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Karyological study on *Dracocephalum* (Lamiaceae) genus in Iran

Mahdiyeh Salehi[1], Seyed Mohsen Hesamzadeh Hejazi*[2]

[1]*Department of Horticulture, Islamic Azad University, Karaj Branch, Iran*

[2]*Department of Biotechnology, Research Institute of Forests and Rangelands, Tehran, Iran*

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Abstract

A karyological study of two taxa (11 populations) of the genus *Dracocephalum* L. from different geographic origins is presented. We found the two usual basic chromosome numbers in the genus. Basic chromosome numbers in all of the populations of *Dracocephalum moldavica* L. were x= 5 (2n=2x=10) and in *Deracocephalum kotschyi* Boiss. were x= 10 (2n=2x=20). Chromosomes in all of populations of *D. moldavica* and *D. kotschyi* were located in 1A and 1B classes respectively. The chromosome lengths were determined between 1.88-2.16µm in *D. moldavica* and 1.37-1.63µm in *D. kotschyi*. Detailed karyotype analysis allows us to group the different populations and to postulate relationships among them.

***Corresponding Author:** Seyed Mohsen Hesamzadeh Hejazi ✉ smhessamzadeh@rifr-ac.ir

Introduction

Lamiaceae family has more than 7000 species and about 282 genera and one of the largest families of plant which has a cosmopolitan distribution (Raymond *et al*., 2004). *Dracocephalum* is a genus of about 186 species (IPNI : International Plants Name Index) of flowering plants in the family Lamiaceae, native to temperate regions of the Northern Hemisphere (Nixon, 2006). They are annual or perennial herbaceous plants or sub shrubs, growing to 15 to 90 centimeters tall. In the flora of Iran, *Dracocephalum* is represented by eight species, which are mainly distributed in the northern and central parts of the country, belonging to the Irano-Turanian phytogeographical region (Rechinger, 1982).

With the exception of the widespread endemic species *D. kotschyi* and the cultivated one *D. moldavica,* the rest of the species (namely *D. polychaetum, D. surmandinum, D. multicaule, D. subcapitatum* and *D. aucheri*) exhibit more or less highly restricted distributional patterns in Iran.

D. kotschyi Boiss, is an endemic herbaceous plant and is known as Badrandjboie-Dennaie and Zarrin-Giah (Ghahreman, 1987; Fattahi *et al*., 2011). Aerial parts of *D. kotschyi* plants are sources of valuable flavonoids and essential oils (Sajjadi *et al*., 1998; Gohari *et al*., 2003; Monsef-Esfahani *et al*., 2007; Saeidnia *et al*., 2007) and its seeds are rich in linolenic, oleic and linoleic acids (Goli *et al*., 2013).
Recently, much attention has been paid to the *Dracocephalum* genus and its chemical constituents because of their diverse activities, such as anticancer, antioxidant, antihypoxic, and immunomodulatory activities (Zeng *et al*., 2010). Medicinal properties and a large variety of specimens in the species increase the importance of diversity studies in this genus.

Although the available literature dealing with plant systematic, biochemical and cytogenetic of *D. moldavica* species and also plant systematic and biochemical available papers of *D. kotschyi* species indicates the importance of these taxa (Ma *et al*.,1984; Yaghmai and Tafazzoli, 1988; Zhang, 1994; Yan *et al*., 2000; Javidnia *et al*., 2005; Semnani and Saeedi, 2005) however , no report to date is available on detailed karyotype of *D. moldavica* populations and endemic species (*D. kotschyi*) from Iran.

The chromosome count and karyotype studies are not only useful in predicting morphological similarity and diversity among *Dracocephalum* species, but also they are valuable sources of taxonomic and biosystematics information. Therefore, the present study consider a mitosis analysis of 11 populations of two *Dracocephalum* species and try to reveal the chromosome numbers and basic cytogenetic information's of these species for the first time.

Materials and methods

Plant materials

The materials used in this study were collected in different areas of Iran. The localities, gene bank codes and species names are shown in Table 1. Vouchers are deposited in gene bank RIFR (Research Institute of Forest and Rangelands from Iran).

Chromosomal studies and data analysis

Root tip meristems obtained from seedlings were pretreated with 0.5% saturated α-Bromo naphthalene at 4 ^{o}C for 4h , fixed in 40% formaldehyde and 1% chromic acid (1:1) for at least 16 h at room temperature, then root tips were rinsed for 3 h in distilled water. Hydrolysis was carried out with 1N NaOH at 60 ^{o}C for 15 min, dyed with Aceto- Iron-hematoxylin for 3-4 h and squashed in a droplet of 45 % acetic acid and lactic acid (10:1). The preparations were observed with an optical microscope (BH_2 Olympus supplemented digital color video camera) at a magnification of 1908x.

The best metaphysical plates were selected and measured by Micro measure 3.3 software (Reeves and Tear, 2000).

In each mitotic metaphase (at least 5 plates) the arm's length of each chromosome was measured and all parameters were estimated in each metaphase plate to characterize the karyotypes, according to the previous studies (Hesamzadeh Hejazi and Rasuli, 2006; Hesamzadeh Hejazi and Ziaei Nasab, 2009, 2010; Javadi *et al.*, 2009; Hesamzadeh Hejazi, 2011;).

Both indices A_1 and A_2 in (Romero Zarco, 1986) formula were independent to chromosome number and size. 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 *et al.* (1964). For each species, karyograms and haploid idiograms were drawn based on length of chromosome size (arranged large to small). In order to determine the variation between species, 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 karytypic parameter to the ordination of populations. Clustering was performed using the single linkage method after calculation of Cophenetic correlation coefficient (*r*) to examine karyotype similarity among populations. Numerical analyses were performed using SAS ver. 6.12, 1996; JMP ver. 3.1.2, 1995; and StatistiXL ver 1.7, 2007 software's.

Results

With relation to the plant materials assayed, Karyotype analyses of two species (11 populations) of *Dracocephalum* were determined. Somatic cells of investigated species had 2n=2x=10 and 2n=2x=20 chromosomes for *D. moldavica* and *D. kotschyi* respectively.

Metaphase plates, ideogram and karyogram of somatic chromosomes are illustrated in Fig.1. Average of total chromosomal length of investigated species ranged from 1.37 to 2.16 µm.

Table 1. Localities of species used in the study.

Species	Gene bank code (RIFR)	Locality
Dracocephalum moldavica	909	Karaj
D. moldavica	1089	Karaj
D. moldavica	1613	Hamedan
D. moldavica	3429	Karaj
D. moldavica	14336	Hamedan
Dracocephalum kotschyi	798	Qazvin
D. kotschyi	18173	Isfahan
D. kotschyi	180	Chalus
D. kotschyi	12938	Qazvin
D. kotschyi	29652	Qazvin
D. kotschyi	H2200	Isfahan-Samirom

The somatic chromosome numbers (2*n*), karyotype formulae and parameters for the studied populations are summarized in Table 2.

The mean value of chromosome's long arm was varied from 0.802 µm in *D. kotschyi* (29652) to 1.246 µm in *D. moldavica* (3429). Averages of chromosome's short arm were different from 0.569 µm in *D. kotschyi* (29652) to 0.914 µm in *D. moldavica* (3429).

The mean value of chromosome's total length was varied from1.371 µm in *D. kotschyi* (29652) to 2.159 µm in *D. moldavica* (3429) and finally the mean value of chromosome's arm ratio was changing from 1.253 in *D. moldavica* (909) to 1.558 in *D. kotschyi* (180) (Tab.2). All chromosomes were metacentric (m) in populations of *Dracocephalum moldavica* and metacentric (m) or sub-metacentric (sm) in populations of *Dracocephalum kotschyi* (Table 2).

Table 2. Karyotype characteristics of 11 populations of *Dracocephalum*.

Population	2n	TL	LA	SA	AR	CI	%LA	%SA	%TF	DRL	A1	A2	DI	SC	K.F.
D. moldavica (909)	10	2.044	1.142	0.902	1.253	0.445	11.173	8.827	44.030	12.457	0.195	0.232	10.775	1A	5m
D. M 1089	10	2.029	1.171	0.858	1.372	0.422	11.547	8.453	42.266	11.580	0.270	0.239	9.794	1A	5m
D. M 1613	10	1.915	1.072	0.843	1.279	0.439	11.195	8.805	44.024	12.412	0.216	0.241	10.630	1A	5m
D. M 3429	10	2.159	1.246	0.914	1.361	0.425	11.537	8.463	42.314	10.933	0.258	0.210	8.252	1A	5m
D. M 14336	10	1.884	1.054	0.829	1.270	0.441	11.194	8.806	44.031	12.095	0.212	0.230	10.124	1A	5m
D. kotschyi(798)	20	1.634	0.940	0.694	1.350	0.427	5.754	4.246	42.458	10.180	0.251	0.309	13.580	1B	10m
D. K 18173	20	1.463	0.867	0.597	1.462	0.407	5.923	4.077	40.767	13.748	0.339	0.425	17.194	1B	10m
D. K 180	20	1.507	0.925	0.582	1.558	0.395	6.138	3.862	38.621	9.819	0.340	0.316	12.825	1B	6m+4sm
D. K 12938	20	1.509	0.892	0.617	1.455	0.408	5.909	4.091	40.911	9.862	0.308	0.319	13.562	1B	10m
D. K 29652	20	1.371	0.802	0.569	1.393	0.420	5.852	4.148	41.476	9.871	0.273	0.315	13.813	1B	9m+1sm
D. K H2200	20	1.424	0.825	0.599	1.389	0.421	5.795	4.205	42.046	10.552	0.270	0.332	14.874	1B	9m+1sm

Somatic chromosome number (2n), ploidy levels, ranges of chromosome length, total length (TL), Long arm (LA), Short arm (SA), Arm ratio (AR), Centromeric index (CI), Long arm percentage (%LA), Short arm percentage (%SA), total form percentage (TF%), difference of range relative length (DRL), asymmetry indexes (A1, A2) of Romero Zarco, dispersion index (DI), symmetry classes (SC) of Stebbins, karyotype formula (K.F.) (m: metacentric) (sm: submetacentric).

The dispersion index is calculated as the proportionate measure of centromeric gradient to the coefficient of variation for chromosome length. The highest value of DI was found in *D. kotschyi* (18173) (17.194) and the lowest value of DI was found in *D. moldavica* (3429) (8.252) species. Symmetry type of (Stebbins, 1971) and asymmetry indices of (Romero-Zarco, 1986) are given in (Table 2). In terms of the Stebbins' system, the karyotype of populations seizes 1A and 1B classes, which are considered majorly primitive classes in this system. All of populations of *Dracocephalum moldavica* are classified as 1A group and all of populations of *Dracocephalum kotschyi* are stand as 1B category (Table 2).

Romero's intrachromosomal asymmetry index (A_1) expresses the arm ratio of each pair of homologous chromosomes.

Table 3. The results of analysis of variance for karyotypic data based on unbalanced CRD design.

Source of variation	Degrees of freedom	TL	LA	SA	AR	CI	LA%	SA%	TF%	DRL	A_1	A_2	DI
populations	10	0.14**	0.07**	0.09**	0.03*	0.002*	0.75**	1.01**	0.013*	0.07*	0.014*	0.02**	0.27**
Error	62	0.02	0.014	0.006	0.013	0.001	0.003	0.005	0.005	0.04	0.007	0.002	0.046
Cv%		9.76	13.96	12.09	10.16	6.44	1.859	2.88	1.63	6.51	20.92	18.63	6.8

*-significant at 5% level of probability, **- significant at 1% level of probability.

The interchromosomal asymmetry index (A_2) corresponds to Pearson's coefficient of dispersion and gives an idea of the asymmetry caused by the different length of the chromosomes. By using the Romero-Zarco asymmetry indices of A_1 and A_2 we can determine the more asymmetric karyotype among the populations which have the similar Stebbins classes of symmetry. In the populations with 1A class, *D. moldavica* (1089) possesses the highest A_1 value (0.27) and the lowest TF% value (42.26), therefore has a more asymmetric karyotype. Similarly in the populations with 1B symmetry class, *D. kotschyi* (180) possessed the highest value for A_1 value (0.34) and the lowest TF% value (38.62) has the highest asymmetric karyotype. The highest VRC (value of relative chromatin) or TL, amongst all populations was obtained for *D. moldavica* (3429) and the lowest was obtained for *D. kotschyi* (29652) (Table 2).

In general, based on intrachromosomal asymmetry (A_1 and %TF), *D. kotschyi* (180) had the most asymmetrical and evolutionary karyo type and *D. moldavica(14336)* , *D. moldavica* (909) had the most symmetrical karyotype in all of the populations. According to interchromosomal asymmetry (A_2 and DRL), *D. kotschyi* (18173) had the most asymmetrical karyotype in all of the populations (Table 2).

Asymmetry index %TF ranged from 38.621 to 44.031 and the intrachromosomal asymmetry index (A_1) varied from 0.212 to 0.340, while the interchromosomal asymmetry index (A_2) ranged from 0.210 to 0.425. (Table 2). A statistical comparison based on unbalanced completely randomized design demonstrates that there are significant differences between the populations based on all karyotypic characteristics among the species for all the measured traits with ($p<0.01$) and ($p<0.05$).

Table 4. Mean of chromosomes analysis of *Dracocephalum* populations.

population	TL	LA	SA	AR	CI	%LA	%SA	%TF	DRL	A_1	A_2	DI
D. M 3429	2.15a*	1.24a	0.91a	1.36ab	0.42ab	11.54a	8.46a	42.31ab	10.9ab	0.258abc	0.21d	8.25d
D. M 909	2.04ab	1.14abc	0.90a	1.25b	0.44a	11.17a	8.83a	44.03a	12.46ab	0.195c	0.23cd	10.78bcd
D. M 1089	2.03ab	1.17ab	0.86a	1.37ab	0.42ab	11.55a	8.45a	42.27ab	11.58ab	0.27ab	0.24bcd	9.79cd
D. M 1613	1.92abc	1.07abcd	0.84ab	1.28b	0.44a	11.19a	8.81a	44.02a	12.41ab	0.22bc	0.24bcd	10.63bcd
D. M 14336	1.88abcd	1.05abcd	0.83ab	1.27b	0.44a	11.19a	8.81a	44.03a	12.09ab	0.21bc	0.23cd	10.12cd
D. K 798	1.63bcde	0.94bcd	0.69bc	1.35b	0.43ab	5.75b	4.25b	42.46a	10.18b	0.25abc	0.31bc	13.58abc
D. K 180	1.507cde	0.93bcd	0.58c	1.56a	0.39b	6.14b	3.86b	38.62b	9.82b	0.34a	0.32bc	12.83abc
D. K 12938	1.51de	0.89bcd	0.62c	1.45ab	0.41ab	5.91b	4.09b	40.91ab	9.86b	0.308abc	0.32bc	13.56abc
D. K 18173	1.43e	0.86cd	0.59c	1.46ab	0.407ab	5.92b	4.07b	40.76ab	13.74a	0.339abc	0.425a	17.19a
D. K H2200	1.42e	0.825d	0.599c	1.389ab	0.42ab	5.79b	4.21b	42.05ab	10.55ab	0.27abc	0.33bc	14.87abc
D. K 29652	1.37e	0.80d	0.569c	1.39ab	0.42ab	5.85b	4.15b	41.48ab	9.87b	0.273abc	0.315bc	13.81abc

TL: total length, LA: long arm, SA: short arm, AR: arm ratio, CI: centromeric index, LA%: relative length of long arm, SA%: relative length of short arm, TF%: Total Form percentage, DRL: Difference of Relative Length, A_1: intrachromosome asymmetry index, A_2: interchromosome asymmetry index, DI: Dispersion Index.* indicated Mean within each column followed by different lowercase letters are significantly different at the 5% level according to the Duncan's test.

This indicated the occurrence of quantitative changes in chromosome size of the studied populations (Table 3). The Duncan's test applied to the chromosome morphometric traits (TL, LA, SA, AR, CI, LA%, SA%, TF%, DRL, A_1, A_2 and DI) showed a highly significant difference among all examined populations (Table 4).

The principal component analysis (PCA), of the karyotypic parameter shows that the first two principal components account for % 94.70 of total variance. component one (% 80) put emphasized on the TL ،SA، %LA ،%SA ، A2 و DI which had the highest coefficients of eigen vectors, while component two(% 14.69) accentuates LA ، AR ، CI ، %TF و A_1 index (Table 5).

Table 5. Eigenvectors from the first two Principal components for 11karyotype parameters to classify 11populations of *Dracocephalum*.

Parameters	First component	Second component
TL	0.30458	0.22294
LA	0.28753	0.32293
SA	0.31567	0.11382
AR	-0.2638	0.40678
CI	0.27609	-0.38417
%LA	0.30734	0.15541
%SA	0.31325	0.06756
%TF	0.26846	-0.39899
A1	-0.22507	0.47133
A2	-0.29698	-0.09343
DI	-0.28777	-0.22697
Eigen Value	9.6009	1.764
Percentage of Variance	80.0076	14.6997
Cum Percentage of variance	80.0076	94.7073

Grouping of the populations are studied based on their relative karyotypic as well as mitotic characteristics (Table 4, Fig. 3). By cutting dendrogram resulted from cluster analysis single linkage methods with Cophenetic correlation coefficient (r=0.86) in metric distance 2.36, the populations classified under four groups.

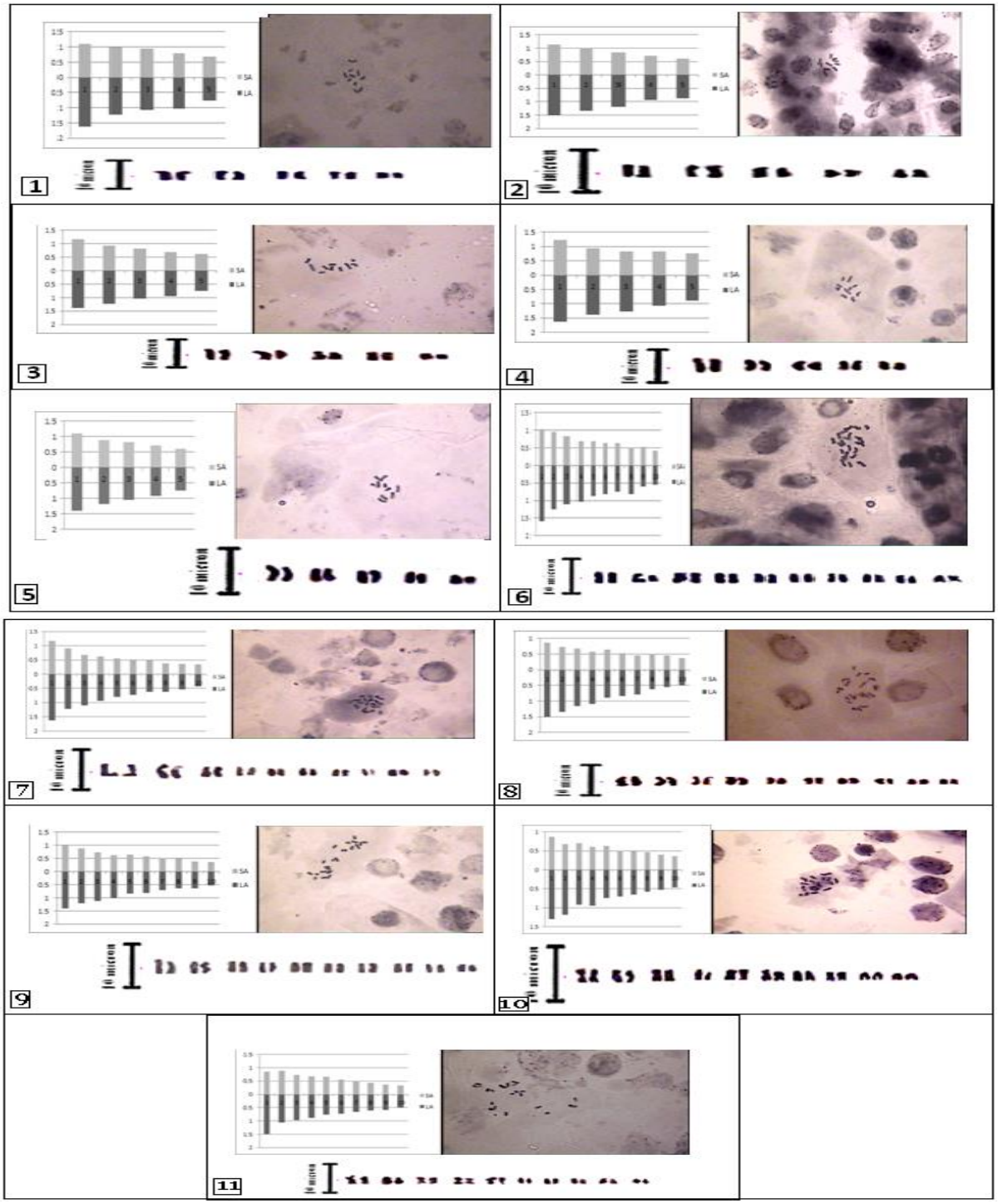

Fig. 1. Metaphase plates, idiogram and karyogram of somatic chromosomes of (1) *D. moldavica* (909), (2) *D. moldavica* (1089), (3) *D. moldavica* (1613), (4) *D. moldavica* (3429), (5) *D. moldavica* (14336), (6) *D. kotschyi* (798), (7) *D. kotschyi* (18173), (8) *D. kotschyi* (180), (9) *D. kotschyi* (12938), (10) *D. kotschyi* (29652), (11) *D. kotschyi* (H 2200), respectively. Bar=10µm.

The highest metric distance was obtained between *D. moldavica* (909) and *D. kotschyi* (4.039) and the lowest metric distance was obtained between *D. moldavica* (1613) and *D. moldavica* (14336)(Fig. 3).

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

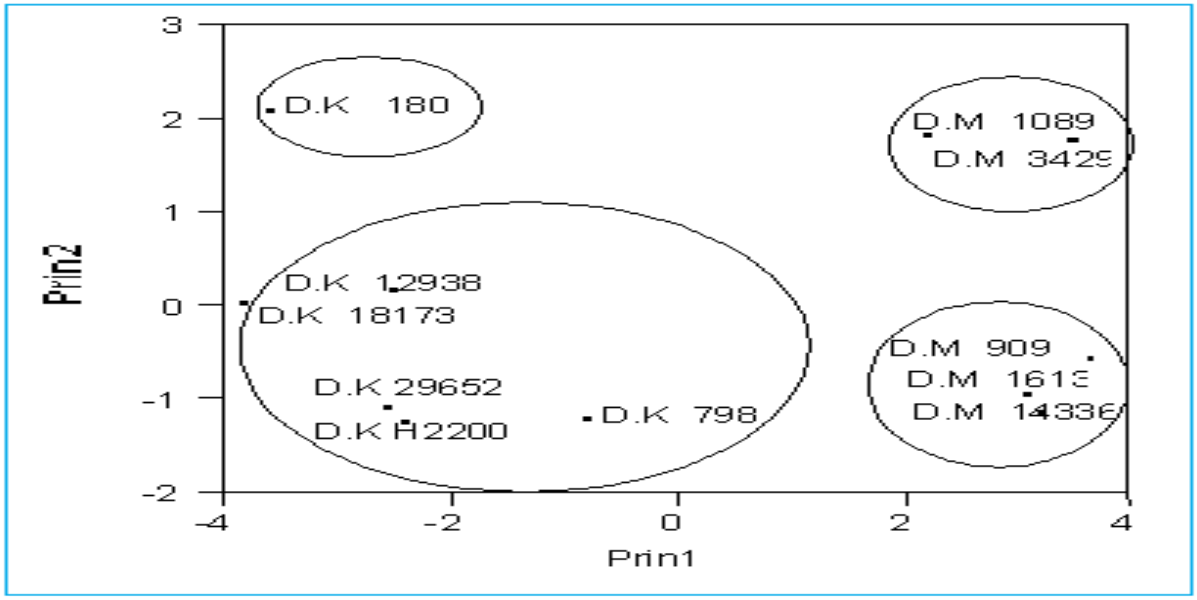

Fig. 2. Scatter plot of 11 populations for the first two principal components.

Discussion

In this study, chromosome numbers and detailed measurements of 11 populations of two species of *Dracocephalum* genus were determined for the first time in Iran. The karyotype concept has been extensively used in characterizing and distinguishing chromosomes of different species. Mitotic karyotype analyses are also helpful in studying evolutionary problems (Gottschalk, 1972). The results showed only minor differences in gross morphology of the karyotypes of *Dracocephalum moldavica* and *Dracocephalum kotschyi* species. In order to refine the measure of karyotype asymmetry, we used Dispersion Index (DI) that has the potential to decipher even the minor karyotypic variations. The DI index plays an important role in arranging the species within the same class of karyotype asymmetry in an advancing order of specialization by permitting further gradations, as depicted by species arrangement within sections. Higher values of DI index would mean an enhanced order of karyotypic specialization (Table 2).

The results of this study reveal a detailed picture of the chromosome features in *Dracocephalum* species. While the DNA sequence can provide valuable data, the knowledge of chromosome numbers, karyotype evolution, ploidy level and genome size can provide additional information that not only gives further insight in to the functioning of the genome, but also have considerable predictive powers. In this study, the basic chromosome numbers were x=5 and x=10 for diploid populations. Analysis of karyotype formulae showed that, generally all of the populations of *Dracocephalum moldavica* and *Dracocephalum kotschyi* had metacentric (m) type chromosomes except of a few chromosomes in some populations of *Dracocephalum kotschyi* which had the sub-metacentric (sm) type. As a result, the species also could be differentiated by the number and type of chromosomes. Karyotype asymmetry, applied in the comparative analysis of *Dracocephalum* species, was used for species discrimination. The ratio of long arm/short arm chromosomes (AR) showed a significant difference ($P<0.05$) between species.

Generally it seems the variation in the size of chromosomes depends on the basic chromosomal number in species. Cluster analysis based on cytological data showed the populations with the lowest metric distance may lead us to use populations in crosses for inducing the highest genetic variations (Fig. 3).

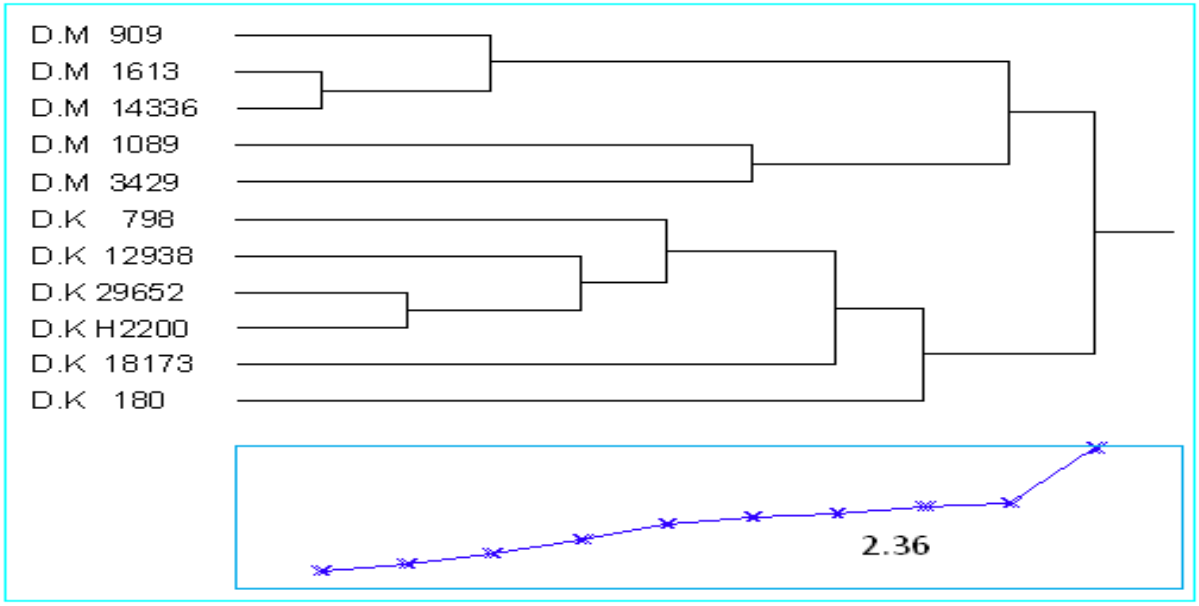

Fig. 3. Dendrogram of 11 populations of *Dracocephalum* by analyzing 11 karyotypic parameters using single linkage cluster analysis method. Cophenetic correlation r=0.86.

Grouping based on karyotypic data indicated *D. kotschyi* (180) stands on distinct group. *D. kotschyi* (180) is significantly different from the other populations through the difference of traits such as AR and A_1, so that is separately classified as an other group.

The present study shows the change in the chromosomal traits as one of the mechanism of inter and intraspecies diversification in the *Dracocephalum* genus as well as the earlier cytological reports (Ma *et al*., 1984; Zhang, 1994; Yan *et al*., 2000).

The differences in karyotype formulae and asymmetric indices found among the species suggest that structural changes of chromosomes may contribute to the diversification of the genus. These genomic differences could be used for breeding purposes. In general, cytological studies of the *Dracocephalum* species growing in Iran indicate the importance of basic chromosome numbers, 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.

Ploidy manipulation especially on endemic species *D. kotschyi* must be considered as an efficient method to increase production potential of medicinally important compounds.

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