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Karyotypic Characteristics of Several *Bromus* Species

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ABSTRACT

Twelve populations of seven locally adopted *Bromus* species were studied to determine and compare their karyotypic characteristics. Karyotypic data were recorded on all chromosomes of five well-prepared cells at metaphase stage containing a complete set of either ploidy levels, for each population. The studied populations of the species differed in their karyotypic characteristics as well as ploidy levels. The populations varied in ploidy level from 2n = 14 to 2n = 84. Within the studied populations, *B. sterilis* was the only diploid species (2n = 14). Size of the chromosomes among the populations and species varied from 2.6 to 7.7 µm. Among the studied *Bromus* populations, the highest total form (TF) percentage (45.05) belonged to one of the *B. tomentellus* populations, (the most symmetric karyotype). The lowest TF% (41.44) belonged to *B. hankegnus*. This species contained 16 sub-metacentric (sm) type chromosomes that made its karyotype asymmetric.

Key Words: *Bromus;* Karyotype; Cytogenetic; Polyploid; Asymmetric

INTRODUCTION

Bromus (L.) is one of the most important rangeland plant genus in Iran, which contains several vide spread species all around the country. The genus comprises about 160 species distributed all over the world (Acedo & Liamas, 2001). Iranian Natural Resources National Gene Bank has collection of a great number of accessions of several locally adopted species from nature. Several perennial species of *Bromus* are very nutritive and palatable and several others are very effective in soil conservation. The palatable species are usually used for grazing. The species vary to some extent in ecological requirements, so that they form different ecotypes with different characteristics. Irrespective of their distribution range, there are limitations for growing the palatable species in dryer areas of central part of Iran.

Interspecific hybridization between the species may end to new genotypes with less demand of ecological requirements, which would be suitable for expanding the ecological zone of the genus in arid environments. For this purpose we performed series of crosses between several tetraploid species of *Bromus*, of which majority of crosses either were un-successful or the produced F1 seeds did not germinate. Since there is still the temptation for the idea, we decided to determine the major factors inhibit the success in interspecific hybridization. Karyotypic non-similarity is one of the main factors influencing interspecific hybridization. Differences in chromosome number, shape and size at metaphase of mitosis division can express genetic variation between the individuals. These differences are much greater at interspecific level. Greater are the differences, lesser is the expected compatibility in interspecific crosses. We may be able to produce more details for the species under investigation with the aid of chromosome analysis procedures, on which the possible compatible karyotypes are more easily recognized. On the other hand, karyotypic data are important in showing the species inter-relationships.

Notwithstanding there are complex relationships between the species of *Bromus*. In some cases, autoploidy, alloploidy, interspecific hybridization and genetic introgression between related populations have contributed to the evolution of species or cultivars. Moreover, presence of possible genetic systems that suppress homologous chromosome pairing would increase the complexity of the relationship (Armstrong, 1991). Thus, all these would make the investigation of the relationships between the species of *Bromus* and its interspecific hybridization more problematic. Researchers have made many attempts for identifying even individual chromosomes of several *Bromus* species, for instance, by using C-banding procedures and chromosome dimensions (Tuna *et al.*, 2001). Martinello and Schifino-Wittmann (2003) studied 14 accessions of *Bromus auleticus*. Their accessions were all hexaploid (2n = 6x = 42) and the high symmetry and homogeneity of the karyotypes made it difficult to detect possible intraspecific differences. Massa *et al.* (2004) proposed a taxonomic realignment within *Bromus* sect. Their plant materials included 28 hexaploid (2n = 6x = 42) accessions and two octaploid (2n = 8x = 56) accessions.

It is assumed that inter- and intraspecific karyotypic differences and similarities within the studied *Bromus* species would make interspecific crosses more problematic. Therefore, this study was performed to determine karyotypic similarities and differences of several *Bromus* species at inter and intra-specific level. Determinations were made for differences in chromosome number, shape and

size at metaphase of mitosis division and several statistical parameters, which compare karyotypic asymmetry of the species.

MATERIALS AND METHODS

Twelve populations of seven *Bromus* species, *B. tomentellus, B. hankegnus, B. sterilis, B. inermis, B. cappadocicus, B. persicus* and *B. biebersteinii,* were either collected from the nature or obtained from National Natural Resources Gene Bank, Iran. Cytogenetic studies were performed on these populations in order to specify their karyotypic characteristics. Preparations were made using fresh grown root tips for the karyotypic studies. Different pre-treatments were used and the best results was obtained for treating the root tips with saturated α-Bromonaphtaline for three hours followed by fixation in a glacial acetic acid and ethanol (1:3 volume ratio) for 17 to 24 h (Mirzaie-Nodoushan & Asadi-Corom, 2002). Root tips were hydrolyzed in 1 N HCl at 60 degree centigrade for 4 - 5 min. Hematoxiline was used as the preferred agent for chromosome staining.

Karyological data such as long arm (L) and short arm length (S) were recorded on all chromosomes of five well-prepared cells at metaphase stage containing a complete set of chromosome, for all populations using photomicroscope equipped with cameralucida and micrometer. Then total length of the chromosomes, long arm to short arm ratio (L/S) and short arm to long arm ratio (S/L) were derived from the recorded data. High number of chromosomes and close morphological similarities of the chromosomes of the species prohibit the exact identification of homologous pairs. However, chromosome pairs were identified and arranged based on their total length and arm ratios. The studied populations of the species differed in their ploidy levels. A haploid set of chromosomes from each population was used for analyzing the data. Chromosomes were described according to Levan terminology (Levan *et al.,* 1964).

Factorial analysis of variance was performed on the data groups based on completely randomized design model for each ploidy level, regarding the populations and their chromosomes as two different factors (Mehrpur *et al.,* 2002). Five series of analysis of variance were performed to test the significance of the difference between the chromosomes and between the populations of tetra to dodecaploid populations. Since there was only one diploid between the studied populations, the analysis of variance was not performed on this ploidy level.

RESULTS AND DISCUSSION

Ploidy level and chromosome number. The somatic chromosome number and details of the karyotypes of the studied population, revealed that *B. sterilis* was the only diploid (2n = 14) species (Table I & Fig. 1). This is in agreement with the results of an investigation recorded by Sheidai and Fadaei (2005). *B. tomentellus* populations possessed three ploidy levels (2n = 42, 70 & 84). Oja and Laarmann (2002) also recorded different ploidy levels within one species of *Bromus*. In this study *B. hankegnus* and *B. cappadocicus* were hexaploid (2n = 42). Both the studied populations of *B. inermis* were octaploid (2n = 56) and both of the populations of *B. persicus* were tetraploid (2n = 28). The only population of *B. biebersteinii* was decaploid (2n = 70). These findings showed that ploidy level in *Bromus* species varied from diploid to decaploid, which confirmed the existence of high levels of ploidy in this genus (Armstrong, 1991; Tuna *et al.,* 2001).

Size of the chromosomes. For a long time difficulties are reported in studying *Bromus* karyotypic characteristics that is due to the large number of chromosomes, small morphological differences between the chromosomes and variability from cell to cell for chromosome length and arm ratio (Tuna *et al.,* 2001). Size of the chromosomes among the populations and species varied from 2.6 µm in *B. inermis* (2) to 7.7 µm in *B. biebersteinii*. Chromosome length grand mean of the populations varied from 3.8 µm in *B. inermis* (2) to 5.7 µm in *B. tomentellus* (2), *B. cappadocicus* and *B. biebersteinii* (Table I).

Analysis of variance. Analysis of variance revealed significant differences between the chromosome dimensions of the populations and between the populations' chromosome grand means (Table II) indicating occurrence of high quantitative changes in chromosome size of the populations studied as well as chromosome number. This justified complementary analysis and description of the karyotypes. Moreover, for all of the ploidy levels, there were significant differences between the populations based on their chromosome short arm length, long arm length and total length means of their chromosomes, except for chromosomes' short arm length of octaploid populations (2n = 70) for which, mean square (MS) was not significant. This indicated that chromosomes short arm length means of the two octaploid populations, *B. tomentellus* (4) and *B. biebersteinii,* are not statistically different. Also there were highly significant differences between the chromosomes based on their short arm length, long arm length and total length means. This indicated that equivalent chromosomes of the populations of all ploidy levels are significantly different. There were rare significant interactions between the population and chromosome means. However, significant interactions indicated that not only the studied characteristics differ between the populations and chromosomes within the populations but also the rate of changes are not constant between different populations.

Karyotypic characteristics. In all of the populations the chromosomes were mainly of m type (centromers at median region). However, five populations possessed several sm type (sub-meta centric) chromosomes (Table II). *B. hankegnus* possessed the most sm type chromosomes (26 m + 16 sm). Among the studied populations the highest TF%

Table I. Mitotic characteristics of the studied populations of *Bromus* species

Species	2n	TF%	M	L	S	S%	S/L	DRL	KF
B. tomentellus(1)	84	43.45	5.4	6.7	3.8	0.84	0.57	0.64	80m+4sm
B. tomentellus(2)	84	44.44	5.7	7.0	4.0	0.84	0.57	0.62	84m
B. tomentellus(3)	42	43.74	4.1	5.3	3.1	1.80	0.58	1.28	42m
B. tomentellus(4)	70	45.05	5.5	6.8	4.1	1.06	0/60	0.71	70m
B. hankegnus	42	41.44	4.6	6.0	3.3	1.71	0.55	1.4	26m+16sm
B. sterilis	14	44.86	4.4	5.4	3.6	5.84	0.67	2.93	14m
B. inermis(1)	56	43.14	4.8	6.0	3.4	1.26	0.57	0.97	54m+2sm
B. inermis(2)	56	42.63	3.8	4.0	2.6	1.22	0/65	0.66	56m
B. cappadocicus	42	44.54	5.7	7.2	4.4	1.84	0.61	1.17	42m
B. persicus(1)	28	43.47	4.9	6.4	3.4	2.48	0.53	2.18	28m
B. persicus(2)	28	43.28	4.5	5.8	3.3	2.62	0/57	1.98	24m+4sm
B. biebersteinii	70	43.06	5.7	7.7	3.8	0.95	0.49	0.98	64m+6sm

TF% = Total form percentage, M= grand mean of chromosome length, L. = length of the longest chromosome, S = length of the shortest chromosome, DRL = Difference between the maximum and minimum relative length of the chromosomes, S% = relative length of the shortest chromosome, S/L = ratio of the shortest to the longest chromosomes, KF = Karyotypic formulae

Table II. Mean squares resulting from factorial analysis of variance on the karyotypic characteristics of eleven populations of *Bromus* species with different ploidy levels

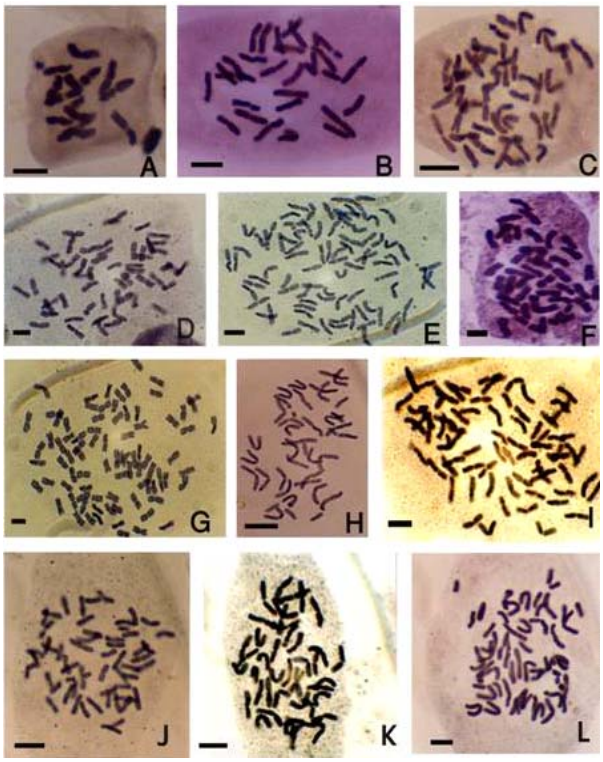
2n	S.O.V.	DF	Long arm (L)	Short arm (S)	Total length	S/L	L/S
	Population (A)	1	1.47*	1.02*	4.94**	0.01ns	0.01ns
	Chromosome(B)	13	1.41**	0.99**	4.53**	0.03ns	0.11ns
28	A*B	13	0.06ns	0.11ns	0.04ns	0.03ns	0.14ns
	Error	84	0.26	0.24	0.75	0.03	0.11
	Population (A)	2	16.88**	17.56**	67.16**	0.33**	1.76**
	Chromosome(B)	20	1.66*8	1.52**	6.09**	0.04*	0.20*
42	A*B	40	0.08ns	0.15ns	0.12ns	0.04**	0.18*
	Error	0.19		0.17	0.46	0.02	0.11
	Population (A)	1	36.57**	24.49**	120.9**	0.02ns	0.21ns
	Chromosome(B)	27	1.86**	1.15**	5.63**	0.04ns	0.15ns
56	A*B	27	0.08ns	0.10ns	0.08ns	0.04ns	0.15ns
	Error	392	0.09	0.11	0.20	0.07	0.12
	Population (A)	1	3.85**	0.15ns	2.49*	0.33**	1.23**
	Chromosome(B)	34	1.36**	1.59**	5.45**	0.05**	0.18**
70	A*B	34	0.15ns	0.18ns	0.14ns	0.05**	0.18**
	Error	350	0.15	0.15	0.38	0.02	0.06
	Population (A)	1	2.56**	5.52**	15.6**	0.12*	0.68**
	Chromosome(B)	41	1.32**	1.38**	5.10**	0.03*	0.11ns
84	A*B	41	0.07ns	0.15*	0.05ns	0.04*	0.15*
	Error	420	0.06	0.10	0.04	0.02	0.10

**, * = significant at 1% and 5% levels of probability respectively, ns = non-significant

was estimated on *B. tomentellus* (4) and then the only populations of *B. sterilis* species (45.05 & 44.84, respectively). These two species possess only m type chromosome that is the main reason for the populations to have the most symmetric karyotype based on TF%. The lowest TF% value was estimated on *B. hankegnus* (41.44). This species contains 16 sm type chromosomes that make its karyotype asymmetric. Difference between the maximum and minimum relative length of the chromosomes (DRL) presents different scheme of asymmetry for the populations. That is due to different basis of the estimation of the two parameters, TF% and DRL. TF% estimates the symmetry based on the ratio of total short arm lengths of the chromosomes of a genotype to its total chromosome length, whereas DRL estimates the symmetry based on the difference between relative length of the shortest and the longest chromosomes of a genotype. However, based on DRL, *B. sterilis* showed the most asymmetric karyotype, (DRL = 2.93), while two populations of *B. tomentellus* (1) and *B. tomentellus* (2) showed the most symmetric karyotype, (DRL = 0.64 & 0.62, respectively). The highest ratio between the shortest to the longest chromosomes (S/L) was observed on *B. sterilis*, the diploid population. In contrast the lowest S/L was observed on *B. biebersteinii*, one of the decaploid species (2n = 70). Different levels of ploidy of the species have made them cytogenetically complex. To get further insight into this issue, more research is necessary on the species to find out the general trend of evolution in the species as well as inter-relationship between the species.

In this genus, the basic chromosome number is x = 7, as noted in related genera of Poaceae, such as *Lolium* and *Triticum*. This 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.

Fig. 1. Representative mitotic plates of *Bromus* species and populations studied. A: *B. sterilis,* 2n = 14, B: *B. persicus,*2n = 28, C: *B. hankegnus,* 2n = 42, D: *B. inermis* (1), 2n = 56, E: *B. tomentellus* (1), 2n = 84, F: *B. cappadocicus,* 2n = 42, G: *B. tomentellus* (2), 2n = 84, H: *B. tomentellus* (3), 2n = 42, I: *B. biebersteinii,* 2n = 70, J: *B. inermis* (2), 2n = 56, K.: *B. tomentellus* (3), 2n = 42, L: *B. tomentellus* (4) 2n = 70. Scale bars = 5µm



As shown in (Fig. 1), considerable numerical instability was met at the individual level. Numerical variations found in *B. tomentellus* (Fig. 1E, 1G, 1H, 1K & 1L) may be caused due to Robertsonian events that lead to an increase in chromosome number (Portugal *et al*., 2002). Such numerical variation would be more frequent in genotypes with higher mitotic rates corresponding to higher growth rates. On the other hand, genotypes with lower growth rates will have less probability of showing numerical variations. In essence, the studied populations are not only highly differentiated from each other based on ploidy level and chromosome numbers but also there are great differences between the populations with similar ploidy levels. These karyotypic data are highly useful in taxonomic studies on the species. Moreover, interspecific hybridisation requires a precise selection of the parents for the crosses.

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