conquest of *H. vulgare* (Table 2). There were 4 pairs of chromosomes with sm constriction in Conquest of *H. vulgare*. Three pairs of chromosomes with sm constriction were observed in BEL-34, BEL-72, BHV-91, BTON-10, BTYN-37, Centinella of *H. vulgare* and *H. nudum*. API-19, BHV-95 and BTYN-8 carried 2 pairs and BEL-4, BEL-36 and BHV-105 of *H. vulgare* carried 1 pairs of sm chromosomes. On the contrary, BHV-1 of *H. vulgare* may be considered as the primitive member because of the presence of highest number (7 pairs) of symmetrical chromosomes.

The line API-19 of *H. vulgare* was supposed to be the most advanced due to having the smallest TF% and BHV-1 of *H. vulgare* possessing the largest TF% was considered as the most primitive one (Table 2). However, in this study, three criteria adopted to determine the primitive and advanced members indicated totally different results. Thus, the study should be taken into consideration following some other criteria, which may reflect a valid result. Generally, it is observed that all the evolutionary tendencies might not go hand to hand in the phylogenetic evolution of different taxa due to their different adaptive values. As such one of the characters might put the line at the level of advancement while the others may be still in primitive stage. It can be visualized in case of BEL-36 of *H. vulgare*. When the total chromatin length is considered, it makes it highly advanced while on the basis of number of sm chromosomes and asymmetrical karyotypes it can be classified as contemporary to the primitive one.

Thus, on the basis of forgoing discussion it can be concluded that the karyotypic evolution within the lines might have been through quantitative or qualitative or both types of changes in the chromosome complement of the lines. Therefore, these findings suggest to trace the karyotypic evolution in any plant species using a quantitative method which may indicate the possible pathway of changes of chromosome in the course of evolution.

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