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Karyotypic and Morphologic Variations in some Hull-less

Barley (*Hordeum vulgare* L.) Genotypes

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Abstract

Hull-Less Barley is a grass belonging to the family Poaceae, the tribe Triticeae and the genus *Hordeum*. The present paper describes the morphologic and karyologic analysis in sixteen genotypes of hull-less barley (*Hordeum vulgare* L.) genotypes. Karyotypic studies were carried out, using squash technique and 2% (w/v) aceto-carmin staining method. Chromosomal parameters examined were long and short arms (L & S), total chromosome length (TL), arm ratio (AR), r-value (S/L), chromosome form percentage (F%), chromosome volume, total chromatin length (X) and the number of satellites. Genotypes tested were diploid ($2n=2x=14$). Satellite numbers were differed, ranging from 0 to 2 pairs. The most diploid chromatin length were detected in G9 (73.4 μm) while G15 demonstrated the least (30.8 μm). The type of chromosomes was determined as "m" in all genotypes, using Levan's chromosome nomenclature. Karyotypes were classified in 1A of Stebbin's classification. In addition to this, to test the karyotypic symmetry in more detail, other parameters, e.g. Romero-Zarco, karyotype total form percentage (%TF), symmetry index (%S), coefficient of variation (%CV), dispersion index (DI) were also considered. For instance, in Romero-Zarco method, the A1 and A2 coefficients were 0.37 (G2) and 0.46 (G9), respectively. The resultant morphological data were tested for normality and then were analyzed with the randomized complete block design (RCBD) with 3 replications. ANOVA indicated high significant differences for most morphological characters. The resultant cytogenetic data were tested for normality and then were analyzed according to completely randomized design (CRD) with 5 replications of cells. ANOVA indicated high significant differences for chromosomal parameters. Correlation determined between genotypes for both morphological and cytogenetical parameters. Cluster analysis

was carried out, showing 2 clusters for either morphological characteristics or chromosomal parameters. Moreover, principal component analysis (PCA) was carried out, demonstrating 8 components for morphological characters justified 91.0% of the total variations and 3 components for chromosomal parameters justified 94% of the total variations. In general, the results of his study proposed that selective genotypes for crossing in plant breeding with the best far morphological variations having the most homology in chromosomes variations.

Keywords: Cytogenetic variation, Karyotype, Chromosome, Hull-less Barley, *Hordeum vulgare* L.

Introduction

Barley is a grass belonging to the family Poaceae, the tribe Triticeae, and the genus *Hordeum* (Nilan and Ullrich, 1993). About 90% of Barley grain is used in alcoholic beverage production and as a livestock feed (Szczodrak *et al.*, 1992). The first report about Hull-less Barley was in Canada that Scout variety were introduced (Lue *et al.*, 2001). Discrimination among these species has frequently been based on external morphology which may change according to biotic and a biotic factors leading to several different classifications. Morphological and cytogenetical studies make the identification genetic variation, in order to supply a reliable basis for selecting Hull-less Barley lines with high yield, and are one of the reasons why genetic and cytogenetic studies are important. Cytogenetic studies of Hull-less Barley are in general limited. Study of genetic and cytogenetic variation is essential for hybridization between genotypes. The first studies number of chromosome was reported by Kihara (1992) and numerical of seven chromosome on Barley proposed by Lewitsky (1931). Ramesh *et al.*, (1998) showed significant difference between length and volume parameters of chromosomes and the chromatin length were detected in 46.02 to 67.23 μm . Chromosomal parameters examined were as follow: long arm (L), short arm (S), total chromosome length (TL), arm ratio (AR), r-value (S/L), form percentage of chromosome (F%), chromosome volume and the number of satellites. As studies reproted by sheidai (2000), Verma (1980), Verna *et al.*, (1991), Bakhshi Khaniki and Ebrahimi (2000), Gennur *et al.*, (1988), Mirzai-Nedoushan *et al.*, (2000). The morphological and chromosomal studies is necessary for induce of genetic variation, transfer the useful characters or collect the useful characters and for studies of genetic and cytogenetic variation useing cluster analysis between genotypes. Morphy *et al.*,

(1992) indicated the cluster analysis for gang of wheat genotypes. Souza and Sorvells (1991) have reported about use of cluster analysis for selection of parent in breeding programs.

This study reports the morphology cytogenetic variation of 16 genotypes of Hull-less Barley introduced at Sistan, Iran in 2003. Also, the intent of this study was selection genotypes that were sampled for crossing in plant breeding with the best far morphological variations having the most homology in chromosomales variations.

Materials and methods

Sixteen Hull-less Barley populations were sampled for this study. The seeds were stored at 4°C until used for study. Seeds from each population were used for the genome size, base composition and cytological analysis. For germination, seeds were put on wet Whatman paper and then placed at room temperature for several hours. Emerging root tips from 30 to 40 h old germinated seeds were used for cytogenetic investigations. To observe metaphase plates, root-tip meristems were immersed in 0.002 M 8- Hydroxyquinoline at room temperature for 3 h and then fixed in ethanol: acetic acid (3:1, v/v) at 60°C for 15 min, and finally stored in 70% ethanol at 4°C. To observe metaphase chromosomal, meristematic tissue was stained with 0.02% (w/v) Aceto carmine. For staining, root-tip were put on Aceto carmin at room temperature for 3-4 days. The roots were then gently squashed in a drop of 45% acetic acid. For the ytological investigation, Images were captured with a BX50 Olympus camera. For numerical karyotype analysis, chromosomes from five metaphase were measured for Hull less Barley were identified and ordered according to their long length, short length, total length (TL), Form percentage ($F\% = \frac{\text{short length}}{\sum \text{total length of all chromosomes}} * 100$), the ratio (r) between the long and short arms, the ratio (R) between the longest and the shortest chromosome pair and hromosome volume. Ideograms were drawn from mean values, and chromosome types were determined according to Levan et al. (1964) and for study symmetric according to Stebbins (ST) and Romero-Zarco method Karyological features were evaluated as number of satellite pair, length chromatin of karyotype, total form percentage where $\%TF = (\frac{\sum \text{short length}}{\sum \text{total length of all chromosomes}} * 100)$, the relative length (RL) of each chromosome ($100 * \frac{\text{total length of each chromosome}}{\sum TL}$), the global asymmetric index (ASI) where $SI\% = (\frac{\sum \text{long}}$

arms/ \sum total length of all chromosomes *100), Difference of range of relative length (DRL; $=\%RL_{Max} - \%RL_{Min}$) and Dispersion Index.

This study was carried out at research station, Zabol University Agricultural Faculty which is located in the Eastern Sistan Region of Iran (61° 54' N Lat., 30° 54' E Long., and 438 m elevation). The experiment field had a soil of silty loam, with pH 8.0-8.3. This location is an arid area characterised by very dry summer and warm temperate with 21.70° C, and 55 mm rainfall. The population of Hull-less Barley (*Hordeum vulgare* L.) originated from ICARDA was established in a randomised complete block design with three replication. Seed was planted in plot with 20 cm between rows. Each plot was fertilised with 90 kg P₂O₅/ha, 60 kg K₂O/ha, 40kg ZnSO₄/ha and 120 kg N/ha. Data were collected from 10 randomly chosen kernels from each plot. The following measurements were made: (1) No. of days to tillering, (2) No. of days to stem, (3) No. of days to spike, (4) No. of days to grain filling, (5) No. of days to doughy, (7) No. of days to maturity, (8) flag leaf length (cm), (9) flag leaf width (cm), (10) No. of node stem, (11) mean of node length, (12) No. of spike/plant, (13) No. of grain/spike, (14) No. of tillers/plant, (15) peduncle length (cm), (16) mean of own length (cm), (17) spike length, (18) flag leaf sheat length (cm), (19) 1000 kernel weight (gr.) and (20) plant yield (gr.).

Grouping morphological characters and chromosomal parameters were performed by using clustering methods (single linkage and WARD) and ordination on the first two principal components axes (PCA). Multivariate statistical analysis was performed on standardized data (mean=0, variance=1) using SAS, Minitab, SPSS and NTSYS. In order to determine the most variable on the species studied, principal components analysis (PCA) was performed. Also corrolation determined between genotypes for both morphological and cytogenetical parameters.

Results

In all metaphase plates analysed in root tips in the seedlings of Hull-less Barley, we observed a diploid chromosome number of $2n=2x=14$ ($X=7$) while those for G9 were $2n=2x=18$ ($x=9$). As viewed by Mohanty *et al.*, (1991) and Ahsan *et al.* (1998) the basic chromosome number some species of Barley was diploid and tetraploid ($2n=2x=14$ and $2n=4x=28$). Satellite numbers was differed, ranging from 1 to 2 pairs that differed in

length it. The chromosomes of the species studied were of m (centromere at median region). Chromosome length varied from 73.37 to 30.85 μm that the most chromatin length were detected in G9 while G15 demonstrated the least. Coefficient of variation were varied from % 46.05 (in No. genotype 9) to % 14.46 (in No. genotype 14). This result determined that genotype No. 14 was more symmetric and wild genotypes of them and influence environment were lower and total form percentage (%TF) were high that determined high systematic position. Also symmetric position determined by difference of form percentage and experiential in genotype of G10 it was high (7.13). Therefore symmetric karyotype were lower and difference of form percentage in genotype of G12 it was lowest (2.44) therefore symmetric karyotype were high of them.

It has been suggested that asymmetrical karyotypes are more advanced than symmetrical ones (Stebbins, 1973) and that the changes in symmetry are usually associated with chromatin loss. Among the species with $2n=14$ and $2n=18$, moving from class 1A (symmetrical class). Dispersion Index indicated symmetry, respectively, were as follows: G13, G12, G11, G8, G1, G14, G3, G15, G4, G2, 7, G5, G10, G6 and G9. Therefore, karyotype in Hull-less barley taxa studied is symmetric. In order to check association between the change in chromosome number, total and mean chromatin length, the coefficient of correlation was showed significant association. Therefore, it may be suggested that during species diversification, the change in genotypes via structural changes has occurred mainly down. However, the number of chromosome observed in this work appear in genotype 9 $2n=2x=18$ ($X=9$) within populations, and probably do not yet reported.

The resultant morphological data were tested for normality and then were analyzed with the randomized complete block design (RBD) with three replications and sixteen genotypes. High significant differences were observed among genotypes for most morphological characters measured. Mean comparisons for morphologic data were carried out using LSD test. The means comparisons confirmed the contrast between genotypes and showed that there are distinct groups and genotypes position were difference thus continue the studies were necessary. For this aim, were carried out principal component analysis and correlation for morphologic and cytogenetic data, then Grouping genotypes were performed by using cluster analysis.

Correlation coefficients are indicating relationship between characters. The developmental traits, namely days to ripe with flag leaf sheath length were maximum significant correlated (0.85) and showed significant negative correlation between plant yield with days to grain filling (-0.73), No. of tillers/plant (-0.64). Plant yield correlated positively with flag sheath length (0.51). In chromosomal parameters, arm ratio high significant correlated with r-value (S/L). Total chromosome length correlated positively with long arm and short arm.

Cluster analysis of morphological and karyological data are presented in Figures 1 and 3. and ordination of morphological and karyological data on the first two PCA are obtainable in Figures 2 and 4. The morphological and karyological cluster analysis, respectively, make up the second and third major cluster, indicating their genotypes distinctness. Ordination of taxa and morphologic based on the first two PCA axes supports the clustering results (Fig. 2&4).

Principal components analysis of morphological and karyological data showed that the first eight components and in PCIII correlation possessed %0.91 from variation in morphological characters and the first three components and in PCIII correlation possessed %0.94 from variation in karyological data.

The study revealed genetic and cytogenetic differences in ANOVA for morphological and karyological data for Correlation coefficient, principal component analysis and cluster analysis. Therefore, the results of this study proposed that selective genotypes with the best far morphological variations having the most homology in chromosomales variations. For this aim, selection for crossing would be effective, genotypes (7, 8, 10 and 13) * genotypes (3, 4, 2, 15, 1, 4, 5, 6 and 12). Cross would be suggest between G7 with G16, G8 with G4, G10 nad G4 because there are best far morphological variations and the most homology in chromosomales variations. Cross don't suggest between G9 with either genotypes because chromosome number of $2n=2x=14$ ($X=7$) while those for G9 were $2n=2x=18$ ($x=9$). Recurrent selection should be effective for improving yield might be improved by simultaneous selection the best genetic variation and the most chromosomales homology, cross between G10 and G12 is suggested.

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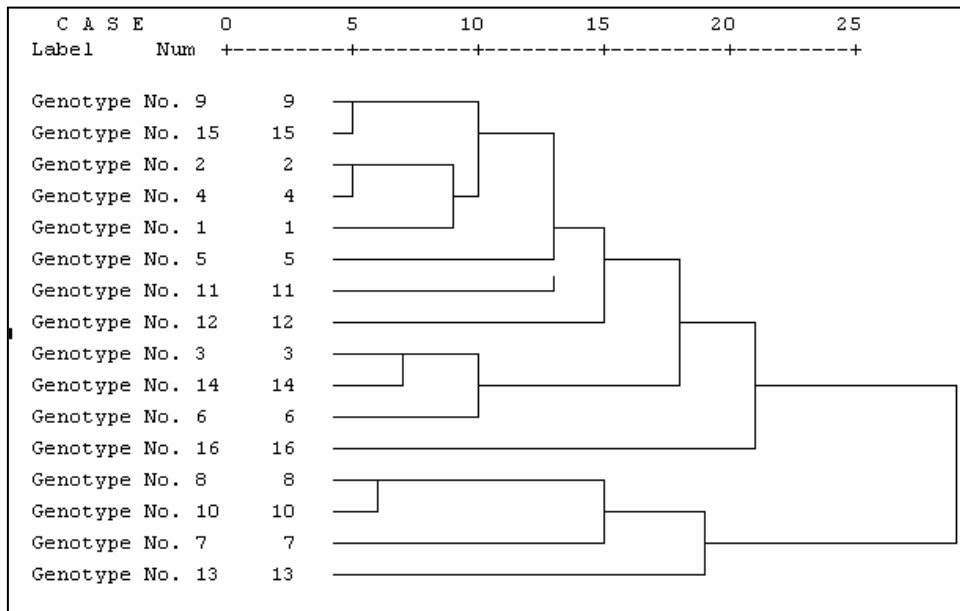


Figure 1. Phylogenetic two obtained by cluster analysis using specimen 16 with basic morphologic analysis. Numbers 1 to 16

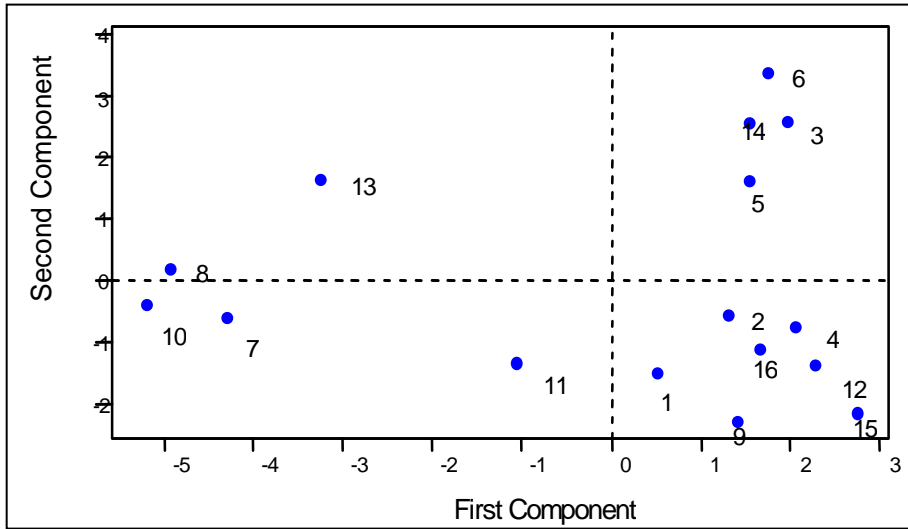


Figure 2. Ordination of 16 genotype Hull-less Barley with basic morphologic analysis.

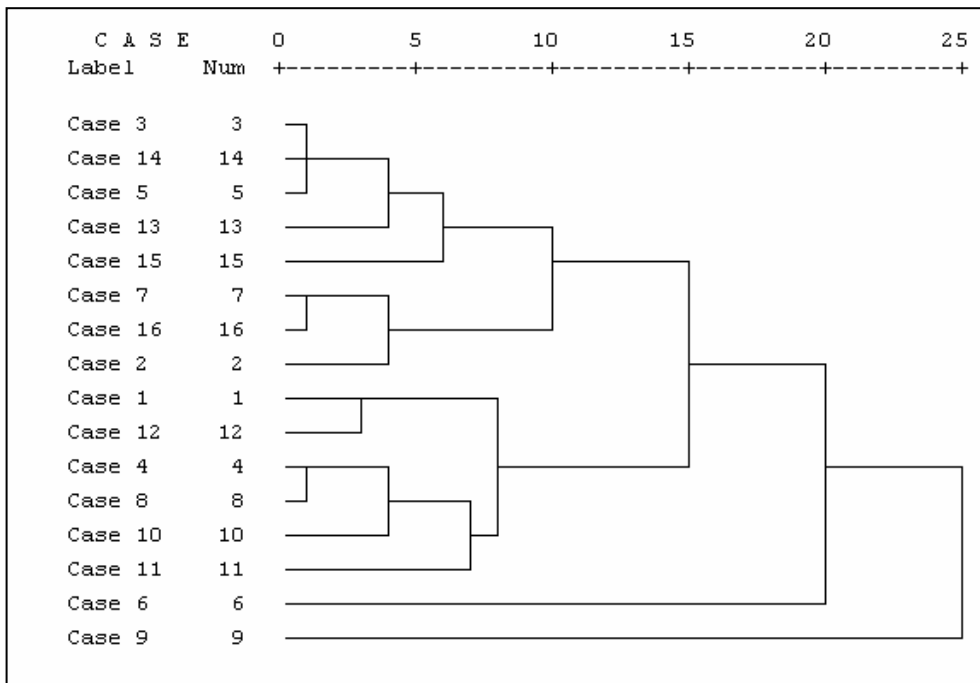


Figure 3. Phylogenetic tree obtained by cluster analysis using specimen 16 with basic karyotype. Numbers 1 to 16.

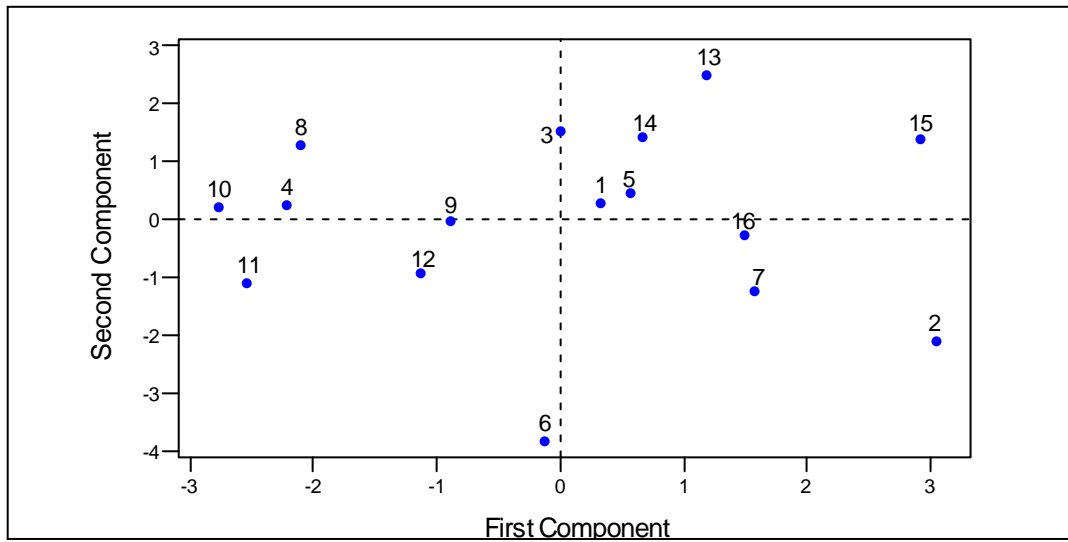


Figure 2. Ordination of 16 genotype Hull-less Barley with basic karyotype.