KARYOTYPIC INVESTIGATIONS OF SOME BROMUS TOMENTELLUS POPULATIONS AND THEIR KARYOTYPIC CORRELATIONS

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Twelve populations of *Bromus tomentellus* collected from different parts of Iran were surveyed for karyotypic characteristics. The data were analysed using a factorial model of analysis of variance. After a confident result of differences between populations for the characters, complementary analysis was performed on the data. The populations were classified for the karyotypic characteristics and correlations between all combinations of the populations were estimated based on the total chromosome length and long arms of the chromosomes. Using principle components analysis the most variable karyotypic characters were identified. The principle components which contained the most amounts of existing variation of the data were also recognized. The two main components were used to produce a scatter plot of the populations. Cluster analysis was used to classify the populations based on the chromosomes total length and all of the measured characters. Regarding the karyotypic characteristics the least and the most similar populations were identified. Several statistics were estimated for assessment of asymmetry of the karyotypes. Regarding the results of the analysis of variance, the populations, chromosomes and their

interactions were significantly different for most of the studied characters. The populations were highly correlated based on the total length of the chromosomes and L/S ratio but some weak correlations were also observed between some combinations of the populations. In principle components analysis, total length of the chromosomes and long arm length had the most influence on the first component, which in turn contained more than 90% of the existing variation in the data. Cluster analysis on both of the total length of chromosomes and all of the recorded attributes produced a similar grouping. The most distant populations based on their karyotypes, which may produce infertile progenies in breeding programs were also identified.

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Key words. Bromus tomentellus, Karyotype, Mitosis, Multivariate Analysis.

مطالعات کاریوتیپی برخی جمعیتهای گونه Bromus tomentellus

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دوازده جمعیت از گونه *Bromus tomentellus* که از نقاط مختلف ایران جمع‌آوری شده بود مورد اندازه گیریهای مختلف کروموزومی قرار گرفت. داده‌های حاصل از این اندازه گیریها ابتدا در قالب طرح آماری فاکتوریل مورد تجزیه و تحلیل قرار گرفته و پس از حصول اطمینان از تفاوتهای معنی دار آماری بین جمعیتها و نیز کروموزومها، اطلاعات حاصل مورد تجزیه و تحلیلهای تکمیلی قرار گرفتند. جمعیتها از نظر ابعاد مختلف کروموزومی دسته بندی شدند و سپس همبستگی بین جمعیتها از نظر ویژگیهای کاریوتیپی مورد بررسی قرار گرفت. با استفاده از روش آماری تجزیه به مؤلفه‌های اصلی مهمترین صفات کاریوتیپی که در کاریوتیپ جمعیتها ایجاد تنوع می نمایند مشخص گردیدند و نیز مؤلفه‌هایی که حجم زیادی از اطلاعات و تنوع موجود در داده‌ها را در بر داشتند بدست آمدند. دو مؤلفه‌ای که بیش از ۹۵ درصد از اطلاعات موجود در داده‌ها را در بر داشتند جهت بدست آوردن پراکنش ژنوتیپها در یک دستگاه مختصات مورد استفاده قرار گرفتند. از روش تجزیه خوشه‌ای نیز جهت دسته بندی جمعیتها بر مبنای صفات طول بازوی بلند کلیه کروموزومهای جمعیتها و نیز طول کل کروموزومها استفاده گردید و جمعیتهایی که از نظر این ویژگیها کمترین و بیشترین تشابه را داشتند شناسایی گردیدند. تعدادی از آماره‌های مورد استفاده در سنجش تقارن کاریوتیپی نیز مورد محاسبه قرار گرفتند و بر مبنای آنها متقارن‌ترین و نامتقارن‌ترین کاریوتیپها شناسایی گردیدند. با توجه به نتایج تجزیه واریانس داده‌ها جمعیتهای مورد مطالعه و نیز کروموزومهای مختلف هر جمعیت از نظر اکثر صفات کاریوتیپی اندازه گیری شده با یکدیگر اختلاف داشتند. همینطور بین این دو فاکتور نیز اثر متقابل مشاهده گردید. جمعیتها از نظر طول کل کروموزوم و نسبت بازوی بلند به بازوی کوتاه با یکدیگر همبستگی بالایی نشان دادند. البته بین تعدادی از ترکیبات دوگانه همبستگیهای ضعیف نیز مشاهده گردید. در تجزیه به مؤلفه‌های اصلی نیز طول کل کروموزوم و نیز طول بازوی بلند بیشترین نقش را در تشکیل اولین مؤلفه که بیش از ۹۰ درصد واریانس موجود در داده‌ها را در برداشت ایفا نمودند. دندروگرامهای حاصل از تجزیه خوشه ای بر مبنای طول کل کروموزوم و نیز کلیه ویژگیهای اندازه گیری شده کروموزومی روند مشابهی در دسته بندی جمعیتها نشان دادند. دورترین جمعیتها از نظر ویژگیهای کاریوتیپی که ممکن است با یکدیگر دارای ناسازگاریهای کروموزومی باشند و در صورت استفاده در پروژه‌های اصلاحی منجر به ایجاد ناباروری نسبی در نتاج گردند نیز تعیین گردیدند.

Introduction

Bromus tomentellus Boiss. is one of the most important palatable forage species in Iran that has not been considered for its cytogenetical characteristics yet. Primary information such as ploidy level and karyotypic characteristics of collected populations from various parts of the country are not known. On the other hand, due to a vast spreading of the species and its palatability, it merits more attention. The first step to be taken for the utilization of the potentials of the species is investigation of karyotypic characteristics of its randomly collected populations. Which populations must be used in the breeding programs so that the least karyotypical incompatibility encountered and the most induced genetic variation gained, is a major question to be answered.

There is little information on cytogenetic aspects of the species in the literature. Different species of *Bromus* have been recorded from diploid (2n = 2x = 14) to dodekaploid (2n = 12x = 84). Hill (1965), recorded 112 chromosomes for *B. erecutus* Hudson. Naganowaska (1993) used genetic distances estimated based on centromeric index and total chromosome length to investigate interrelationships of several species of *Bromus*. Based on this study, *B. sterilis* L. and *B. tectorum* L. do not have any relationship with *B. fasciculatus* Presl., *B. rubens* L. and *B. madritensis* L. Yang and Dunn (1997) also recorded various levels of polyploidy in *B. inermis* Leyss.

Univariate and multivariate statistical methods are widely used in karyological studies. Mirzaie-Nodoushan and Fayazi (1998) have used cluster analysis to classify several populations of sainfoin (*Onobrychis sativa* Lam.) in order to find the homogeneity of the populations. Sheidai & al. (1996), have used cluster analysis to investigate cytological data of Iran zira from three genus *Bunium, Carum* and *Cuminum*. Sheidai & al. (1998) studied several cultivars of cotton (*Gossypium hirsutum*) using various statistical methods to investigate karyotypic variations of the cultivars.

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

Materials and Methods

Twelve populations of *Bromus tomentellus* Boiss. randomly taken from collected populations of the species were used in this study. Preparations were made using fresh grown root tips for karyological studies. Root tips were treated with 0.002 8-Hydroxyquinoline for 2-3 hr followed by fixation in a glacial acetic acid and absolute alcohol mixture (1:3 volume ratio) for 17 to 24 hr at room temperature. Root tips were then hydrolysed in 1N HCL at 60°C for 2-3 minutes. 2% aceto orcein was used for chromosome staining. Karyological data such as long (L) and short (S) arms and total length (TL) of the chromosomes, L/S and S/L ratios were recorded on all chromosomes of five well prepared cells at metaphase stage for each population. Chromosome pairs were identified and arranged on the basis of total length and arm ratio. Factorial analysis was performed on the data in a completely randomized design regarding the populations and chromosomes as the two factors with 12 and 21 levels respectively. The least number of recorded cells was regarded as replications. Pearson correlation coefficient was estimated for all paired combinations of the populations based on the total length, and L/S ratio of the congruent chromosomes of the populations. Several statistics such as total form percentage (TF%), (Forni-Martinn & al. 1994), DRL (Maximum relative length - Minimum relative length) , coefficient of correlation (CV%) and S/L were estimated to assess the karyotype asymmetry. Principle components analysis (PCA) was performed on the data to find the importance of the mitotic characteristics on the existing variation and also to use the first two components for producing a scatter plots of the populations. Different methods of cluster analysis performed on the original karyotypic data using JMP software were UPGMA, single linkage and complete linkage. Cophenetic correlations were estimated to judge on the goodness of fit of the clusters to the original data (Romesburg 1984).

Results and Discussion

Analysis of variance showed highly significant differences between the populations, chromosomes and their interactions for the most of the measured characters recommending further analysis of the characters (Table 1). The significant interactions between the populations and

Table 1. Mean squares resulting from factorial analysis of variance on the mitotic characteristics. SOV=stands for source of variation. DF=stands for degree of freedom. **, * Significant at the 0.01 and 0.05 probability levels. ns = Non-significant

SOV	DF	Total length	Long arm (L)	Short arm (S)	L/S	S/L
Populations(A)	11	1.613**	0.946**	4.534**	0.087**	0.098ns
Chromosomes(B)	20	8.494**	3.490**	22.660**	0.040**	0.082ns
AB	220	0.045**	0.260**	0.301*	0.029**	0.081ns
Error	504	0.006	0.209	0.212	0.006	0.069

chromosomes indicated that not only the studied traits differ between the populations and between chromosomes within the populations but also the rate of changes is not constant between different populations. In the case of significant differences between the factors further analysis is justified. Since the populations were collected from various and far distant locations of the country in which they were isolated for a long time, a significant chromosome structural change between the populations would be expected. These may lead to evolution of *Bromus* species. These genomic differences, which may indicate the role of environment on the structural changes of chromosomes of the studied populations, would be used for breeding purposes. Karl Pearson correlation coefficient for total length of the chromosomes was highly significant for most of the combinations of the populations but not for L/S ratio in some combinations of the populations (Table 2). Lower values of correlation based on L/S ratio indicate occurrence of structural changes of chromosomes among the populations and point towards their distinctness (Sheidai & al. 1996). This would suggest significant structural changes within chromosomes of the isolated populations that may cause incompatibility between the pairs of populations with none significant correlations. This is important when in a breeding program, F1 progenies of the crosses or their hybrids are concerned. Particularly in the cultivated seed crops, this may lead to some incompatibility, infertility and resultantly less seed yield.

Table 2. Correlation coefficients between all pairs of populations based on L/S ratio (over diagonal) and total length of the chromosomes (below diagonal).

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	1.000**	0.445*	0.648**	0.347ns	0.401ns	0.059ns	-0.465*	-0.203ns	-0.202ns	0.414ns	0.400ns	-0.121ns
G2	0.978**	1.000**	0.665**	0.509*	0.634**	-0.292ns	0.036ns	-0.503*	-0.275ns	0.459*	0.814**	0.250ns
G3	0.434*	0.329ns	1.000**	0.385ns	0.560**	-0.236ns	-0.167ns	-0.290ns	-0.254ns	0.455*	0.663**	0.076ns
G4	0.977**	0.991**	0.360ns	1.000**	0.703**	-0.262ns	0.120ns	-0.496*	-0.079ns	0.730**	0.566**	0.062ns
G5	0.951**	0.969**	0.346ns	0.977**	1.000**	-0.217ns	0.145ns	-0.525*	-0.414*	0.579**	0.744**	0.118ns
G6	0.989**	0.979**	0.431*	0.979**	0.964**	1.000**	-0.316ns	0.567**	0.195ns	-0.149ns	-0.190ns	-0.509*
G7	0.914**	0.938**	0.291ns	0.955**	0.982**	0.936**	1.000**	-0.242ns	0.028ns	-0.130ns	0.031ns	0.307ns
G8	0.982**	0.963**	0.429ns	0.965**	0.952**	0.982**	0.922**	1.000**	0.659**	-0.206ns	-0.408ns	-0.764**
G9	0.992**	0.977**	0.457*	0.980**	0.955**	0.989**	0.927**	0.987**	1.000**	-0.091ns	-0.340ns	-0.681**
G10	0.951**	0.962**	0.313ns	0.972**	0.992**	0.963**	0.981**	0.954**	0.955**	1.000**	0.673**	-0.184ns
G11	0.965**	0.986**	0.318ns	0.990**	0.992**	0.974**	0.977**	0.959**	0.968**	0.989**	1.000**	0.172ns
G12	0.966**	0.971**	0.354ns	0.978**	0.990**	0.973**	0.974**	0.977**	0.971**	0.990**	0.988**	1.000**

Population numbers and assigned codes, karyotype formulae, total length (TL) and grand means of the chromosomes, DRL, CV%, S/L ratio and TF% for all of the populations are presented in Table 3. All populations possessed 21 metacentric chromosomes. Based on the estimated statistics, the populations showed different patterns of asymmetry. The population G3 that showed a weak correlation with other populations showed one of the most asymmetric karyotypes based on DRL and S/L. It had the second longest grand mean of the chromosomes. The larger the DRL value the more differences exist between the shortest and longest chromosomes. Smaller S/L value also implies the same. This would indicate that a great deal of structural changes has occured on the chromosomes of the population.

In principle components analysis due to high correlation between some characters such as S/L and L/S, four components were produced. The first two components contained more than 99 percent of the variations. Total length, long arm and short arm length, had the most important influence on the first component (Table 4), which in turn contained more than 90% of the existing variation of the data. Using the first two important components, scatter plot of the populations were produced (Fig. 1). Several distinct groups of populations could be classified based on the scatter plot. For instance population number 3 may form a separate distinct group that conformed the results of other analysis.

Although the cophenetic values in different methods of cluster analysis were less than 0.7, but similar grouping indicated distinctness of clusters. Cluster analysis showed the populations with the most genetic distance (Fig. 2 and 3) which may lead us not to use populations number 1 and 12 in breeding programs as breeding parents. This is due to the possibility of infertility in their progenies. A cluster analysis based on morphological characters is recommended. In that case the combinations of populations with the most genetic distant would produce more genetic variation. The best result would be gained when the combinations of populations have the most distance based on the clustering for morphological characters but the least distance based on clustering for mitotic characters.

Table 3. Statistics estimated on mitotic characteristics. TF% = Total Form Percentage. DRL = Difference between the maximum and minimum relative length of chromosomes. TL = Total length of haploid set of chromosomes. CV% = Coefficient of variation. S/L = The ratio of the shortest to longest chromosome of the karyotype.

Population	Code	TF%	DRL	MEAN	TL	CV%	S/L	Karyotype
G1	92	44.0	3.0	4.76	100	19.4	0.50	21m
G2	400	42.5	2.7	4.95	104	15.3	0.54	21m
G3	1302	45.7	3.5	5.24	110	14.8	0.49	21m
G4	677	43.8	3.2	5.19	109	18.7	0.48	21m
G5	123	43.6	2.6	5.19	109	15.8	0.55	21m
G6	205	45.4	2.6	4.48	94	15.6	0.58	21m
G7	525	43.5	2.4	5.10	107	14.8	0.57	21m
G8	754	43.0	2.9	4.95	104	19.7	0.52	21m
G9	23	44.2	3.0	4.76	100	20.5	0.51	21m
G10	490	44.4	2.6	5.19	109	15.8	0.54	21m
G11	73	43.8	2.7	5.19	109	15.5	0.54	21m
G12	777	44.0	2.5	5.48	115	15.8	0.56	21m

Table 4. Latent vectors in descending order

Characters	PRIN 1	PRIN 2	PRIN 3	PRIN 4
Total length	0.813	0.064	-0.006	-0.051
Long arm (L)	0.469	-0.522	-0.115	0.414
Short arm (S)	0.344	0.585	0.106	-0.428
L/S	0.025	-0.387	0.878	-0.281
S/L	0.002	0.481	0.452	0.751

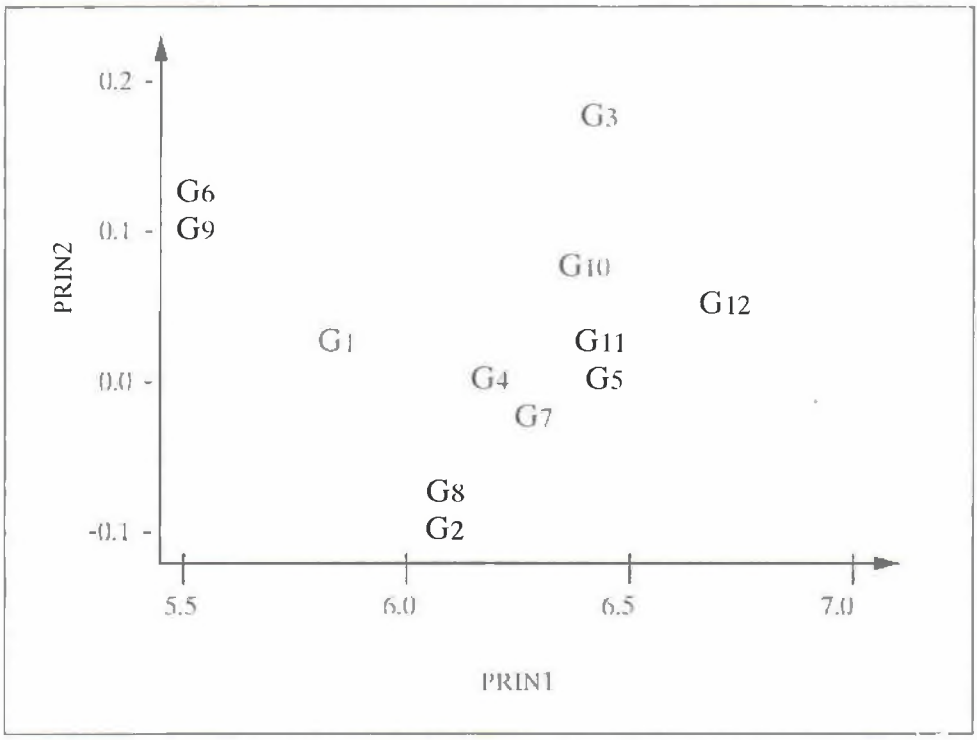

Fig. 1. Ordination of *Bromus* populations on first two principle components.

References

Forni-Martins, E. R., Franchi-Tanibata, M. & Cardelli-de-Lucena, A. 1994: Karyotypes of species of Sesbania Scop. - Cytologia, 59: 13-18.

Hill, H. D. 1965: Karyology of species of Bromus, Festuca and Arrhenatherum (Gramineae). - Bull. Torrey, Bot. Club, 92: 192-197.

Mirzaie-Nodoushan, H. & Fayazi M. A., 1998: Karyological studies in sainfoin (Onobrychis sativa) populations. -The Nucleus, 41: 142-144.

Naganowaska, B. 1993: Karyotypes of five Bromus species of Genea section. -Genetica Polonica, 34: 197-213.

Romesburg, H. C. 1984: Cluster Analysis for Researchers. - Lifetime Learning Publications, California. 335 p.

Sheidai, M., Ahmadian, P. & Poorseyedy, S.

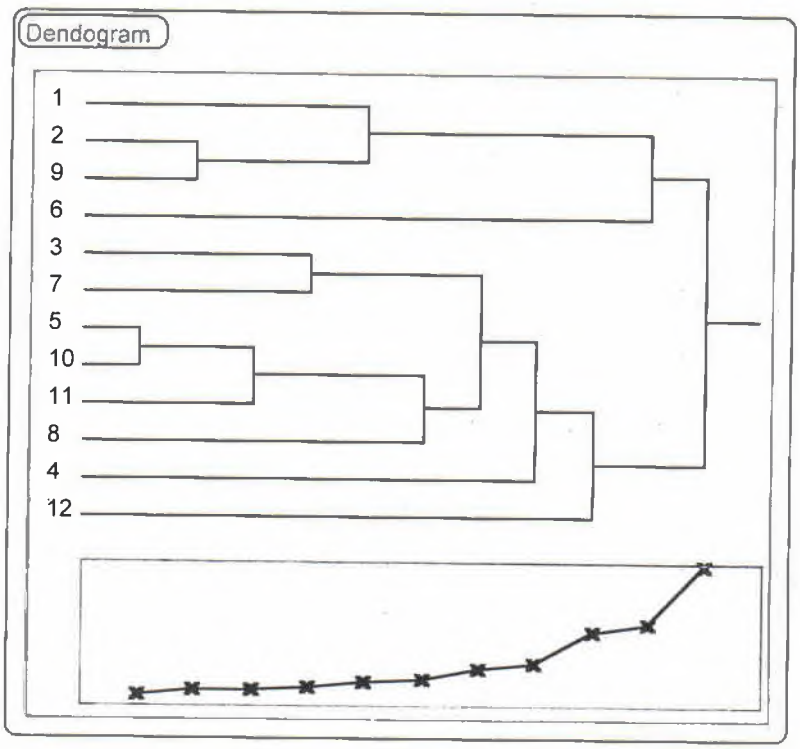

Fig. 2. UPGMA cluster analysis of *Bromus* populations based on the total length of the chromosomes. The plot beneath the dendogram has a point for each cluster. The distance and curvature between the points represents the distance between clusters.

1996: Cytological studies in Iran Zira from three Genus (genera): Bunium, Carum and Cuminum. -Cytologia, 61: 19-25.

Sheidai, M., Vafaietabar, M., Mirzaie-Nodoushan, H., Hosseininejad, Z. 1998: Cytogenetical studies in Gossypium hirsutum L., cultivars and hybrids. -Cytologia, 63: 41-48.

Yang, G., Dunn, G.M. 1997: Mitotic instabilities in tetraploid, hexaploid and octaploid Bromus inermis. -Can. J. Genet. Cytol., 19: 550-553.

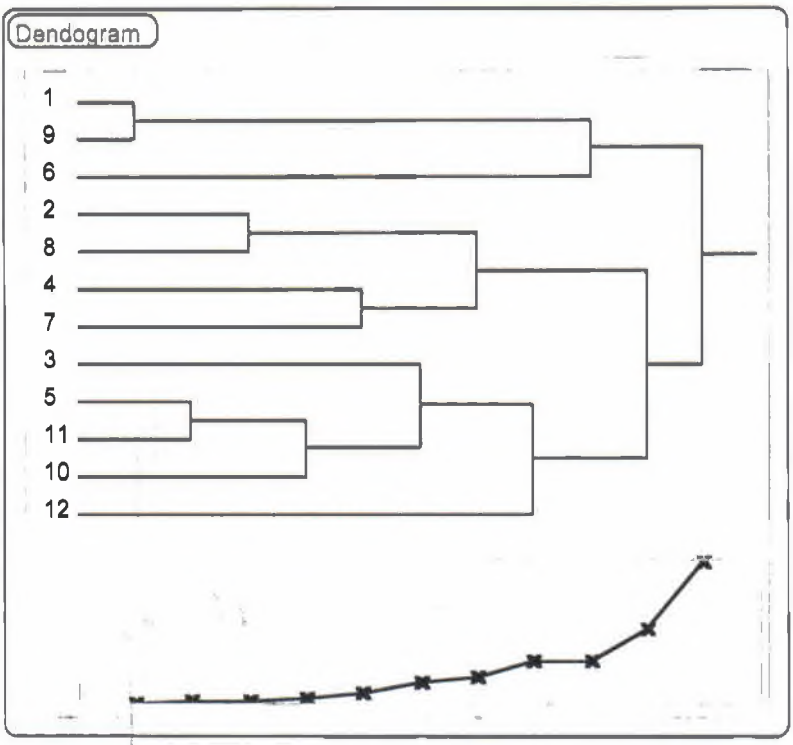

Fig. 3. UPGMA cluster analysis of *Bromus* populations based on all recorded mitotic characteristics. The plot beneath the dendogram has a point for each cluster. The distance and curvature between the points represents the distance between clusters.