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Cytogenetic Studies in Some Species of *Bromus* L. in Iran

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Abstract: The genus *Bromus* L. belongs to tribe Bromeae (Poaceae, family). The taxon includes about 160 annual and perennial species distributed all over the world. It has important rangeland plant species in Iran, which are placed in 6 sections. Nine populations of three *Bromus* species: *B. danthoniea* (Three populations), *B. sterilis* (three populations) and *B. tectorum* (three populations) were selected based on their morphological characteristics to investigate their karyotypes. Preparations were made using fresh grown root tips. Saturated α-bromonaphtaline, formaldehyde and chromium trioxide (1:1), 1 N NaOH and hematoxiline were used for pre- treatment, fixative, hydrolyser and chromosome staining agent, respectively. The chromosomal studies were done using photomicroscope equipped with micrometer. Karyological data such as long arm, short arm and total length were recorded on all chromosomes of 5 well-prepared cells at metaphase stage containing a complete set of chromosomes. Finally, Pearson correlation coefficient was estimated for all paired combinations of the karyotypic characteristics to investigate their inter-relationships. Stebbins table was used for determining evolutionary state and karyotypic symmetry of the studied genotypes. All populations had mainly metacentric type chromosomes. *Bromus danthoniea* was placed in A1 and had the highest values of inter-asymmetry chromosomal index. It had the lowest %TF. Based on Stebbins table all species were placed in class 1A and primitive evolutionary state.

Key words: Cytogenetic, studies, species, *Bromus*

INTRODUCTION

The genus *Bromus* L. belongs to tribe Bromeae, (Poaceae, family). The taxon includes about 160 annual and perennial species (Acedo and Liamas, 2001) distributed all over the world. It has important rangeland plant species in Iran, which are placed in 6 sections (Bor, 1970).

Bromus species are known as the species with various intra-specific ploidy levels. There is little information on cytogenetic aspects of the species in the literature (Hill, 1965) recorded up to 112 chromosomes for *B. erectus* (Naganowaska, 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).

Various levels of polyploidy in *B. inermis* (Devesa *et al*., 1990) indicates the importance of cytological studies for understanding the evolution of the genus *Bromus*. Mirzaie-Nodoushan *et al*. (2000) recorded karyotypic investigation of some

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Bromus tomentellus population in Iran. Sheidai and Fadaei (2005) studied ten population of 6 *Bromus* species. Also, Mirzaie-Nodushan (2000, 2006) recorded evolutionary karyotypic variation in *Bromus tomentellus* populations in Iran.

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

MATERIALS AND METHODS

Plant Material

We studied nine populations of three *Bromus* species: *B. danthoniea* (three populations), *B. sterilis* (three populations) and *B. tectorum* (three populations). For this purpose complete specimens were collected in different regions of Fars Province (Iran) in several field trips during their period of fruiting (Spring and Summer from 2005 to 2009) and dried with the specific methods as herbarium specimens. Voucher specimens are deposited in the Herbarium of Natural Research Center of Fars, Iran.

Cytological Preparations and Mitotic Analysis

Preparations were made using fresh grown root tips for the karyotypic studies. Different pre-treatments were tested and the best results were obtained for treating the root tips with saturated α-bromonaphtaline for 4 h followed by fixation in formaldehyde and chromium trioxide (1:1) for 16 to 20 h. Then the root tips were hydrolyzed in 1 N NaOH at 60°C for 20-25 min and used hematoxiline for chromosome staining. The chromosomal studies were done using photomicroscope equipped with micrometer. Karyological data such as long arm, short arm and total length were recorded on all chromosomes of 5 well-prepared cells at metaphase stage containing a complete set of chromosomes, for all of the populations using photomicroscope equipped with micrometer. High number of chromosomes and close morphological similarities of the chromosomes of the population prohibited the exact identification of homologous pairs. However, we identified and arranged chromosome pairs based on their total length and arm ratios.

The studied populations differed in their ploidy levels and karyotypic characteristics. The chromosomes were described according to Levan terminology (Levan *et al*., 1964). Several statistical parameters such as total form percentage (Huziwara, 1962), chromosome length, relative length percentage of each chromosome, length of long arm, relative length percentage of long arm, length of short arm, relative length percentage of short arm, arm ratio (length of long arm/length of short arm) and the centromeric index which indicates the ratio between length of short arm and total length of chromosome, were estimated on the data to compare the karyotypes of the populations. Finally, Pearson correlation coefficient was estimated for all paired combinations of the karyotypic characteristics to investigate their inter-relationships. Stebbins table was used for determining evolutionary state and karyotypic symmetry of the studied genotypes (Stebbins, 1971).

RESULTS AND DISCUSSION

The results confirm no differences were found among the different species for the number of chromosomal stocks (x = 7). All species were diploid with 2n = 2x = 14 (Fig. 4) and had metacenteric type chromosome that indicate karyotypic symmetry (Fig. 1). Species and

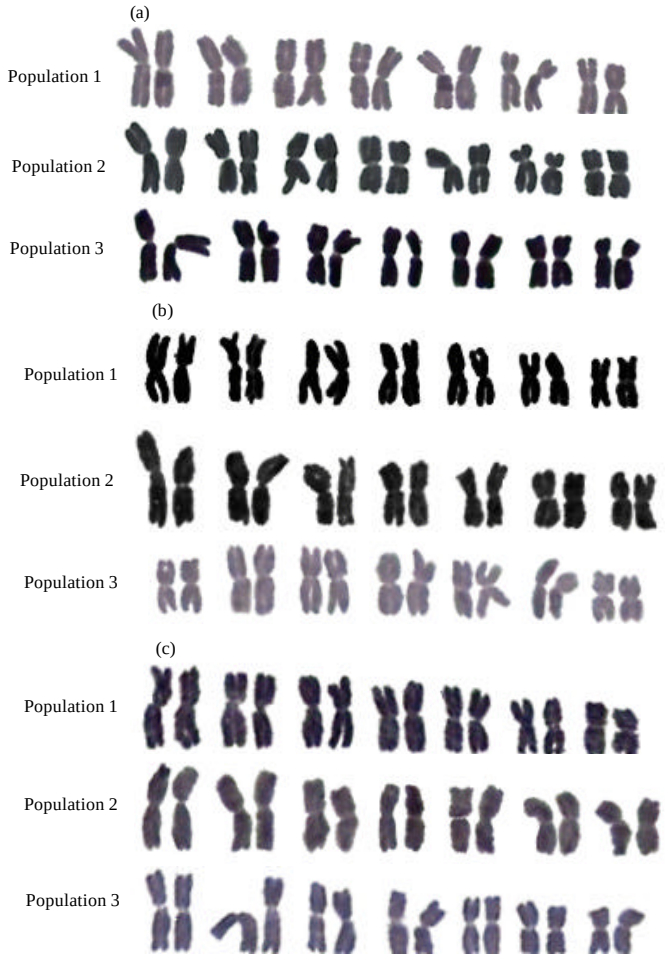

Fig. 1: Karyogram of *Bromus* species based on chromosomal length (x1880). (a) *Bromus danthoniea* (b) *Bromus sterilis* and (c) *Bromus tectorum*

genotypes were placed in the related symmetry classes using Stebbins table. Also karyotypic details were studied (Table 1, 2). The TF% and A1, DRL and A2 were compared (Fig. 2, 3). Difference in the relative length percentage of the biggest and the smallest chromosomes (DRL) varied from 6.37 μm in *B. tectorum* (pop-3) to 4.68 μm in *B. tectorum* (pop-1). According to Table 1, *Bromus danthoniea* (population 1) was placed in A1 and had the highest values of inter-asymmetry chromosomal index. It had the lowest %TF.

Results showed %TF and A1 had inverse ratio (Fig. 2). It presented different scheme of symmetry for the populations. The lower DRL showed more symmetric karyotypes.

Chromosome Number and Size

Nine populations of three species of *Bromus* possessed n = 7 (2n = 2x = 14) chromosome number (Fig. 4a-i). Three populations of *B. danthoniae* possessed n = 7 (2n = 2x = 14) chromosome number (Fig. 4a-c). Supporting an earlier report (Sheidai and Fadaei, 2005).

Table 1: Karyotypic details in *Bromus* species studied

Species	Population	2n	x	A1	A2	SC	DRL	%TF	VRC	KF
Bromus danthoniea	1	2n = 14	7	0.22	0.11	1A	5.22	43.24	9.35	7m
	2	2n = 14	7	0.21	0.13	1A	6.02	43.11	7.31	7m
	3	2n = 14	7	0.18	0.11	1A	4.80	43.68	8.65	7m
Bromus sterilis	1	2n = 14	7	0.16	0.11	1A	4.83	44.32	6.66	7m
	2	2n = 14	7	0.20	0.12	1A	5.15	43.39	8.50	7m
	3	2n = 14	7	0.20	0.15	1A	6.21	43.37	6.85	7m
Bromus tectorum	1	2n = 14	7	0.18	0.11	1A	4.68	44.09	7.13	7m
	2	2n = 14	7	0.15	0.14	1A	5.73	45.06	6.89	7m
	3	2n = 14	7	0.17	0.14	1A	6.37	44.25	6.50	7m

A1: Intra-asymmetry chromosomal index, KFL Karyotype formula, A2: Inter-asymmetry chromosomal index, VRC: Value of relative chromatin, SC: Symmetry classes, %TF: Total form percentage, DRL: Difference in range relative length

Table 2: Karyotypic characters in *Bromus* species studied

Species	Population	TL	LA	SA	AR	CI
Bromus danthoniea	1	9.35	5.15	4.04	1.30	0.42
	2	7.31	4.01	3.15	1.33	0.42
	3	8.65	4.64	3.78	1.25	0.43
Bromus sterilis	1	6.66	3.55	2.95	1.19	0.44
	2	8.50	4.63	3.69	1.25	0.43
	3	6.85	3.71	2.97	1.27	0.43
Bromus tectorum	1	7.13	3.86	3.14	1.23	0.43
	2	6.89	3.66	3.10	1.18	0.44
	3	6.50	3.47	2.88	1.21	0.44

TL: Total length, LA: Length of long arm, SA:Length of short arm, AR: Arm ratio, CI: Centromere index

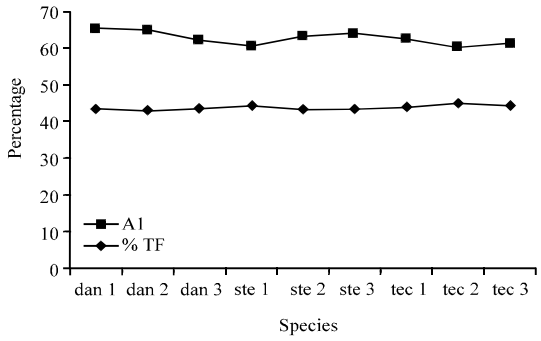

Fig. 2: Intra-asymmetry chromosomal index and total form percentage trend in different *Bromus* species

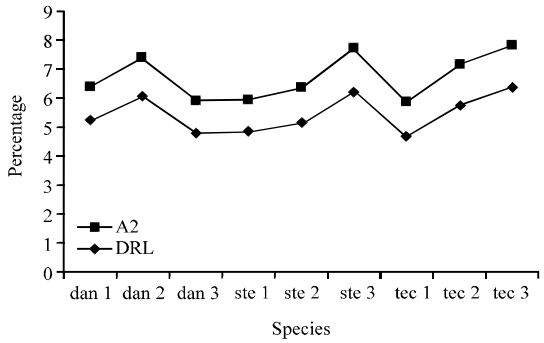

Fig. 3: Difference in range relative length and inter-asymmetry chromosomal index trend in different *Bromus* species

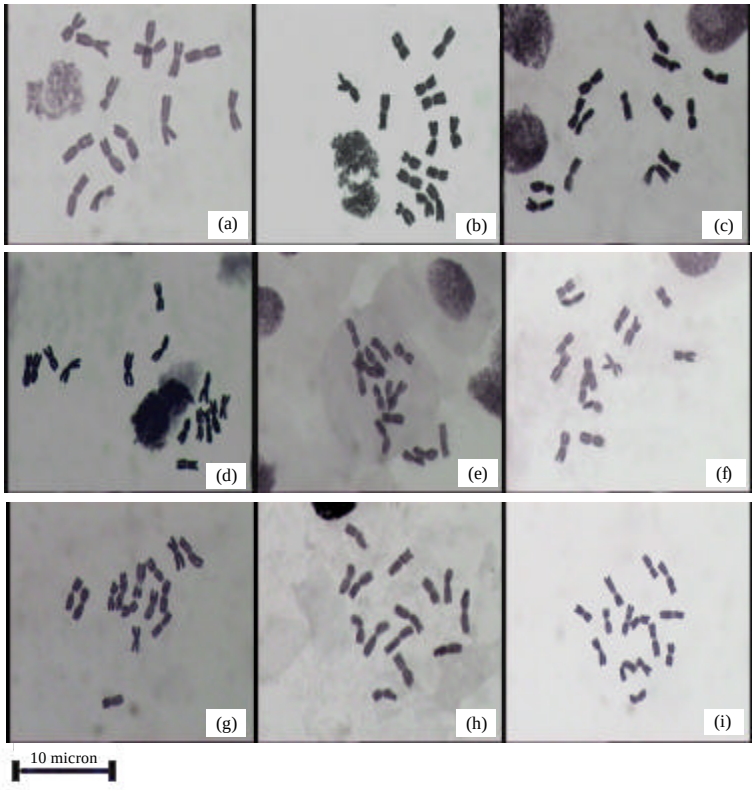

Fig. 4: Representative mitotic plates of *Bromus danthoniae*, *Bromus sterilis* and *Bromus tectorum* populations studied. (a) *Bromus danthoniae*, (pop-1), 2n = 14, (b) *Bromus danthoniae* (pop-2), 2n = 14, (c) *Bromus danthoniae* (pop-3), 2n = 14, (d) *B. sterilis* (pop-1), 2n = 14, (e) *B. sterilis* (pop-2), 2n = 14, (f) *B. sterilis* (pop-3), 2n = 14, (g) *B. tectorum* (pop-1), 2n = 14, (h) *B. tectorum* (pop-2), 2n = 14 and (i) *B. tectorum* (pop-3), 2n = 14, Scale bars = 10 micron

Three population of *B. sterilis* possessed n = 7 (2n = 2x = 14) chromosome number (Fig. 4d-f). The earlier study on this species reports the somatic chromosome number of 2n = 28 (Goldblatt, 1994). Therefore, this is a new report for ploidy level of *B. sterilis*.

Three populations of *B. tectorum* possessed n = 7 (2n = 2x = 14) chromosome number (Fig. 4g-i). This is in agreement with the report of Darlington and Wylie (1955) and Lövkvist and Hultgard (1999).

The results confirm no differences were found among the different species for the number of chromosomal stocks (x = 7). All species were diploid with 2n = 2x = 14. The earlier study on this species showed different ploidy levels within species of *Bromus* (Armstrong, 1991; Tuna *et al*., 2001; Oja and Laarmann, 2002).

Difference in the relative length percentage of the biggest and the smallest chromosomes (DRL) varied from 6.37 μm in *B. tectorum* (pop-3) to 4.68 μm in *B. tectorum* (pop-1).

Also the highest chromatin observed in *B. danthoniae* (pop-1) and the lowest chromatin observed in *B. tectorum* (pop-3).

Karyotypic Characteristics

All populations had mainly metacentric type chromosomes (centromers at median region) (Fig. 1).

Based on Stebbins table all species were placed in class 1A and primitive evolutionary state.

Dispersion diagram of *Bromus* species according to intra-asymmetry and inter-asymmetry chromosomal index showed species placed in two groups. The %TF and A2 (inter-asymmetry chromosomal index) were compared.

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