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Cytological study of *Hordeum bulbosum* L. in Iran

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Abstract
Hordeum bulbosum L. (Poaceae) is considered to be sources of useful alleles which can be used in cereal improvement. Thirty two native Iranian *H. bulbosum* were collected from different localities and were studied by karyotype analysis. We assessed the karyotype asymmetry of the Iranian bulbous barley populations and analyzed the data to look for their geographic distribution correlations. All of the studied populations were tetraploid (2n=4x=28) and the analysed parameters of karyotype of *H. bulbosum* support the autopolyploidy origin of the species with nearly symmetric karyotype. The results showed the most asymmetric karyotypes within northeast (Golestan) and northwest (Gardane-e Heiran) populations and the most symmetric karyotyps in populations from the west of Iran. Therefore, it can be assumed that the oldest populations are in the slopes of Zagros Mountains and the youngest germplasms occur in the northeast of this country. It can be concluded that the species originated from the west of Iran and distributed towards east and northeast.
Key words: *Hordeum bulbosum* L., Iran, Karyotype symmetry, Tetraploid

Introduction

The genus *Hordeum* consists of 32 species (45 taxa in total, including subspecies and cytotypes) including diploid (2n=2x=14), tetraploid (2n=4x=28) and hexaploid (2n=6x=42) cytotypes with a basic chromosome number of x=7 (Bothmer *et al.,* 1995). The genus is classified into five genome groups, namely H, I, X, Y and XI (Taketa *et al.,* 1999). In this study, genome designation followed that of Taketa *et al.* (2001), namely, *H. vulgare* and *H. bulbosum* both carry the H genome, so that *H. marinum* carries the X genome, while *H. murinum* has the Y genome, and the 25 remaining species share variants of the I genome (Taketa *et al.,* 2005). *H. bulbosum* has been recognized as one of the two separate allogamous species of the genus, possessing a sporophytic incompatibility system (Bothmer *et al.,* 1995). This species include two well-known cytotypes, diploid and tetraploid, with the latter being more widespread. The tetraploid cytotype is commonly considered as an autopolyploid (HHHH) (Xu and Snape, 1988; Chin, 1941; Papes and Bosiljevac, 1984).

The populations of bulbous barley grow widely in the mountainous and sub mountainous regions of Iran in the north, northeast, northwest, west, southwest and the south (except in

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the Central Plateau, northern Persian Gulf and southern Caspian Sea shores) (Bor, 1970) with different and under stressful environmental conditions.

Symeonidis *et al.,* (1985) claimed that the chromosome set of bulbous barley originated from Greece which contains 16 metacentric including 4 satellited, 8 sub-metacentric and 4 telo-centric chromosomes. Nasirzadeh and Mirzaie Nadoushan (2005) reported that bulbous barley in north of Fars province is tetraploid with karyotype formulae (6m+1sm).

The aim of the present work was the evaluation of the cytotypes of *H. bulbosum* in Iran, characterization of the cytological and karyotypic details (numerical parameters) and their correlations with the geographic distribution of *H. bulbosum.*

Materials and Methods

Plant materials

A total of 32 specimens of *H. bulbosum* were randomly collected from various regions of Iran by the authors and were identified morphologically according to Bothmer *et al.,* (1995) and analysed cytologically (Table 1).

Chromosome spread preparation

The seeds were germinated on paper tissue in pertidishes and the root tips selected for cytological experiments. Somatic chromosomes of meristematic root tip cells were treated from germinated seeds based on Agayev (1996) protocol with minor modifications. Briefly, pretreatment was carried out in saturated solution of Monobromonaphthalene, washed in distilled water for 30 min, fixed in Chromic Acid/Formaldehyde mixture (1/1) at about 4 ºC for 24 h, and finally washed under tap water for 3 h. Then the materials were transferred into 70% ethanol solution and kept refrigerated till staining process. For staining, the materials were transferred into distilled water for about 5-6 min and treated with 1N NaOH at 60 ºC for 10 min, washed in distilled water thoroughly for 30 min then stained in aceto-iron hematoxylin at 30 ºC for 24 h, washed in distilled water for at least 30 min, and macerated for 10-15 min in cellulase-pektinase enzyme solution at 37 ºC.

The roots were gently squashed in 45% acetic acid, on a slide glass and were observed and photographed under an Olympus AX-40 light microscope. At least, five cells were screened and the cells with good spread were used for analyzing and constructing karyograms. In order to characterize the karyotypic asymmetry, 5- 10 chromosome spreads from different individuals of each accession were examined. All chromosome sizes were measured with computer–aided program Image Tool 3.0. The parameters measured for each metaphase chromosome spread included Total Chromosome Length of the haploid complement (TCL), Mean Chromosome Length of the haploid complement (MCL), and Total Form percent (TF%: Ratio between the shortest arms of the chromosomes and their total length); the TF% value was considered to be close to 50% in most symmetric karyotypes and less than 50% based on the degree of asymmetry, (Huziwara, 1962), R (Ratio between the longest and the shortest arms of the chromosomes, Siljak-Yakovlev, 1986), S% (equals to length of the shortest chromosome dividede on length of the longest chromosome, Stebbins, 1971), Asymmetry index (AsI%=100 × ΣL/ΣTCL; where l is long arms in chromosome set and TCL is total chromosome length in chromosome set, Arano and Saito, 1980) and Karyotype formulae: according to their arm ratios (long/short) designated by the position of the centromere: 1 (metacentric; M), 1-1.7 (metacentric; m), 1.7-3 (submetacentric; sm), 3-7 (subtelocentric; st), and 7-39 (telocentric; t)] (Levan *et al.,* 1964).

Table 1. Accessions of *H. bulbosum* (HB) collected from different places in Iran.

Accession no.	Region	Altitude (m)	Locality
HB2W	W	690	Ilam, Darehshahr, Shahre bastani
HB3W	W	642	Ilam, Darehshahr, Gharatmalgeh
HB6W	W	1509	Lorestan, Dorud,Siahkoleh
HB14W	W	1931	Lorestan, Khoramabad toward Borujerd, Zagheh
HB22W	W	1703	Ilam, 45 Km Islamabad-e-gharb toward Eivan
HB23W	W	1580	Kermanshah, 40 Km Eivan toward Islamabad-e-gharb
HB24W	W	1292	Ilam, Darehshahr toward Ilam, Mishkhas
HB30SW	SW	2100	Chaharmahal-va-Bakhtyari, Felard, Aboueshagh, Kahriz
HB73SW	SW	1690	Fars, Eghlid to Marvdasht, Dorudzan
HB76SW	SW	1702	Fars, Shiraz, Roknabad
HB77SW	SW	1975	Fars, Shiraz toward Kazeroon, Hoseinieh
HB79SW	SW	2051	Fars, Shiaz toward Kazerun, Dashte Arjan
HB81SW	SW	1050	Fars, Noorabad-e-Mamasany
HB84SW	SW	2050	Kohgiluie-va-Boyerahmad, Babameidan toward Yasooj
HB87SW	SW	1695	Kohgiluie-va- Boyerahmad, 25 KmYasooj toward Isfahan
HB90SW	SW	1752	Chaharmahal-va-Bakhtyari,Broojen toward Yasooj, Felard
HB91SW	SW	2240	Chaharmahal va Bakhtyari, Broojen toward Yasooj
HB95N	N	1640	Tehran, Boomehen
HB105NE	NE	1775	Golestan Azadshahr toward Shahrood, Khoshyeilagh
HB106NE	NE	700	Golestan, National Park of Golestan
HB109NE	NE	993	Khorasane Shomali, Bojnourd, Baba aman park
HB202W	W	1193	Ilam, Darehshahr toward Ilam, Pakal-e-Gerab
HB207W	W	1360	Kermanshah, Kermanshah toward Kamyaran, Vermenje
HB208W	W	1741	Kurdistan, Kamyaran toward Sanandaj, Morvarid
HB209W	W	1581	Kurdistan, Sanandaj
HB211W	W	1257	Kurdistan, 15 Km Sarvabad toward Sanandaj
HB212W	W	1222	Kurdistan, Sarvabad
HB213W	W	1249	Kurdestan, around of Zarivar lake
HB215W	W	1587	Kurdistan, Marivan toward Saghez, Sarshio
HB216W	W	1423	Azarbaijane Gharbi, Boukan, Kanitoomar
HB217NW	NW	1822	Azarbaijane Gharbi, Boukan,Mohabad, Gharehbolagh
HB221NW	NW	1537	Gilan, Astara, Heiran

Results and Discussion

All of the studied populations were tetraploid (2n=4x=28) and the results of the analyzed parameters of karyotype of *H. bulbosum* supported the autopolyploidy origin of the species with nearly symmetric karyotype combining four homologous or near homologous genomes that were in accordance with previous reports (Chin, 1941; Morrison, 1959; Xu and Snape, 1988). Karyotype was nearly symmetrical with chromosomes varying in mean total chromosome lengths from 5.22 (in HB90SW from Dasht-e Felard at Chaharmahal va Bakhtiari province) to 15.04 μm (in B3W from Darrehshahr in Ilam province) (Table 2). The descriptions of karyotype formulae and their analyzed parameters results are shown in Tables 2 and 3, respectively.

Table 2. Karyotype analysis of the different populations of *H. bulbosum* species (n=chromosome number, TL=Total haploid chromatin length, MCL=Mean Chromosome Length, SE=Standard Error, TF%=Total Form percent, S%=Length of the shortest chromosome divided on length of the longest chromosome, R=ratio between the longest and the shortest arms of the chromosomes, AsI%=Asymetry index, *=Satellite.

Population	2n	TCL	MCL ± SE	TF%	S%	R	AsI%	Karyotype formulae
HB2W	28	63.68	9.1±1.117	45.1	68.56	1.23	54.89	2M + 5m*
HB3W	28	92.53	13.22±1.56	41.22	70.61	1.45	58.77	6m* + 1sm
HB6W	28	53.79	7.68 ±0.74	41.68	76.57	1.45	58.31	6m* + 1sm
HB14W	28	73.2	10.5±1.341	44.03	70.77	1.33	55.96	1M + 4m* +2sm
HB22W	28	76.84	10.1±1.288	44.15	73.92	1.28	55.84	2M + 5m*
HB23W	28	84.03	12.0 ±1.235	44.34	77.31	1.27	55.658	7m*
HB24W	28	67.6	9.66±0.933	42.42	76.88	1.39	57.573	1M* + 5m + 1sm
HB30SW	28	81.48	11.64±1.01	42.26	79.12	1.397	57.731	6m* + 1sm
HB73SW	28	55.97	7.1±1.261	43.79	59.3	1.297	56.208	1M + 5m* + 1sm
HB76SW	28	76.28	10.9±1.5	40.53	67.12	1.53	59.465	2M* + 4m + 1sm
HB77SW	28	54.38	7.77±1.055	41.28	65.29	1.467	58.716	1M* + 5m + 1sm
HB79SW	28	74.8	10.69±1.344	42.78	67.01	1.397	57.22	1M* + 5m + 1sm
HB81SW	28	59.26	8.47±0.908	39.7	73.52	1.655	60.3	5m* + 1sm + 1st
HB84SW	28	57	8.14±1.234	38.57	63.35	1.616	61.42	5m* + 2sm
HB87SW	28	57.79	8.26±0.94	41.18	71.48	1.56	58.81	5m* + 2sm
HB90SW	28	44.88	6.41±0.7	43.6	71.21	1.345	56.39	2M + 4m + 1sm
HB91SW	28	51.26	7.32±0.85	41.02	72.35	1.541	58.97	1M + 5m* + 1sm
HB95N	28	76.73	10.961±1.278	42.42	74.43	1.395	57.578	6m* + 1sm
HB105NE	28	55.7	7.96±1.04	40.68	68.6	1.491	59.317	6m* + 1sm
HB106NE	28	63.02	9.002±1.41	37.81	64.17	1.687	62.186	4m + 3sm*
HB109NE	28	59.77	8.538±1.147	40.48	68.65	1.543	59.511	6m* + 1sm
HB202W	28	53.08	7.582±0.79	39.44	70.97	1.57	60.55	1M + 3m + 3sm*
HB207W	28	70.06	10.01±1.03	40.1	74.32	1.53	59.006	1M* + 4m + 2sm
HB208W	28	85.41	12.201±1.55	40.86	71.03	1.493	59.138	1M + 5m* + 1sm
HB209W	28	48.86	6.98±0.69	41.17	76.41	1.533	58.821	1M + 4m* + 2sm
HB211W	28	59.86	8.55±1.74	38.79	51.3	1.6	61.209	5m* + 2sm
HB212W	28	53.45	7.64±0.8	42.37	75.57	1.467	57.623	5m* + 2sm
HB213W	28	85.61	12.23 ±1.27	42.42	73.33	1.438	57.575	1M + 5m* +1sm
HB215W	28	67.07	9.58 ±2.1	40.15	48.58	1.565	59.847	1M* + 4m + 2sm
HB216W	28	73.01	10.43 ±1.4	42.5	69.15	1.366	57.498	5m* + 2sm
HB217W	28	66.91	9.56 ±0.85	41.72	81.32	1.432	58.272	1M*+ 5m + 1sm
HB221W	28	62.61	8.94 ±.91	37.93	72.53	1.683	62.066	1M* + 3m + 3sm

The morphological characteristics of chromosomes are shown in Figure 1. As presented in Table 2, the metacentric (M and m) chromosomes dominated the observed karyotypes with 79.46% and the second frequency belongs to the submetacentrics (20.09%). Only one population (HB81SW from Noorabad –e Mamasany in Fars province) had a sub-telocentric (st) chromosome with karyotype formulae (5m* + 1sm + 1st). No telocentric chromosome was observed (see Table 2).

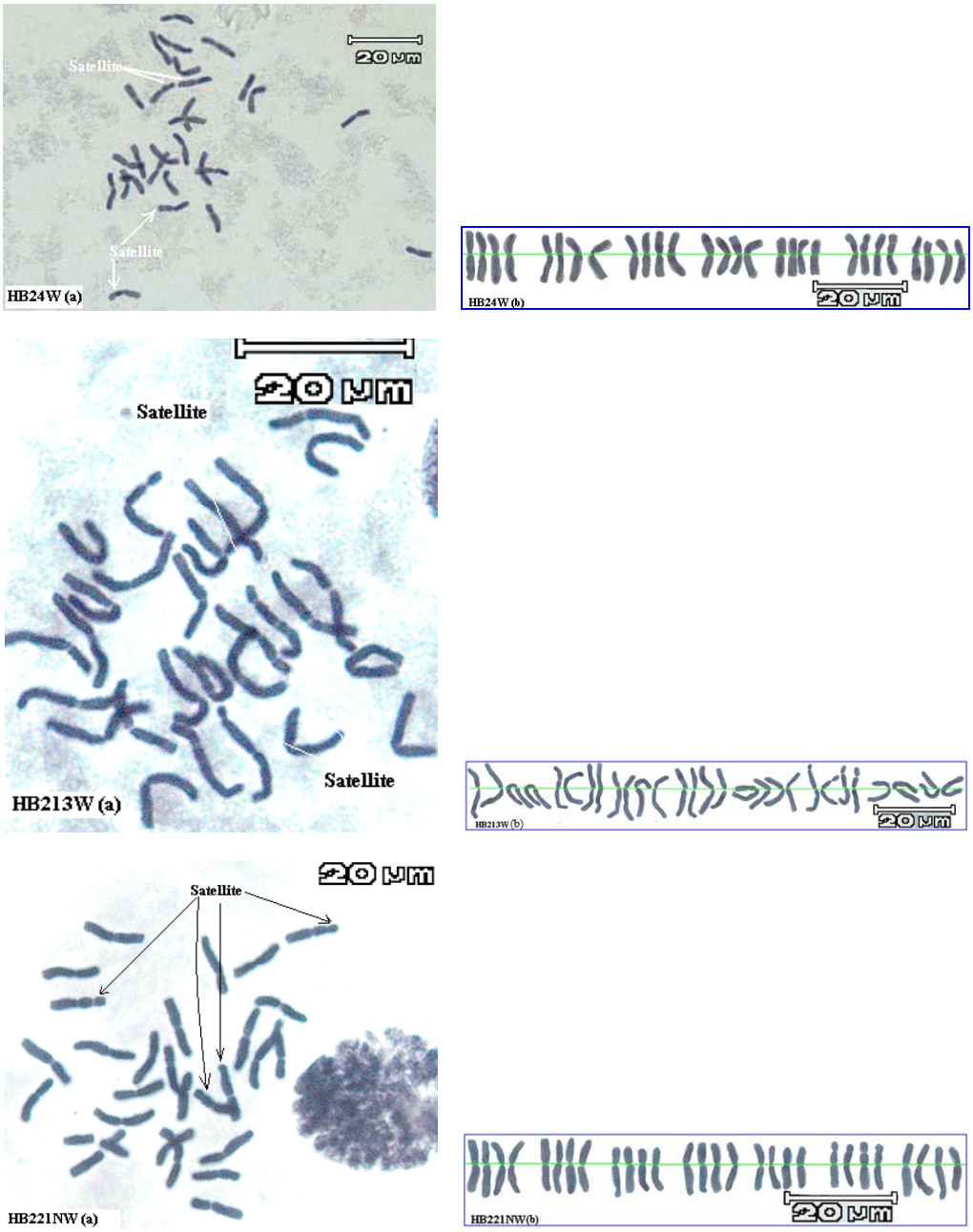

Figure 1: Somatic chromosomes (karyotype) of 32 Iranian *H. bulbosum* (HB) populations (2n=4x=28). Mitosis squash photograph for accessions: HB24W, HB213W and HB221NW with showing Satellited chromosoms are presented. Scale bar: 20 µm.

The populations HB106NE, HB201W and HB221W had 3 sub-metacentric, HB2W, HB22W (2M + 5m*) and HB23W (7m*) without sub-metacentric chromosome and other remaining populations (56.25%) had karyotype formulae of 6m+1sm including six metacentric and one sub-metacentric that were in accordance with Chin (1941), Linde-Laursen *et al*. (1990), Morrison (1959) and Vahidy and Jahan (1998) (Table 2). Nasirzadeh and Mirzaie Nadoushan (2005) have analyzed the karyotype parameters of *H. bulbosum* populations and have suggested that they originated from Fars province and showed that their karyotype formulae were 6m+1sm which was partly in agreement with the results of

this study. Symeonidis and Lazaros (1985) reported that the karyotype of Greece populations of bulbous barely was 4m+2sm+1t. In this study, we have not found telocentric chromosome in tetraploids indicating that the karyotype of Iranian tetraploid bulbous barley is different from Greece populations. Our results showed that all populations have one metacentric or sub-metacentric satellited chromosome, except for HB90SW (from Dasht-e Felard in Chaharmahal va Bakhtiari province). Two populations (HB106NE from National Park of Golestan and HB202W from Ilam) had one submetacentric satellited chromosome with karyotype formulae (4M+3sm*). The presence of typical SM satellited chromosomes occurred more frequently among the studied populations of the *Hordeum bulbosum* (Rajhathy *et al.,* 1964; Vosa, 1976; Coucoli and Symeonidis, 1980; Chin, 1941; Linde-Laursen *et al*., 1990; Morrison, 1959). As noted by Heneen (1977) and the different origin of the materials should be a logical explanation for the observed differences since SAT chromosomes in the Triticeae are well known to evident morphological variation the of shape and the indices among different populations or varieties of one species. The karyotype formulae polymorphism in homologous chromosomes of *H. bulbosum* could be correlated with their out-breeding nature. No B chromosome was observed among the materials studied.

The highest TL variation was found in HB215W population [SE (standard error) of MCL=2.1μm], and the lowest chromosome length variation was scored in HB209W population (SE of MCL=0.69 μm) (Table 2). The ratio between the longest and the shortest arms (R) ranged from 1.23 HB3W accession to 1.69 in HB106NE accession (Table 2). Asymmetry Index (AsI%) ranged from 54.89 in HB2W population to 62.19 in HB106NE population (Table 2). The degree of karyotype asymmetry as indicated by TF% values ranged from 37.1% (HB106NE and HB221NW accessions) to 45.1% (HB2W) (Table 2). As the TF% values were near to 50%, we can conclude that type of chromosomes were metacentric to submetacentric. Also the mean of S% (Stebbins 1971) indicating symmetry index was from 48.58% (HB215W) to 81.32 (HB217W) with mean of 70.1% indicating nearly symmetrical karyotype for *H. bulbosum*.

Based on the results of this study (the factors studied and the resulted asymmetry indices) HB221NW proved to have the most asymmetric karyotype (with the formulae of 1M* + 3m + 3sm) among the populations studied. Regarding the asymmetry indices observed in HB221NW it could be suggested that the karyotype asymmetry in this population was mainly affected by the place of the centromers rather than length of the chromosomes. HB2W with the least chromosomal arm ratio variability, showed the most symmetric karyotype (with the formulae of 2M + 5m*). Regarding all the analyzed factors, a high similarity were found between HB2W, HB14W, HB22W and HB23W (see Table 2).

The karyotype asymmetry can be a fine appearance of the general morphology of karyotype in plants (Romero Zarco, 1986). As Sharma (1990) has mentioned, symmetrical karyotypes are more primitive than asymmetrical ones and longer chromosomes than shorter ones; median centromers with chromosome arms of equal length are more primitive than chromosomes with arms of unequal length. From the chromosome length point of view, the longest chromosomes were found in HB3W that could be considered as most primitive population. We observed that the most asymmetric karyotypes within northeast populations (e.g. Golestan) and populations of the west of Iran had the most symmetric karyotypes. Therefore considering the above notions and the results of this study, it could be assumed that the oldest populations are in the slopes of Zagros Mountains (west of Iran) and the youngest ones occurred in the northeast of the country (Figure 2).

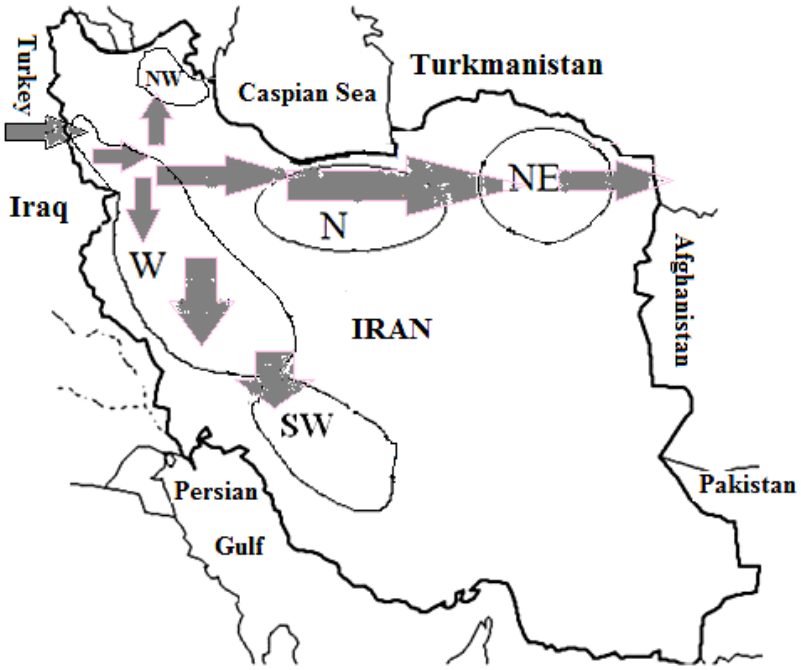

Figure 2: Distribution of collected accessions of *Hordeum bulbosum* (W=west, SW=southwest, N=north, NE=northeast, NW=northwest). Arrows indicate the distributions direction of *H. bulbosum* in Iran.

This suggestion is in accordance with the conclusion reported by Bothmer, *et al.* (1995) namely the *H. bulbosum* (4x) has originated from Greece and then distributed eastwards. Based on these results it can also be concluded that the Western populations (e.g. HB2W, HB14W, HB22W, HB23W and HB90SW) generally possessed the highest chromosomal length and the highest mean TCL (9.87 µm) and the most symmetric karyotypes are the oldest populations and the northeast populations with mean TCL of 8.5 µm are the youngest populations of *H. bulbosum* in Iran (Figure 2).

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مطالعه سیتولوژیک *Hordeum bulbosum* L. در ایران

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چکیده

گونه *Hordeum bulbosum* همواره به عنوان یکی از منابع آللی مفید که می‌تواند در اصلاح غلات زراعی استفاده شود مد نظر است. در این تحقیق، کاریوتیپ ۳۲ نمونه جمعیتی این گونه جمع‌آوری شده از نقاط مختلف ایران مورد بررسی قرار گرفت. تقارن کایوتیپی جمعیت‌ها و ارتباط آن با مناطق جغرافیایی ارزیابی شد. تمامی جمعیت‌های مطالعه شده تتراپلوئید با کاریوتیپ، متقارن بودند و شاخص‌های بررسی شده نشان از اتوتتراپلوئید بودن این گونه دارد. مشاهدات نشان می‌دهد که جمعیت‌های شمال شرقی (گلستان) و شمال غربی (گردنه حیران) نامتقارن‌ترین و جمعیت‌های غربی، متقارن‌ترین کاریوتیپ را دارند. بر اساس این نتایج می‌توان گفت که قدیمی‌ترین جمعیت‌های این گونه در کوه‌های زاگرس و جوان‌ترین آنها در شمال شرق ایران قرار دارند. این گونه احتمالاً از غرب ایران وارد شده و به سمت شرق گسترش یافته است.

واژه‌های کلیدی: *Hordeum bulbosum* L.، ایران، تقارن کاریوتیپ، تتراپلوئید

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