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Karyotypic investigation concerning five *Bromus* Species from several populations in Iran

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Abstract. Karyotypes of five taxa (fourteen populations) of the genus *Bromus* from different geographic origins is presented: *B. scoparius, B. japonicus*, *B. madritensis*, *B. rubens* and *B. tomentellus*. The ploidy levels were different. *B. scoparius* and *B. japonicus* were found 2n=2x=14, *B. madritensis* and *B. rubens* were found 2n=4x=28 and *B. tomentellus* were found 2n=6x=42. Detailed karyotype analysis allows us to group the different populations and to postulate relationships among them.

Keywords. *Bromus*, Chromosome, Karyology, Iran.

INTRODUCTION

The genus *Bromus* L. belongs tribe *Bromeae* and Poaceae family. The taxon includes about 160 annual and perennial species (Acedo and Liamas, 2001) distributed all over the world. *Bromus* species are distributed in temperate regions and are always exist with rangeland species (Verloove, 2012). It is an important rangeland plant species in Iran, which are placed in 6 sections; *Bromus*, *Genea*, *Nevskiella*, *Neobromus*, *Ceratochla* and *Pnigma* (Bor, 1970) (Table 1). The Genea section is the widest section of the *Bromus* genus in terms of geographic distribution (sales, 1994).

Bromus species are known as the species with various intra-specific ploidy levels and form different ecotypes with various characteristics. Hill (1965) recorded up to 112 chromosomes for *B. erectus*. Devesa *et al.* (1990) indicates the importance of cytological studies for understanding the evolution of the genus *Bromus*. Naganowas ka (1993) used genetic distances estimated based on centromeric index and total chromosome length to investigate interrelationships of several species of *Bromus*. Yang and Dunn (1997) recorded various levels of polyploidy in *B. inermis* Leyss. Martinello and Schifino-Wittmann (2003) studied 14 accessions of *Bromus auleticus*. Their accessions were all hexaploid and the high symmetry and homogeneity of the karyotypes made it difficult to detect possible intraspecific differences.

Massa *et al.* (2004) proposed a taxonomic treatment within *Bromus* sect. *Ceratochloa* of South America. Their plant materials included 28 hexaploid

(2n=6x=42) populations and 2 octaploid (2n=8x=56) populations. Oja and Laarmann (2002) also recorded different ploidy levels within species of *Bromus* (2n=14, 28, 42 and 56). Sheidai and Fadaei (2005) studied ten populations of six *Bromus* species and the species possess karyotypes varying from 2n = 2x = 14 (diploid) to 2n = 4x = 28 (tetraploid).

Mirzaie-Nodoushan *et al*. (2006a) investigated karyotypic of some *Bromus* species in Iran and indicated that populations of the species were differed in their karyotypic characteristics and ploidy levels of the populations were varied from 2n=14 to 2n=84. Mirzaie-Nodoushan *et al*. (2006b) also reported evolutionary karyotypic variation in *B. tomentellus* populations in Iran and confirmed the existence of high levels of ploidy as well as existence of dodecaploid karyotypes in the species. Sadeghian and *et al*. (2010) studied nine populations of three *Bromus* species (*B. danthoniae*, *B. sterilis* and *B. tectorum*) and reported that all species were diploid with 2n=2x=14. Artico *et al*. (2017) also reported that the chromosomal number of *B. Linnaeus* was 2n = 6x = 42.

Since the karyological information is the basic requirement of a breeding program, in this study, 14 populations of *Bromus* were surveyed for the karyological data as a part of an ongoing work on the populations.

Cytogenetic studies play an important role in determining the relationship between species especially wild and native plants and as a first step in the analysis of the phylogeny and evolution of species is relative. Considering that the species studied in different climates of southwest Iran and Fars province are abundant they are considered as the main vegetation cover of these areas. Therefore, to investigate the relationship between species, these species have been used in this study.

MATERIALS AND METHODS

Fourteen populations of five *Bromus* species: *B. tomentellus* (three populations) belong to *Pnigma* section, *B. madritensis* (two populations) and *B. rubens* (three populations) belong to *Genea* section and *B. scoparius* (three population) and *B. japonicus* (three population) belong to *Bromus* section were studied (Table 1). Voucher specimens were deposited in the Herbarium of Fars Research and Education Center for Agriculture and Natural Resources and in gene bank RIFR (Research Institute of Forest and Rangelands) of Iran.

Root tip meristems from seedling obtained by the germination of ripe seeds collected from natural populations (14 populations, representing 5 species) on wet filter paper in Petri dishes and left at 22°C temperature. When they reached 1-1.5 cm in length, rootlets were collected. The material was pretreated in %0.5 saturated α-Bromo naphthalene at 4°C for 4 h, fixed in %10 formaldehyde and chromium trioxide (1:1 volume ratio) for 16 to 20 h at 4°C. Then, the roots tips were rinsed for 3 h in distilled water. Hydrolysis was carried out with NaOH (1 Normal) at 60°C for 20-30 min (Sadeghian *et al*. 2010)) and used hematoxylin-iron for chromosome staining for 1-2 h. Squashed in a droplet of %45 acetic acid and lactic acid (10:1) (Wittmann 1965). At least, five well-spread metaphase plates from different individuals were analyzed per population. The best metaphasical

Table 1. The origin of materials used in chromosome studies of *Bromus*.

Species (population)	Section	Origin	Altitude	Herbarium code
B. japonicus (16462)	*Bromus*	Golestan, Maraveh tapeh, station	430 m	16462
B. japonicus (16525)	*Bromus*	Golestan, Gomayshan, seidabad	-15 m	16525
B. japonicus (16587)	*Bromus*	Golestan, Tooskasetan	1216 m	16587
B. madritensis (3668)	*Genea*	Fars, Shiraz, Dasht-e Arjan	2000 m	3668
B. madritensis (Arjan)	*Genea*	Fars, Shiraz rosd of Dasht-e Arjan to Tang- e Abolhayat, about Kandee village	1300 m	-
B. rubens (15169)	*Genea*	Fars, Kazeroon, kotal dokhtar	1400 m	15169
B. rubens (15317)	*Genea*	Fars, Fasa, Mianjangal	1750 m	15317
B. rubens (2125)	*Genea*	Fars, Kazeroon	530 m	2125
B. scoparius (5983)	*Bromus*	Gilan, Talesh, subatan yelagh	1800 m	5983
B. scoparius (5984)	*Bromus*	Gilan, Talesh, khotbesara, lapehkara	1800 m	5984
B. scoparius (5985)	*Bromus*	Gilan, Masal	1900 m	5985
B. tomentellus (Bavanat)	*Pnigma*	Fars, Bavanat, Simakan, Lakposhti range	2300 m	-
B. tomentellus (Simakan)	*Pnigma*	Fars, Simakan, Lakposhti range	2350 m	-
B. tomentellus (Eghlid)	*Pnigma*	Fars, Eghlid, Dozkord, Pasahlaki	2200 m	-

plates were selected and measured by Micromeasure 3.3 software (Reeves *et al*. 2000). In each mitotic metaphase (at least 5 plates) the arm's length of each chromosome was measured.

The following parameters were estimated in each metaphase plate to characterize the karyotypes numerically: long arm (LA), short arm (SA), total length (TL), relative length percentage (RL %), arm ratio (AR), centromeric index (CI) (Huziwara, 1962), value of relative chromatin (VRC). Karyotype asymmetry was estimated by three different methods namely, total form percentage (TF %) (Huziwara, 1962); difference of relative length (DRL), intra-chromosomal asymmetry index (A_1) and inter-chromosomal asymmetry index (A_2). Both indices (A_1 and A_2) (Romero Zarco, 1986) were independent to chromosome number and size. Also karyotypic evolution has been determined using the symmetry classes of Stebbins (SC) (Stebbins, 1971). Karyotype formula was determined by chromosome morphology based on centromere position according to classification of Levan (Levan *et al*. 1964). For each population, karyograms were drawn based on length of chromosome size (arranged large to small).

In order to determine the variation between populations, one-way unbalanced ANOVA was performed on normal data and parameter means were compared by Duncan's test. The principal components analysis (PCA) was performed to evaluate the contribution of each karyotypic parameter to the ordination of species. Clustering was performed using the unweighted pair group method with arithmetic (UPGMA) after calculation of Cophenetic correlation coefficient (r) to examine karyotype similarity among populations. Numerical analysis was performed using SAS ver. 6.12 (1996), JMP ver. 3.1.2 (1995) and StatistiXL ver. 1.7 (2007) softwares.

RESULTS

There was no different among basis chromosome number of the species (x=7). The somatic chromosome numbers (2n), karyotype formula and parameters for the studied species are summarized in Table 2. Two species as *B. scoparius* and *B. japonicus* were diploid, two species as *B. madritensis* and *B. rubens* were tetraploid and one species as *B. tomentellus* was hexaploid.

The studied species included metacentric (m) and sub-metacentric (sm) chromosomes regarding the chromosomal types (Table 2). Satellites were observed in one chromosomes pair in *B. scoparius* and *B. japonicus* and two chromosomes pairs in *B. madritensis* and *B. rubens* and for *B. tomentellus* species which has three chromosomes pairs having satellites (Fig 1). According to the Stebbin's bilateral table, populations of *B. rubens* (15317) included the highest value regarding the intra-chromosomal asymmetry index (0.288) and was classified as group 1B and population of *B. japonicus* (16462) included the lowest value regarding the intra-chromosomal asymmetry index (0.180) and was classified as group 1A.

The results of analysis of variance indicated that there was a significant difference (P≤1%) between the populations in terms of chromosomal traits (TL, LA,

Table 2. Karyotypic characters of different *Bromus* taxa and population.

Taxon (population)	2n	A1	A2	%TF	DRL	VRC	SC	K.F.
B. japonicus (16462(	2x=14	0.180	0.129	45.174	5.369	9.605	1A	12m+2sm
B. japonicus (16525(	2x=14	0.197	0.145	44.700	6.131	8.704	1A	14m
B. japonicus (16587)	2x=14	0.250	0.144	42.950	6.193	8.249	1A	14m
B. madritensis (3668)	4x=28	0.206	0.213	43.022	4.353	5.358	1A	28m
B. madritensis (Arjan)	4x=28	0.238	0.199	41.988	4.757	5.329	1A	28m
B. rubens (15169)	4x=28	0.252	0.241	41.486	5.583	6.114	1B	28m
B. rubens (15317)	4x=28	0.288	0.256	38.797	5.332	6.013	1B	28m
B. rubens (2125)	4x=28	0.257	0.230	39.904	5.389	6.390	1B	24m+4sm
B. scoparius (5983)	2x=14	0.233	0.116	42.493	4.722	8.166	1A	14m
B. scoparius (5984)	2x=14	0.227	0.124	42.113	5.722	7.929	1A	12m+2sm
B. scoparius (5985)	2x=14	0.214	0.126	44.062	5.479	6.866	1A	14m
B. tomentellus (Bavanat)	6x=42	0.215	0.110	42.523	1.698	6.271	1A	38m+4sm
B. tomentellus (Simakan)	6x=42	0.204	0.122	44.354	2.278	6.894	1A	42m
B. tomentellus (Eghlid)	6x=42	0.225	0.140	41.278	2.146	6.878	1A	42m

2n: Diploid chromosome numbers A_1: intrachromosome asymmetry index, A_2: interchromosome asymmetry index, TF%: total form percentage, DRL: difference of relative length, VRC: value of relative chromatin, symmetry classes (SC) of Stebbins and karyotype formula (K.F.).

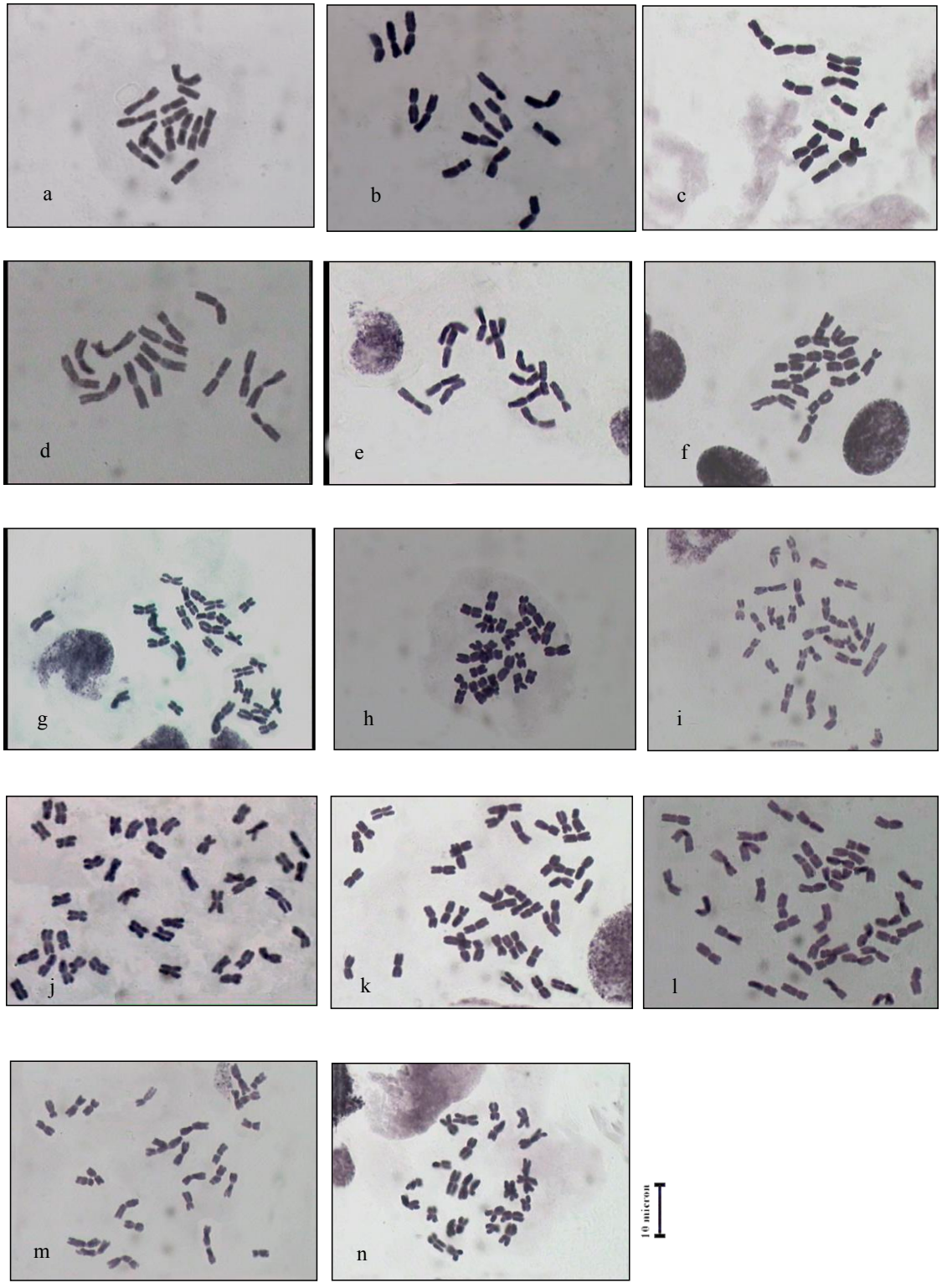

Figure 1. Representative mitotic plates of *Bromus* – (a) *B. scoparius* (5983), 2n=2x=14, (b) *B. scoparius* (5984), 2n=2x=14, (c) *B. scoparius* (5985) 2n=2x=14, (d) *B. japonicus* (16462), 2n=2x=14, (e) *B. japonicus* (16525), 2n=2x=14, (f) *B. japonicus* (16587), 2n=2x=14, (g) *B. rubens* (15169), 2n-4x=28, (h*) b. rubens* (15317), 2n=4x=28, (i) *B. rubens* (2125), 2n=4x=28, (j) *B. tomentellus* (Bavanat), 2n=6x=42, (k) *B. tomentellus* (Simakan), 2n=6x=42, (l) *B. tomentellus* (Eghlid), 2n=6x=42, (m) *B. madritensis* (3668). 2n=4x=28, (n) *B. madritensis* (Arjan), 2n=4x=28.

SA) which revealed large variations among the germplasms in regard to studied traits. Symmetry type of Stebbins (1971) and asymmetry indices of Romero-Zarco (1986) are given in Table 2.

Difference in the relative length percentage (DRL) of the highest and the smallest chromosomes varied from 6.19 in *B. japonicus* (16587) to 1.69 in *B. tomentellus* (Bavanat). According to Table 2, *B. rubens* (15317) was placed in 1B and had the highest values of intra- chromosomal asymmetry index. Similarly, high DRL value leads to more changes in the construction of chromosomes. It had the lowest TF%. The TF% and A_1 values had inverse ratio (Table 2).

The mean value of chromosome's long arm was varied from 5.27 in *B. japonicus* (16462) to 2.92 in *B. madritensis* (3668). Averages of chromosome's short arm were different from 2.24 in *B. madritensis* (Arjan) to 4.34 in *B. japonicus* (16462). The total length of the chromosome was varied from 9.61 in *B. japonicus* (16462) to 5.17 in *B. madritensis* (Arjan) and the mean value of chromosome's arm ratio was in range from 1.41 in *B. rubens* (15317) to 1.22 in *B. japonicus* (16462) (Table 3).

The results showed that the highest VRC amongst all populations was obtained for *B. japonicus* (16462) and the lowest was obtained for *B. madritensis* (Arjan). Based on intra-chromosomal asymmetry, some populations had the most asymmetrical and evolutionary karyotype. According to inter-chromosomal asymmetry, *B. rubens* (15317) had the most asymmetrical karyotype in all of the populations.

The ratio of long arm/short arm chromosomes (AR) showed a high significant difference among some species belong to different sections, while other species are not clearly distinct (Table 3). Diploid species of *B. japonicus* (16462) for instance, had the lowest AR value (1.22), the highest TF% value (45.17) and the lowest A_1 value (0.18), exhibiting the most symmetrically karyotypes, while *B. rubens* (15317) with the highest AR value (1.41), the lowest TF% value (38.80) and the highest A1 value (0.29) were introduced as the most asymmetrical karyotypes (Table 3). The pattern of variation of A_1 and A_2 values has been compared with the pattern of Stebbins' system.

The statistical comparison based on completely randomized design showed that there were significant differences among the populations for TL, LA and SA traits ($P \leq \%1$) (Table 4).

The principal component analysis (PCA) of the karyotypic parameter shows the first two principal components account for 0.81% of total variance. Component one (0.59%) put emphasized on the A_1, A_2 and DRL. While component two (0.23%) accentuates, chromosome total length, long arm length, short arm length and TF% values which had the highest coefficients of Eigen vectors (Table 5).

The diagram of the population's dispersion, based on two first components showed that the populations separated in four groups, which completely fits with the results obtained through the average grouping analysis method (Fig. 2).

The dendrogram obtained from the cytogenetic studies of 14 populations of *Bromus* indicated the for-

Table 3. Mean of chromosomes analysis of *Bromus* population.

Populations	TL	LA	SA	AR	CI	DRL	%TF	A1	A2
B. japonicus (16462)	9.61	5.27	4.34	1.22	0.46	5.37	45.17	0.18	0.13
B. japonicus (16525)	8.71	4.82	3.90	1.24	0.45	6.13	44.70	0.20	0.15
B. japonicus (16587)	8.24	4.71	3.55	1.32	0.43	6.19	42.95	0.25	0.14
B. madritensis (3668)	5.23	2.92	2.31	1.27	0.44	4.35	43.03	0.21	0.21
B. madritensis (Arjan)	5.17	2.94	2.24	1.32	0.43	4.76	41.99	0.24	0.20
B. rubens (15169)	5.91	3.38	2.54	1.33	0.42	5.58	41.49	0.25	0.24
B. rubens (15317)	5.62	3.29	2.34	1.41	0.41	5.33	38.80	0.29	0.26
B. rubens (2125)	5.97	3.42	2.55	1.35	0.42	5.39	39.90	0.26	0.23
B. scoparius (5983)	8.02	4.55	3.48	1.31	0.43	4.72	42.49	0.23	0.12
B. scoparius (5984)	7.62	4.29	3.34	1.29	0.43	5.72	42.11	0.23	0.12
B. scoparius (5985)	6.87	3.84	3.03	1.27	0.44	5.48	44.06	0.22	0.13
B. tomentellus (Bavanat)	6.08	3.42	2.67	1.28	0.43	1.69	42.52	0.22	0.11
B. tomentellus (Simakan)	6.90	3.84	3.06	1.26	0.44	2.28	44.35	0.20	0.12
B. tomentellus (Eghlid)	6.52	3.68	2.84	1.30	0.43	2.15	41.28	0.22	0.14

TL: total length of chromosome, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, DRL: difference of relative length, TF%: total form percentage, A_1: intra-chromosome asymmetry index, A_2: inter-chromosome asymmetry index.

Table 4. The results of variance analysis for karyotypic data based on CRD design.

S.O.V	D.F	TL	Mean of squares							
			LA	SA	AR	CI	DRL	TF	A_1	A_2
Populations	13	11.50**	2.677**	2.032**	0.014**	0.001**	10.383**	9.7ns	0.004ns	440.55**
Error	56	0.50	0.131	0.115	0.009	4.64E-04	3.987	6.869	0.002	86.161
%C.V.		2.57	0.61	0.48	0.01	5.51E-04	5.19	7.40	2.57E-03	152.93

**: Significant at 1%.

Table 5. Specific values of variance percentage and coefficients of specific vectors in analysing main components.

Name of traits	First component	Second component
SA	0.93	0.32
LA	0.87	0.44
TL	0.90	0.38
AR	0.05-	0.72
CI	0.89	-0.27
A1	0.82-	0.52
A2	-0.77	0.22
DRL	0.09	0.79
TF	0.89	-0.23
Specific values	5.28	2.07
Percentage of Variance	0.59	0.23
Cum Percentage of Variance	0.59	0.81

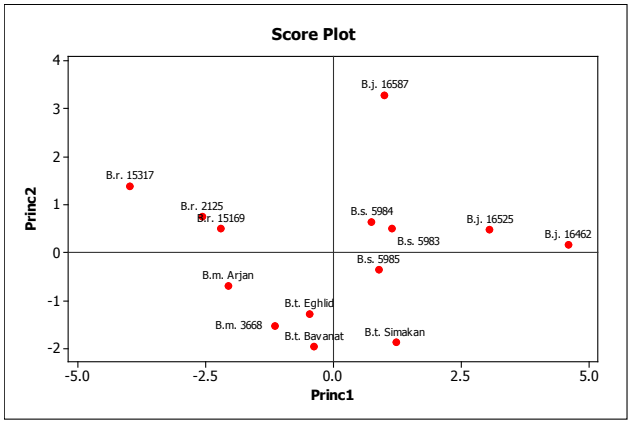

Figure 2. Scatter plot of 14 populations for the first two principals.

mation of five clusters in the Euclidean distance of 0.05. The first cluster consists of the *B. tomentellus* populations (Simakan, Eghlid and Bavanat). The populations of Bavanat and Eghlid showed the most kinship, and the population of Simakan is located in a relatively short distance to these two populations. The second cluster consists of *B. rubens* (15317) and *B. rubens* (2125) populations which showed a close kinship. The third cluster consists of the populations *B. madritensis* (3668, Arjan) and *B. rubens* (15169), the two populations of 3668 and Arjan are closely related to each other, with a relatively short distance from the population of 15169. The populations of *B. scoparius* (5983, 5984, 5985) and *B. japonicus* (16587) formed the fourth cluster. The populations of 5983 and 5984 are located close to each other with a relatively short distance from the population of 5985. The population of 5985 is located in a longer distance than the three mentioned populations. The two populations of *B. japonicus* (16462 and 16525) with a short distance from each other, formed the fifth cluster (Fig. 3).

DISCUSSION

This study reveals a detailed picture of the chromosome features in five *Bromus* species of Iran. The knowledge of chromosome numbers, karyotype evolution, ploidy level and genome size can provide additional information that not only gives further insight into the functioning of the genome, but also have considerable predictive powers.

In this genus, the basic chromosome number is x=7, as were found for fourteen populations of five species of *Bromus* (2n=2x=14, 2n=4x=28 and 2n=6x=42). This study confirmed that the *Bromus* species show great variations in the number of chromosomes. At the interspecific level, quantitative and qualitative data allowed us the differentiation of several of the taxa studied. Among species, the most variable characters were the number of "m", and "sm" chromosomes, as well as the number and position of satellites (Table 2; Fig. 1). As a result, the species also could be differentiated by the number, type and position of satellites.

This study revealed that three populations of *B. rubens* and *B. madritensis* were tetraploid (2n=4x=28) species (Table 2 and Fig. 1). This is in agreement with the results of an investigation recorded by Sheidai and Fadaei (2005). Three populations of *B. tomentellus* was the only hexaploid (2n=6x=42), but Mirzaie-Nodoushan *et al.*

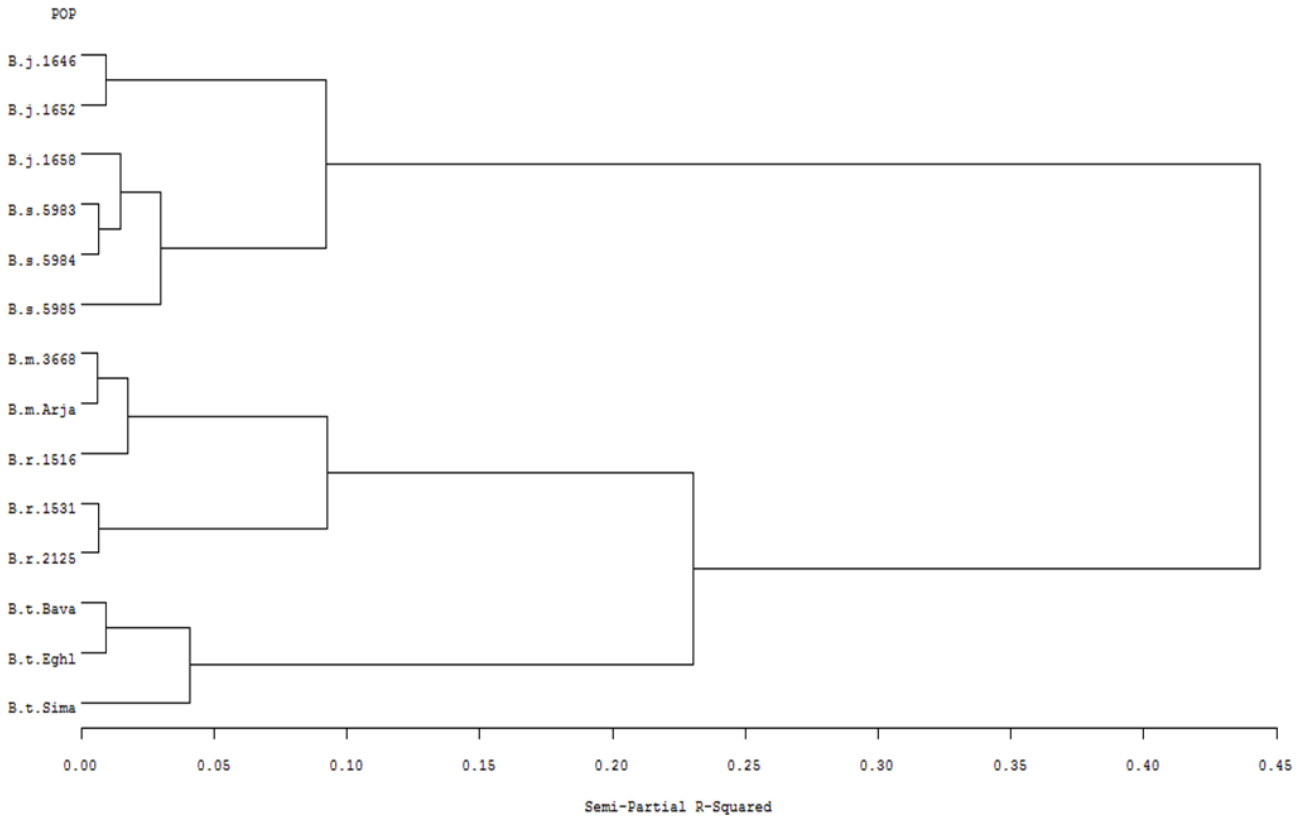

Figure 3. Dendogram of 14 populations of *Bromus* by analyzing nine karyotipic parameters using Ward 's cluster analysis method.

(2006b) recorded different ploidy levels within this species (2n=42, 70 & 84). Three populations of *B. japonicus* and *B. scoparius* species studied were diploid (2n=2x=14), supporting the earlier report of Safari *et al*. (2017). The present study confirmed that the *Bromus* species show great variations in the number of chromosomes both at inter and intra-specific levels. This kind of genetic and cytogenetic variability can confer an adaptive advantage against variable climate and other ecological elements in the region (Mirzaie-Nodoushan *et al*. 2006a).

The Duncan's test applied to the chromosome morphometric traits (LA, SA, TL, AR, DRL, TF%, A_1 and A_2) showed a highly significant difference among all examined populations of different sections (Table 3). The study revealed cytogenetic differences (P≥1%) in ANOVA for karyological data as well as the ratio of long arms to short arms among populations. So these results indicate a significant quantitative change in amount of chromatin in *Bromus* species diversification (Tables 2 and 4).

Considering the changes of intrachromosome asymmetry index (A_1) among diploid and tetraploid species, the lowest value exists in the diploid (*B. japonicus*, 16462) and the highest value exists in the tetraploid species (*B. rubens*, 15317) (Table 2).

The results of analysis of variance showed that except for A_1 and TF%, there was a significant difference (P≤1%) between genotypes in terms of chromosomal traits (LA, SA, TL, AR, DRL and A_2) which indicated large variations among the germplasms in regard to studied traits.

Cluster analysis based on chromosomal characteristics separated, the fourteen investigated populations of *Bromus* species into two major groups consistent of statistical analysis of chromosome morphometric traits (Fig. 3). The first group has eight populations of *Bromus* species (*B. madritensis, B.rubens and B. tomentellus*) which are tetraploid and hexaploid (2n=4x=28 & 2n=6x=42) and belong to *Genea* and *Pnigma* sections. The second group has six populations of *Bromus* species (*B. scoparius* and *B. japonicus*) that are diploid (2n=2x=14) and belong to *Bromus* section. Cluster analysis based on cytological data showed that the populations with the lowest metric distance may lead to use populations in crosses for inducing the highest genetic variations (Fig.3). However, grouping of the *Bromus* populations based on karyotypic data, agrees with either the taxonomic treatment of the genus *Bromus* of the same species based on morphological characters.

These genomic differences could be used for breeding purposes. In general, cytological studies of the *Bromus* species growing in Iran indicate the importance of polyploidy, chromosome structural changes, presumably quantitative changes in the amount of DNA and probably the role of growing sites in species diversification and suggest that such data may be used in the taxonomy and phylogenetic consideration of the genus (hesamzadeh and Ziaei Nasab 2010).

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