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ARTICLE

Cytogenetic study of some *Hordeum* L. species in Iran

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ABSTRACT Karyotypic and meiotic studies were performed in 17 populations of seven *Hordeum* species and subspecies showing the occurrence of 2n = 2x = 14, 2n = 4x = 28 and 2n = 6x = 42. Tetraploid level for *H. distichon* and hexaploid level for *H. leporinum* are new reports. The chromosomes were mostly metacentric and sub-metacentric. Significant differences in the size of chromosomes and their arm ratios indicated the occurrence of quantitative changes in the chromatin (DNA) material during species diversification. The species also differed in their karyotypic formulae possibly due to the occurrence of chromosome structural changes. Comparison of the total haploid chromatin length among populations of *H. bulbosum* and *H. glaucum* having diploid and tetraploid chromosome numbers, indicated that the occurrence of polyploidy is not associated with a significant increase in the amount of DNA and almost the same DNA content has been distributed among the chromosomes of the tetraploid population. The relative karyotypic data were used for multivariate analysis, which showed karyotypic variations among *Hordeum* species and populations studied. **Acta Biol Szeged 51(2):107-112 (2007)**

KEY WORDS

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The genus *Hordeum* L. (barley) of the family Pooideae (subfamily Hordeae) is comprised of about 40 annual or perennial species including diploid (2*n* = 2x = 14) and polyploid (2*n* = 4x = 28, 2*n* = 6x = 42) species or cytotypes (Bothmer et al. 1995), mainly distributed in the North temperate and South America but also available in South-West and central Asia. The *Hordeum* are commonly adventive, mesophytic, or xerophytic species of open habitats; halophytic and glycophytic. They usually grow in open weedy or sandy places and mostly in dry soils (Bothmer et al. 1991; Shewry 1992, Watson and Dallwitz 1992). The genus *Hordeum* possesses some significant weed species like *H. jubatum*, *H. leporinum*, *H. marinum*, *H. murinum* (the fruiting inflorescence parts of this causing eye and other damage to livestock, and problems with wool) and some grain crop species including *H. vulgare* (Barley).

The genus originated about 12 MYA (Blattner 2004) in western Eurasia from where it colonized its extant distribution area in Europe, central Asia, North America, South America, and South Africa. The monophyly of the genus is well supported by morphological (Seberg and Frederiksen 2001) and molecular phylogenetic studies (Petersen and Seberg 1997; Blattner 2004), whereas the intrageneric phylogeny is still a matter of dispute (Sabine and Blattner 2006).

The species of *Hordeum* growing in Iran varies according to different authors, Parsa (1950), Mobayyen (1981) and Bor (1970) reported the occurrence of 11 *Hordeum* species in Iran, but the species they report vary from each other. Moreover, Termeh (1986) reported *H. bogdanii* from Iran which is not reported by the previous authors. These species grow wild mostly in the north, west-north and south-west of Iran and are considered as important forage plants of the country.

According to Flora Iranica (Bor 1970), the *Hordeum* species of Iran, are distributed in 4 different sections of 1- *Bulbohordeum*, 2- *Crithe*, 3- *Hordeastrum* and 4- *Stenostachys*. The present study considers karyotypic details of 14 populations of 7 *Hordeum* species and subspecies as well as meiotic analysis of 3 populations of two species for the first time.

Materials and Methods

Plant materials

Karyotypic studies were performed on 14 populations of 6 *Hordeum* species and 1 sub-species namely: 1- *H. bulbosum* L. (two populations), from the section *Bulbohordeum*, 2- *H. distichon* L., 3- *H. spontaneum* C. Koch (two populations) and 4- *H. vulgare* L. (two populations), from the section *Crithe*, 5- *H. glaucum* Steud. (five populations), 6- *H. leporinum* Link., and 7- *H. marinum* Huds. subsp. *Marinum*, from the section *Hordeastrum*, while the meiotic studies were performed on 3 populations of *H. bulbosum* and *H. spontaneum*.

Cytological studies

For karyotypic studies freshly grown root tips were collected from the seeds of at least 10 randomly selected plants in each species, pretreated with 0.002 mol 8-hydroxyquinolin (1–2 hrs.) and fixed in ethanol: acetic acid (3:1) for 24 hrs. The

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Table 1. Karyotypic details of *Hordeum* species and populations studied.

A2	A_1	ST	TF%	No. sat	Sat.ch	S	L	T.L	2n	Polidy level	Locality	Sp. code	Species
16.49	0.26	1A	41.90	-	-	13.75	20.5	34.25	14	2x	Tehran	H.b1	*H. bulbosum*
18.04a	0.32	2A	40.13	2	11,14	12.2	23.4	35.6	28	4x	Arasbaran	H.b2	*H. bulbosum*
16.75	0.29	2A	40.34	3	1, 2, 3	14.49	22.87	37.36	14	2x	Dorood	H.d	*H. distichon*
16.49	0.31	2A	40.20	2	3,6	12	20.25	32.25	14	2x	Sangsefid	H.g1	*H. glaucum*
25.03	0.32	2B	39.27	1	1	8.2	18.5	27.32	14	2x	Sabzevar	H.g2	*H. glaucum*
24.49	0.28	1B	42.09	1	1	11.74	24	35.75	14	2x	Ardebil	H.g3	*H. glaucum*
19.09	0.36	2A	38.75	5	1,3,4,5,7	11.35	20.65	32	14	2x	Abadan	H.g4	*H. glaucum*
19.60	0.44	2A	35.09	-	-	13	23.25	36.25	28	4X	Tehran	H.g5	*H. glaucum*
22.97	0.30	2B	40.11	3	11,13,15	11	23.25	24.25	42	6X	Shazand	H.l	*H. leporinum*
20.64	0.36	2A	38.25	2	5,7	10.25	19.18	29.43	14	2x	Abadan	H.m	*H. marinum ssp. marinum*
16.04	0.34	1A	39.28	3	1,6,7	12.93	20.20	32.95	14	2x	Tehran	H.s1	*H. spontaneum*
16.54	0.27	1A	41.97	2	5,7	12.49	19.33	31.82	14	2x	Darake	H.s2	*H. spontaneum*
25.96	0.36	1B	38.86	5	2,4,5,6,7	14.55	31.7	46.25	14	2x	Ahvaz	H.v1	*H. vulgare*
10.87	0.36	1A	38.86	2	3,5	16.66	22.83	39.49	14	2x	Abadan	H.v2	*H. vulgare*

TL = Total haploid chromatin length (μm), L = Longest chromosome (μm), S = Shortest chromosome (μm), Sat = Sat-chromosome, TF = Total form percentage, ST = Stebbins class, A_1 & A_2 = Romero-Zarco indices.

fixed tips were then washed thoroughly in distilled water and macerated in 60°C 1N HCl for about 30 seconds. Squash technique was used for cytological studies with 2% aqueous aceo-orcein as the stain. The somatic chromosome number and karyotypic details were studied in at least 5 well-prepared metaphase plates. The chromosomes were sketched with the use of a Camera Lucida and measurements were performed accordingly from such sketches. The chromosomes were identified according to Levan et al. (1964), karyotype symmetry was determined according to Stebbins (1971) and A1 and A2 symmetry indices of Romero-Zarco (1986), while other karyotypic parameters like total form percentage (TF%) and coefficient of variation of the chromosome size were also determined (Sheidai et al. 2000).

Young flower buds were collected from 10 randomly selected plants of each species/ population and fixed in glacial acetic acid: ethanol (1:3) for 24 hrs. Flower buds were washed and preserved in 70% ethanol at 4°C until used (Sheidai et al. 2002, 2003). Cytological preparations used squash technique and 2% aceto-orcein as the stain.

Fifty to one hundred pollen mother cells (PMCs) were analysed for chiasma frequency and distribution at diakinesis. metaphase stage and 500 PMCs were analysed for chromosome segregation during the anaphase and telophase stages.

Statistical analyses

The analysis of variance (ANOVA) and the least significant difference test (LSD) were performed to reveal significant differences in the size of chromosomes among the populations of each species as well as among species with similar somatic chromosome numbers (Sheidai et al. 2000). In order to group the species studied based on similarity in their karyotypic features different clustering methods of UPGMA (Unweighted Paired Group with Arithmetic Average) and WARD (minimum spherical cluster method) as well as ordination based on principal coordinate analysis (PCO) were performed. Since the species studied possess different somatic chromosome numbers, relative karyotypic and meiotic parameters were used in clustering and PCO ordination (Table 1). NTSYS Ver. 2.02 (1998) was used for clustering and PCO analyses.

Results and discussion

The somatic chromosome number and karyotypic details of the *Hordeum* species and populations studied are presented in Table 1 and Figs. 1 & 2. Tehran population of *H. bulbosum* possessed 2n = 2x = 14 while, Arasbaran population possessed 2n = 4x = 28 chromosome number supporting the earlier reports (Strid and Franzen 1981; Semenov 1986). Dorood population of *H. distichon* possessed 2n = 2x = 14 chromosome number, supporting the earlier report (Zhang et al. 1990). Among 5 populations of *H. glaucum* studied, Tehran population possessed 2n = 4x = 28 chromosome number while, the other populations were diploid. The earlier studies reported only diploid chromosome number for this species (Farugi 1985, Avagian et al. 1989) therefore tetraploid level is new for *H. distichon*.

Shazand population of the species *H. leporinum*, possessed 2n = 6x = 42 chromosome number. The earlier studies reported only diploid and tetraploid chromosome numbers for this species (Hatch 1980; Valdés et al. 1999) therefore hexaploid level is new for *H. leporinum*.

Populations of *H. marinum subsp. marinum*, *H. spontaneum* and *H. vulgare* possessed 2n = 2x = 14 supporting the earlier reports (Nicoloff et al. 1977; Chen and Wang 1988; Spies et al. 1999). Tetraploid level has been reported for *H. marinum subsp. Marinum* by Avagian et al. (1989). Both

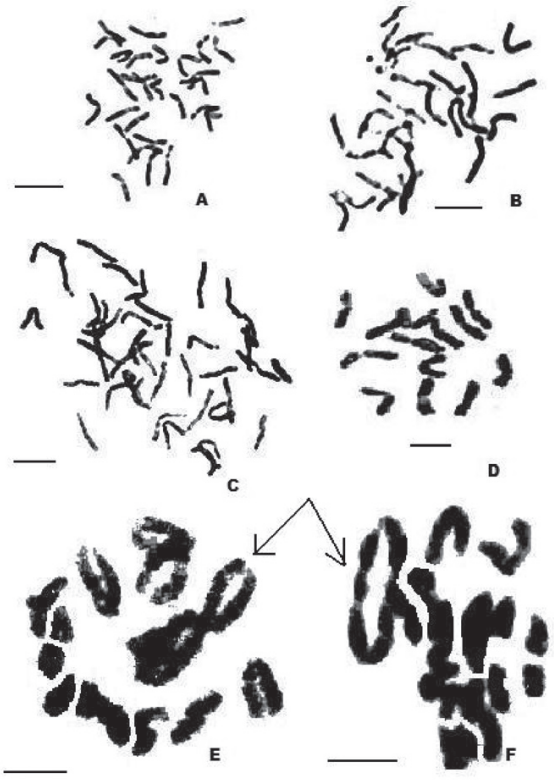

Figure 1. Representative somatic and meiotic cells in *Hordeum* species studied. A-D = Metaphase cells in Arasbaran population of *H. bulbosum* (2n = 28), Tehran population of *H. glaucum* (2n = 28), Shazand population of *H. leporinum* (2n = 42) and Tehran population of *H. spontaneum* (2n = 14). E & F = Meiotic cells in Tehran population of *H .bulbosum* showing quadrivalents (arrow). Scale bar = 10 µm.

tetraploid and hexaploid level have also been reported for *H. vulgare* (Koba 1993; Chen and Wang 1998).

Polyploidy and inter-specific hybridization is considered to be of high importance in the evolution of Gramineae (Stebbins 1982, 1986), the occurrence of different polyploidy levels in different *Hordeum* species and also among populations of a single species, indicates the role of polyploidy in the evolution and adaptation of these species.

The *Hordeum* species and populations studied mainly possessed metacentric (m) and sub-metacentric (sm) chromosomes, however, sub-telocentric chromosomes (st) occurred in Tehran population of *H. glaucum*and Shazand population of *H. leporinum* (Table 1). Variations observed in the karyotypic formulae, the number of SAT-chromosomes and the chromosomes carrying secondary constriction in different populations of each species and also among different species studied, indicate the occurrence of chromosomes structural changes.

The highest value of haploid total chromatin length (46.25 µm) occurred in Ahvaz population of *H. vulgare* which is diploid, while the lowest value of the same (24.25 µm) occurred in hexaploid *H. leporinum*, indicating that the increase in the chromosome number is associated with some degree of DNA loss possibly in the repetitive parts of the genome. Similarly the highest value of the size of longest chromosome (31.70 µm) occurred in Ahvaz population of *H. vulgare*, while the lowest value of the same (18.50 µm) occurred in Sabzevar population of *H. glaucum*.

Comparison of the total haploid chromatin length among two populations of *H. bulbosum* having diploid (34.25 µm) and tetraploid chromosome number (35.60 µm, Table 1), indicates that the occurrence of polyploidy is not associated with a significant increase in the amount of DNA and almost the same DNA content has been distributed among the chromosomes of the tetraploid population. A similar situation exists in diploid and tetraploid populations of *H. glaucum* (except for Sabzevar population, Table 1). The ANOVA and LSD tests performed for the size of chromosomes among *Hordeum* species and populations studied, showed a significant difference ($p < 0.05$) in the size of chromosomes among different *Hordeum* species but not among populations of a single species. Therefore a significant change in DNA content has possibly been associated with the species diversification in the genus *Hordeum*.

Genome size variation in plants is thought to be correlated with cytological, physiological, or ecological characters. However, conclusions drawn in several studies are often contradictory (Kalendar et al. 2000). To analyze nuclear genome size evolution in a phylogenetic framework, Blattner (2004), studied DNA contents of 134 accessions, representing all but one species of the genus *Hordeum* by flow cytometry. The 2C DNA contents obtained were in a range from 6.85 to 10.67 pg in diploids (2n = 14) and reached up to 29.85 pg in hexaploid species (2n = 42). The smallest genomes were found in taxa from the New World, which became secondarily annual, whereas the largest diploid genomes occur in Eurasian annuals. Genome sizes of polyploid taxa equaled mostly the added sizes of their proposed progenitors or were slightly (1% to 5%) smaller. The analysis of ancestral genome sizes on the base of the phylogeny of the genus revealed lineages with decreasing and with increasing genome sizes. Correlations of intraspecific genome size variation with the length of vegetation period were found in *H. marinum* populations from Western Europe but were not significant within two species from South America. On a higher taxonomical level (*i.e.*, for species groups or the entire genus), environmental correlations were absent. This could mostly be attributed to the superimposition of life-form changes and phylogenetic constraints, which conceal ecogeographical correlations.

The highest value of coefficient of variation (CV) for the size of chromosomes occurred in Ahvaz population of *H. vulgare* (25.96) indicating the highest amount variation in the size of its chromosomes. The lowest value of CV (10.87) occurred in Abadan population of *H.* vulgare.

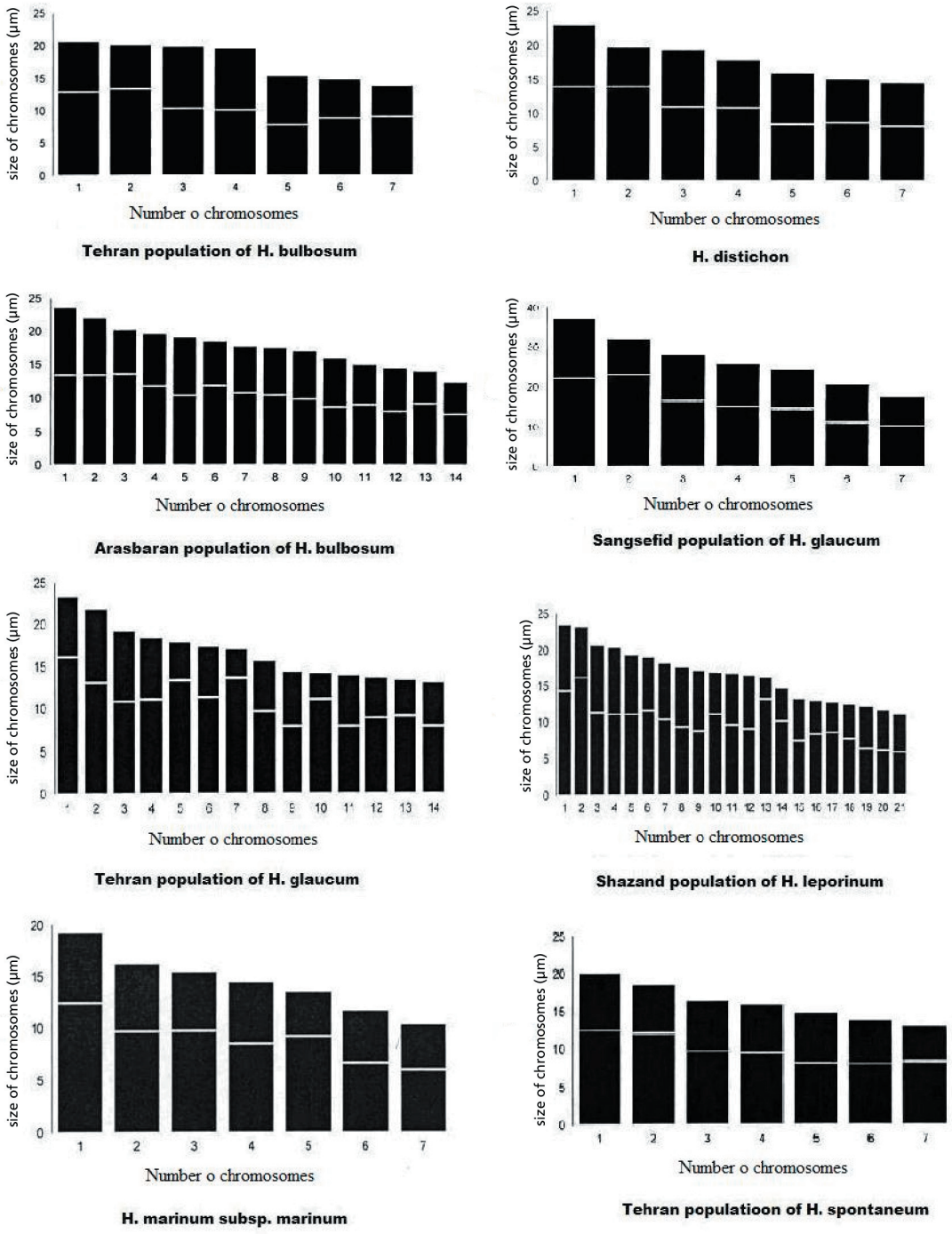

Figure 2. Representative ideograms of *Hordeum* species studied.

The highest value of total form percentage (TF %) occurred in the Ardebil population of *H. glaucum* (42.09) indicating the presence of a symmetrical karyotype, while the lowest value occurred in Tehran population of *H. glaucum* (35.09) having a more asymmetrical karyotype compared to Ardebil population. In terms of the Stebbins two system of karyotype symmetry, the *Hordeum* species studied mostly occupy 1A, IB and 2B classes, which are considered rather primitive classes in this system.

Different populations of the *Hordeum* species studied occupy different classes of karyotype symmetry due to the occurrence of structural changes in their chromosomes (Table

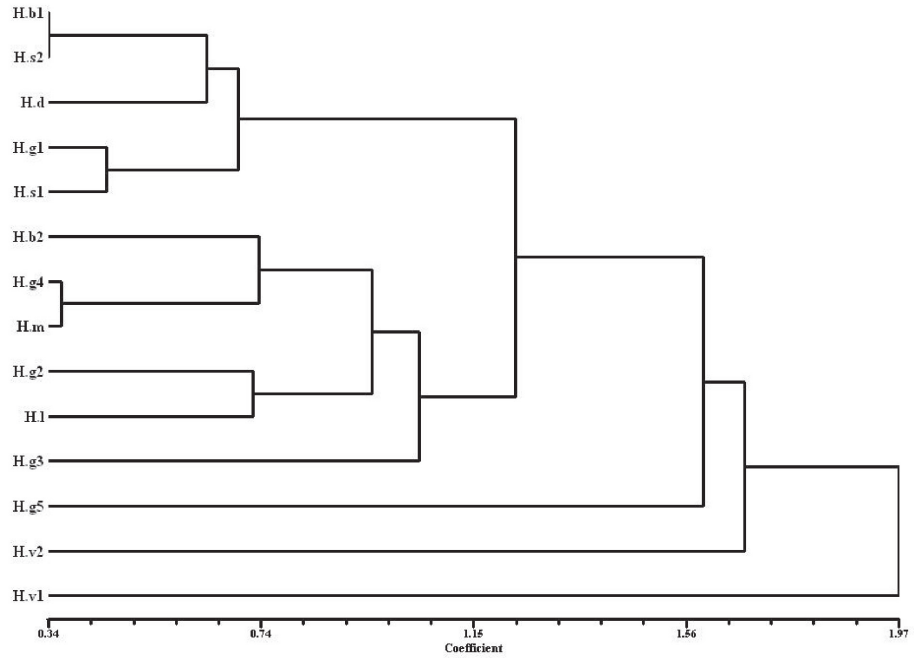

Figure 3. UPGMA clustering of *Hordeum* species studied. (Species code as in Table 1.).

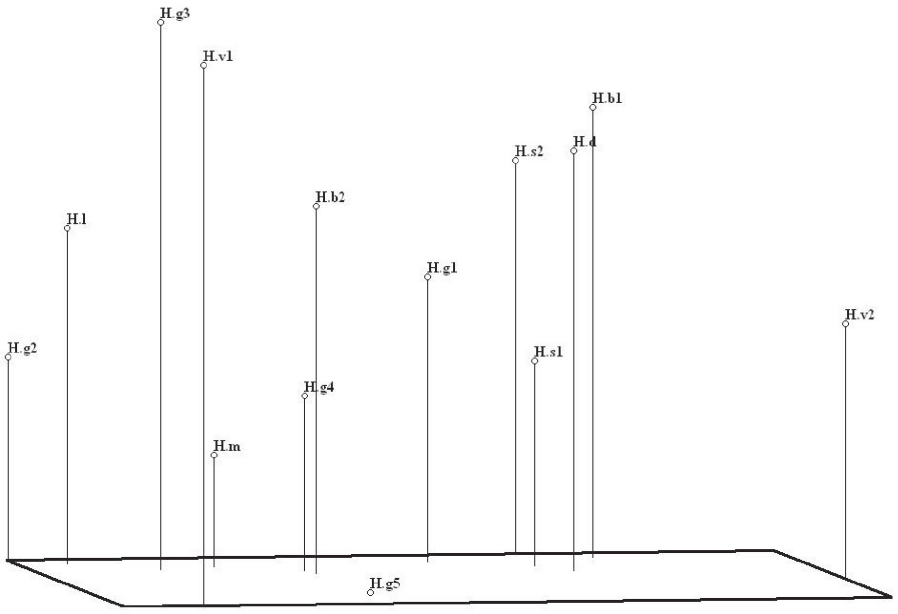

Figure 4. PCO ordination of *Hordeum* species studied. (Species code as in Table 1.).

1). By using the Romero-Zarco symmetry indices of A1 and A2 we can determine the more asymmetric karyotype among the species having similar Stebbins classes of symmetry. For example among the species with the 1A class, Abadan population of *H. vulgare* possesses the highest A1 value (0.36) and a more asymmetric karyotype. Similarly, among the species with the 2A symmetry class, Tehran population of *H. glaucum* possessed the highest value for A1 (0.44) and the highest asymmetric karyotype and among the species with 2B symmetry class, Sabzevar population of *H. glaucum*

possesses the most asymmetric karyotype.

The grouping of the *Hordeum* species and populations based on relative karyotypic data is presented in Figs. 3 & 4. Both UPGMA clustering and PCO ordination produced similar results revealing karyotypic variation among populations of each species, as these populations have been placed in different clusters or groups. For example, Tehran population of *H. bulbosum* is placed in the first major cluster while, Arasbaran population of this species has been placed in the second major cluster with some distance from the first cluster. The same holds true for different populations of *H. glaucum*, *H. vulgare* and *H. spontaneum*, revealing the role of chromosomal changes along with polyploidy in the evolution of the *Hordeum* species.

Meiotic analysis

Meiotic studies of 3 populations of *H. bulbosum* and *H. spontaneum*, showed that Tehran and Darake populations of *H. bulbosum* possess 2n = 4x = 28 and *H. spontaneum* possesses 2n = 2x = 14, supporting our karyotypic results. In all populations the chromosomes mainly formed bivalents and in Tehran population of *H. bulbosum* 1-2 ring or chain quadrivalents were formed (Fig. 1). Chromosomes segregation during anaphase was normal in most of the anaphase and telophase cells in all 3 populations, except few cases of laggard chromosomes formation and chromosome stickiness.

References

Avagian IG, Atayeva GM, Romanova AB, Ghandilian PA (1989) To the question on systematic situation of some representatives of the genus *Hordeum* L. Analysis of C-stained chromosomes of taxons of Murina Nevsky series. Biologicheskii Zhurnal Armenii 42:621-629.

Blattner FR (2004) Phylogenetic analysis of Hordeum (Poaceae) as inferred by nuclear. rDNA ITS sequences. Mol Phylogenet Evol 33.289-299.

BoR NL (1970) *Hordeum*. In Reschinger KH, ed. Flora Iranica 70:105-141. Akademische Druck, Verlagsanstalt, Graz, Austria.

Bothmer R von, Jacobsen N, Baden C, Jorgensen RB, Linde- Laursen I (1991) An ecogeographical study of the genus *Hordeum.* IBPGR (International Board for plant Genetic Resources), Rome.

Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum.* 2nd ed. Rome, Italy, IPGRI.

Chen, YR, Wang LQ (1988) Morphological and cytogenetical studies on F1 of *Hordeum vulgare* L. and *H. bulbosum* L. J Zhejiang Agric 14(2): 142-148.

Farugi SA (1985) Studies of Libyan grasses IX. Breeding system in *Hordeum glaucum* Steud. Pakistan J Bot 17:305-307.

Hatch SL (1980) Chromosome numbers of some grasses from the southwestern United States and Mexico. The Southwestern Naturalist 25: 278-280.

Sabine SJ, Blattner FR (2006) A Chloroplast Genealogy of *Hordeum* (Poaceae): Long-Term Persisting Haplotypes, Incomplete Lineage Sorting, Regional Extinction, and the Consequences for Phylogenetic Inference. Mol Biol Evol 23(8):1602-1612.

Kalendar R, Tanskanen J, Immonen S, Nevo E, Schulman AH (2000) Genome evolution of wild barley (Hordeum spontaneum) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc Natl Acad Sci USA 97:6603-6607.

Koba T (1993) Phytotechnology no Kokoromi (19). Agriculture and Horticulture 68:515-523.

Levan A, Fredga K, Sandberg A (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220.

Mobayyen S (1981) Seedlings of Iran and Flora of Vascular Plants. Vol. 1. Tehran University Publication, Tehran, Iran.

Nicoloff H, Anastassova-Kristeva M, Kunzel G, Rieger R (1977) The behaviour of nucleolus organisers in structurally charged karyotypes of Barley. Chromosoma 62:103-109.

Parsa A (1950) Flora de L'Iran. Vol. 4. Publications du ministere de l'education. Mseun d'historic naturelle de Tehran.

Petersen G, Seberg O (1997) Phylogenetic analysis of the Triticeae (Poaceae) based on *rpo*A sequence data. Mol Phylogenet Evol 7:217-30.

Romero-Zarco C (1986) A new method for estimating karyotype asymmetry. Taxon 35:526-530.

Seberg O, Frederiksen S (2001) A phylogenetic analysis of the monogenomic Triticeae (Poaceae) based on morphology. Bot. J. Linn. Soc., 136:75-97.

Semenov VI (1986) Vnutrikhromosomnaja topografija geterokhromatina u zlakovykh. Bjulleten' Glavnogo Botaniceskogo Sada 140:68-73.

Sheidai M, Nasirzadeh A, Kheradnam M (2000) Karyotypic study of *Echinops* (*Asteraceae*) in Fars Province of Iran. Bot J Lin Soc 134: 453-463.

Sheidai M, Arman M, Zehzad B (2002) Chromosome pairing and B- chromosomes in some *Aegilops* species and populations of Iran. Caryologia 55(3):261-271.

Sheidai M, Noormohamadi Z, Kashani N, Ahmadi M (2003) Cytogenetic study of some rapeseed (*Brassica napus* L.) cultivars and their hybrids. Caryologia 56(4):387-397.

Shewry PR, ed. (1992) Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. CAB International, Oxford.

Spies JJ, Burger TH, van Wyk SMC (1999) Chromosome studies on African plants. 12. The tribes of subfamily Pooideae. Bothalia 29(2):335-341.

Stebbins GL (1971) Chromosomal Evolution in Higher Plants. Edward Arnold, London.

Stebbins GL (1982) Major trends of evolution in the Poaceae and their possible significance. In Estes JR, Tyrl RJ, Brunken JN, eds. Grasses and Grasslands: Systematics and Ecology. University of Oklahoma Press, pp. 1-36.

Stebbins GL (1986) Grass Systematics and Evolution: Past, Present and Future. In Soderstrom TR, ed. Grass Systematics and Evolution.Smithsonian Institution Press.

Strid A, Franzen R (1981) In Chromosome number reports LXXIII. Taxon 30:829-842.

Termeh F (1986) New Grasses of Iran and Their Geographical Distribution. Vol. 2. (17). Research Institute of Pest & Diseases of Plants, Tehran, Iran.

Valdés, B, Parra R, Sánchez AM, Díaz MD (1999) Números cromosómicos de plantas de Marruecos, IV. Lagascalia 21(1):235-240.

Watson L, Dallwitz MJ (1992) onwards. The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Version: 28th November 2005. http://delta-intkey.com.

Zhang Zp, Zhang YM, Yuan JZ (1990) A study of the chromosomes of *Triticum aestivum* and *Hordeum distichon* by silver staining. Acta Agric Univ Henan 24:82-85.