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Cytological and Karyotypic Analyses in Some Populations of *Leonurus cardiaca*

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Abstract *Leonurus cardiaca* (Motherwort) is one of the most resistant plants to biotic and abiotic stresses with a number of medicinal properties. In order to investigate the karyotypic variation between six populations of motherwort, this experiment was performed. Root-tip meristems were used for karyotypic evaluation. 8-hydroxy quinoline and ethanol–acetic acid (3:1 V/V) solution were used as pretreatment and fixative solutions, respectively. Chromosomes were stained by 2 % aceto-orcein. Long arm length (L), short arm length (S), total chromosome length (TL), L/S and S/L, overall genome length, the ratio of the longest to the shortest chromosome, and centromeric index were recorded. Result showed that the best root size for chromosome study of this species is 0.50–1.50 cm long and the best time for cutting the roots is about noon time. All the six studied populations were diploid ($2n = 2x = 18$), and metacentric chromosomes were dominant in all population. In some populations, a few submetacentric chromosomes were also observed. Cluster analysis based on karyotype traits revealed four groups of populations using Euclidean distance.

Keywords Karyotypic study · *Leonurus cardiaca* · Diploid · Population

Introduction

The chromosome number and the chromosome morphology are increasingly being used in plant taxonomy. These data are also important to elucidate the origin, speciation, and phylogenetic relationships of plants [2, 11, 12]. Also, chromosomal information is an important key for evolution, genetics, and breeding in plants.

Cytogenetic is the correlated study of genetics and cytology including chromosome structure, function, evolution, and behavior during mitosis and meiosis in individuals, populations, or hybrids. Cytogenetics also deals with aspects of chromosome manipulation for crop

improvement and development of special cytogenetic stocks for genome mapping projects. The standard karyotype descriptions of relative length and arm ratio work well where chromosomes can readily be paired. They were designed for comparisons within species where there is little variation between genotypes. However, these measurements are difficult to be used where chromosomes do not pair or for comparisons between related taxa with different karyotypes. Changes in chromosome morphology reflect evolution, and it would, therefore, be advantageous to have a system whereby karyotypes of related genotypes and species could be compared. Assessment of differences in chromosome morphology within taxa would help elucidate the inheritance of genetic material and the relationships between species [1].

Motherwort, *Leonurus cardiaca*, is one of the many introduced members of the Mint family (Lamiaceae). Iran has developed one of the largest germplasms of this plant in the world [6]. The names of the plant include the words that show connection with the heart. Among other biochemical constituents, it also contains bitter iridoid

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glycosides, diterpenoids, flavonoids (including rutin and quercetin), tannins, volatile oils, and vitamin A. *Leonurus cardiaca* herbs synthesize flavonoids, alkaloids, iridoids, diterpenoids, cardenolids such as glycosides, tannins, and other constituents in lower amounts [8, 9].

Our bibliographical studies showed that there is no prior chromosome counting for *L. cardiaca* species, an endemic species from Iran. Thus, the aim of this research was to contribute to the general knowledge of chromosome number in different Iranian populations of this species that grow in different ecological conditions. We present the first report of the chromosome numbers, ploidy levels, and chromosome characters of *L. cardiaca*. Our results are suitable for a better understanding of its taxonomy and breeding purposes such as intraspecific hybridization and genetic variation induction.

Materials and Methods

Seeds of *L. cardiaca* were collected from six natural regions of Iran (Table 1). Seed germination was carried out on wet Whatman paper in the Petri dish, and then the germinated seeds were placed at room temperature (25 °C) under (14-h darkness and 10-h light) photoperiod.

For chromosome morphology and banding, seedlings were pretreated before fixation with 0.002 M 8-hydroxy-quinoline at 16 °C during 4 h. 0.5- to 1.5-cm-long root was separated at 10:30–11 in the morning and put on pretreatment solution. For observation of mitosis, after performing pretreatment, the root was rinsed in distilled water for 5 min, then dehydrated by Whatman paper, and finally fixed in ethanol–acetic acid (3:1 V/V) for 24 h at 4 °C. In order to produce clear and stable chromosomal samples, staining was carried out with 2 % aceto-orcein that was more selective than aceto-carmum for 24–48 min in room temperature. Preparation of dye solution was done following Ostergren and Hansen's technique [7]. In order to produce dispersed and single cells, fixed roots were rinsed in distilled water, dehydrated by Whatman paper, and then put on 1 N HCL for 10–11 min in room temperature. None of the other antimitotics tested (colchicine,

1-chloronaphthalene, oryzalin, cold water, or their combinations) could improve chromosome spreads. Before banding, hydrolysis was performed with 45 % acetic acid. Squashed preparations were made permanent following Conger and Fairchild [3].

Optical microscope (Axiophot Zeiss Germany) was used to observe metaphase chromosome. Images were captured with a (D450, Canon Eos, Japan) digital camera. For numerical karyotype analysis, the minimum of 10 metaphase cells (repeat) of each population was selected, the images were captured, and then the chromosome number and ploidy level were determined.

For investigating cytogenetic variations, eight metaphase cells of each population were measured for karyotypic traits which consisted of Long arm (L), Short arm (S), total chromosome length (ATL), total chromosome short length (SL), total chromosome long length (LL), and overall genome length (TL). The ratio of the longest to the shortest chromosome (L/S) and Centromeric index (CI) is indicating the ratio of short arm to total chromosome length which was measured by Micro Measure version 3.3.

Ideogram of haploid chromosome of each population was plotted using arm's length and centromere position. The difference of range length of two chromosomal arms was performed according to Levin's method [4]. In this survey, in order to determine evolutionary position, karyotypic symmetry index (A1), and karyotypic symmetry, five parameters were used that were composed of interchromosomal asymmetry index (A2) calculated by formulas

$$A_1 = 1 - \left(\sum q_i/p_i \right) / n$$

$$A_2 = Sd/X,$$

where q_i is the mean length of short arms of each pair of homologous chromosome and p_i is the mean length of long arms of each pair of homologous chromosome.

A new asymmetry index (AI) is proposed to measure karyotype asymmetry, and a new parameter, the CV_{CI} , is offered, which precisely assesses the relative variation in centromere position in a complement. The AI index, the CV_{CI} , and the CV_{CL} ($= A_2 \times 100$) have the potential to display even minor karyotypic variations [10].

Table 1 Locations of studied *L. cardiaca* populations in Iran

No.	Population	Province	Altitude (m)	Longitude (E)	Latitude (N)
1	Kerman	Kerman	2,600	56° 50' 31"	29° 18' 36"
2	Dargaz	South-Khorasan	2,194	58° 42' 1"	37° 34' 42"
3	Taleghan	Alborz	1,850	50° 45' 34"	36° 10' 27"
4	Khansar	Isfahan	2,210	50° 17' 57"	33° 15' 50"
5	Sarab	Ardabil	1,687	47° 31' 40"	37° 55' 59"
6	Sari	Mazandaran	2,170	53° 12' 3"	36° 3' 37"

$$CV_{CL} = A2 \times 100 = \frac{S_{CL}}{X_{CL}} \times 100$$

$$CV_{CI} = \frac{S_{CI}}{X_{CI}} \times 100$$

$$AI = \frac{CV_{CL} \times CV_{CI}}{100}$$

The cluster analysis was constructed based on karyotypic traits using SPSS software according to Euclidean distance.

Results and Discussion

In the selection of ploidy-level studies on motherwort, it was detected that the longest radicle was 1–1.50 cm. Different times of the day effect on cell division were based on numerous reports of best time of sampling variously. Preliminary hours of morning were the best time of cutting radicles and performing pretreatment which was recognized from 12 to 12:30. In this study, staining was carried out by aceto-orcein; the most suitable time of staining was 36–48 h. Microscopic observation and counting the chromosomes of 10 metaphase cells indicated that the basic chromosome number was $x = 9$ and diploid level in collected populations was $2n = 18$ (Fig. 1). Determination of chromosome number in different populations of Iranian motherwort is being reported for the first time.

Ideograms

Ideograms related to each population with graphical representations of arranged chromosomes (big to small) including content of long arm, short arm, and centromeres

showed linear diagram of haploid chromosome figure in a page and is inspired drawing of actual karyotype of these populations. The drawing caused our studies to be more suitable and precise in many sides so that it could recognize some parameters like chromosome number, chromosome size and figure, centromere location, and ratio of chromosomal length and arm (Fig. 2).

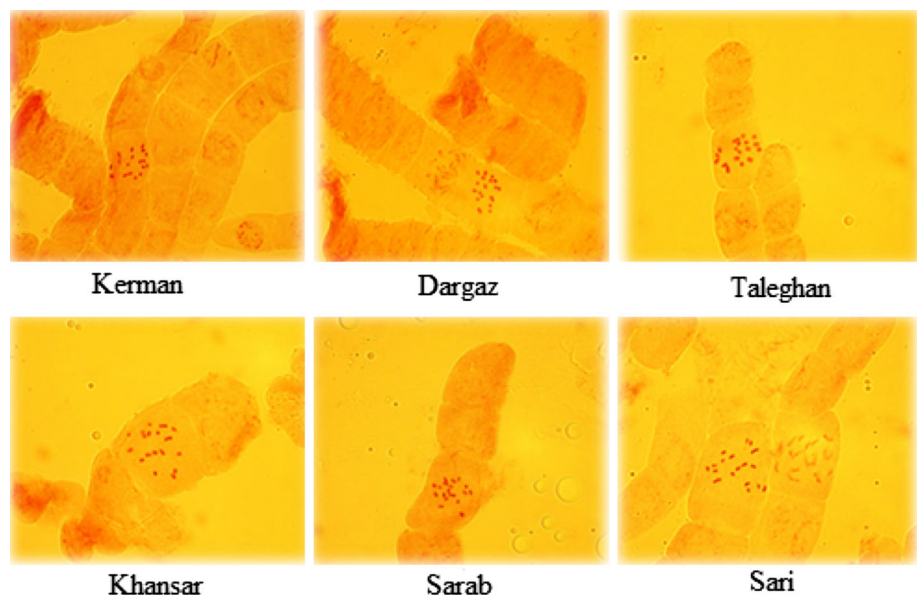
Chromosomal Index

Based on Levin's method [4] and according to the ratio between the long and short arms and differences of two chromosomal arm's lengths, karyotypic formula in populations was determined. The type of chromosome was completely metacentric and submetacentric near to median region, and the ratio of long arm to short arm was in 1.7–3 class.

According to the drawn ideogram, morphologic characters of chromosomes and also metacentric and submetacentric types of chromosomes could result that individuals of this species moved to evolutionary changes in chromosomes and had nearly asymmetrical karyotype.

The result obtained from [5] research on Hellebore's genus in Ranunculaceae family showed that individuals in this genus had metacentric chromosome. The study revealed that the genus has been considered to be primitive whereas evolutionary genera in the family and centromere location could be different. Probably, asymmetric karyotypes have preferences over symmetric karyotypes and show more adaptive capabilities. In general, intrachromosomal asymmetry was expressing variations between arm's lengths of chromosomes in populations; on the other hand, interchromosomal asymmetry was expressing very low variation among the studied populations.

Fig. 1 Metaphase chromosomes of the studied *L. cardiaca* populations



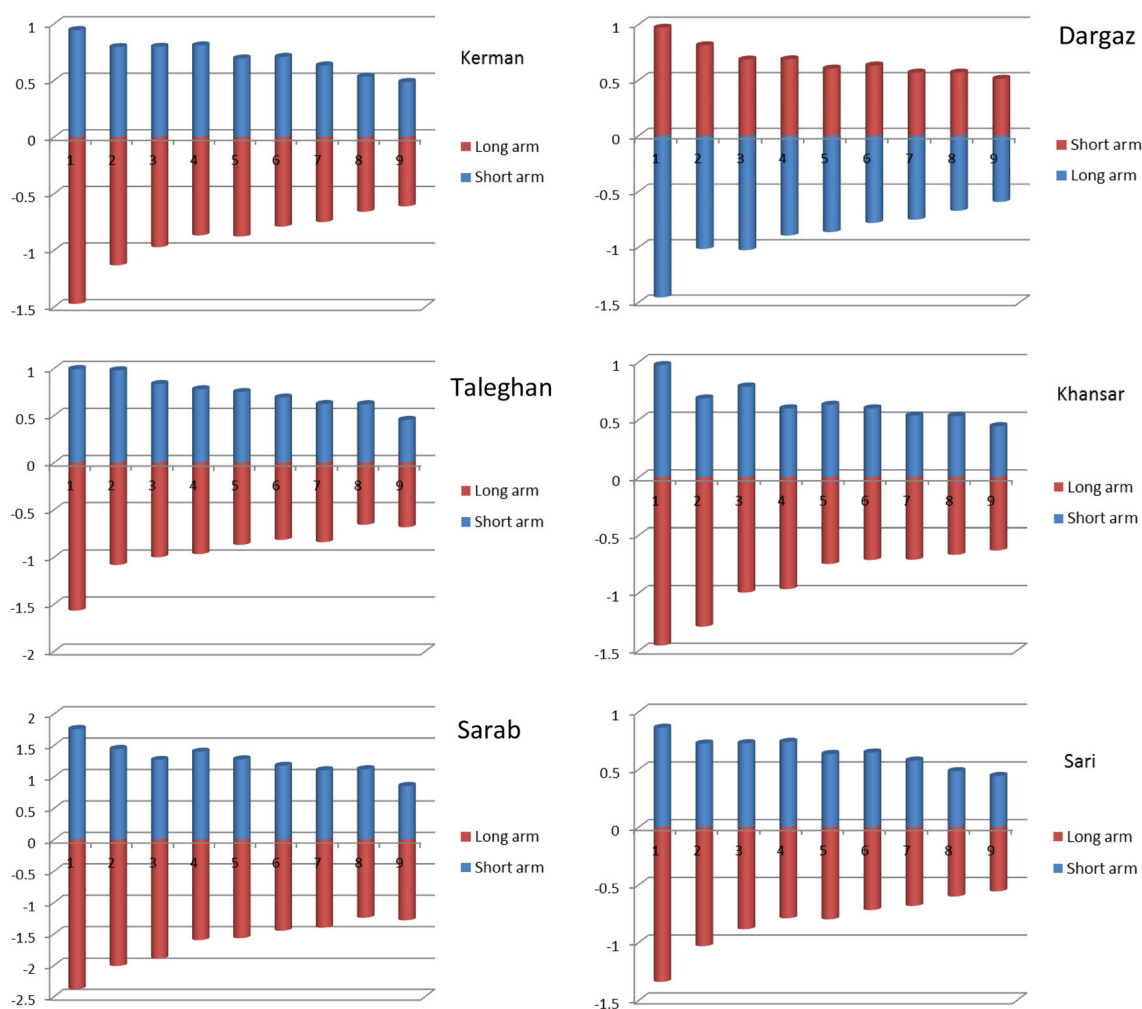


Fig. 2 Ideograms of the studied *L. cardiaca* populations

The highest value of A1 was related to Taleghan population (0.659), and the lowest value of A1 was 0.136 in Sarab population. The highest and the lowest value of A2 was respectively 1.499, 1.231, and 0.129 for Sarab, Dargaz, and Taleghan populations. Hence, Dargaz and Sarab populations had the lowest value of A1 but A2 values were the highest ones. So, Taleghan and Khansar populations were recognized as the most symmetric karyotypes and Dargaz and Sarab were the most asymmetric ones (Table 2).

Coefficient of variation in centromeric index (CV_{CI}) is expressing heterogenic value in centromeric location of chromosomes, and coefficient of variation in chromosome length (CV_{CL}) is expressing heterogenic value of chromosome in length. The result determined that Sarab and Dargaz populations had the highest value of coefficient of variation in centromeric index and chromosomal length whereas Taleghan and Khansar populations had the lowest value in these parameters. According to interchromosomal asymmetry index and interchromosomal asymmetry index and also karyotypic asymmetry parameter, symmetric

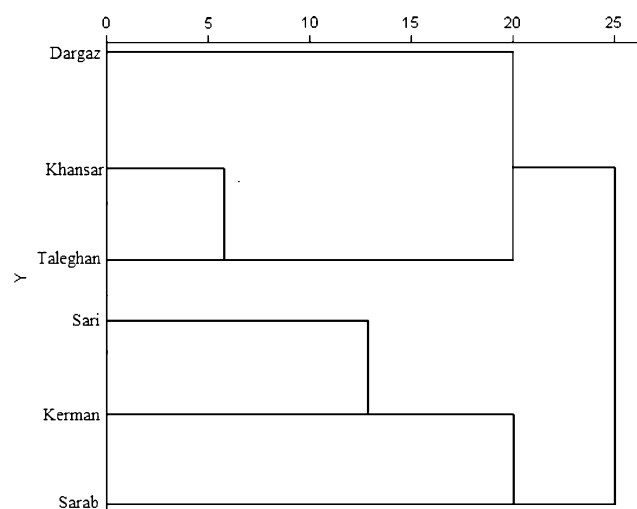
karyotypes were Khansar and Taleghan populations and asymmetric karyotypes were Dargaz and Sarab ones.

In order to determine the role of each chromosomal parameter on variations, the principal component analysis (PCA) was performed on all of the measured traits and classified them into two components that explained 98.06 % of total variations among data so that in the first component, short arm's length, the average of the ratio between long arm and short arm, centromeric index, the mean of chromosome total length, the total value of short arm's length, and genome length possessing the highest vector coefficient had the most important role on variation among population. In the second component, long arm length and total value of long arm's length were the main traits that had significant role in variance among populations.

According to interchromosomal asymmetry index and interchromosomal asymmetry index and also karyotypic asymmetry, the most symmetric karyotypes were Taleghan and Khansar populations. Dargaz and Sarab populations

Table 2 Karyotypic asymmetry measuring parameters in the studied *L. cardiaca* populations

Population	CN (2n)	KF	CI	A1	A ₁	CV _{CI}	CV _{CL}	AI
Kerman	18	16M + 2SM	0.429	0.325	0.468	15.864	0.0381	0.049
Dargaz	18	14M + 4SM	0.435	0.237	1.231	18.519	0.0458	0.079
Taleghan	18	16M + 2SM	0.446	0.659	0.129	12.922	0.0289	0.037
Khansar	18	16M + 2SM	0.426	0.477	0.175	14.551	0.0239	0.042
Sarab	18	14M + 4SM	0.433	0.136	1.499	25.946	0.0499	0.062
Sari	18	16M + 2SM	0.431	0.382	0.521	14.955	0.0322	0.051

**Fig. 3** Cytogenetic classification of the studied *L. cardiaca* populations

were recognized as asymmetric karyotype. Grouping of populations based on measured karyotypic traits was performed according to Ward method.

Dendrogram produced from cluster analysis of karyotypic traits among populations is presented in Fig. 3. Fitting of dendrogram in 21 Euclidean distance divided total populations into two separated groups and with reduction distance from 21 to 12, population classified to 5 subgroups. The first group consisted of Dargaz, Taleghan, and Khansar populations, and the second group is composed of Sari, Kerman, and Sarab populations. The average of studied traits revealed that there was a significant difference between short arm length and total short arm chromosome length among populations that was the reason of separation in two groups.

Karyotypic trait investigations indicated that Sarab population obtained the highest value of total chromosome length and arm length. Separation of Dargaz population from others in coefficient variation of centromeric index

and similarity of Taleghan and Khansar populations in A1 and A2, chromosome length, and arm's length were clearly recognized.

In conclusion, our results showed that the basic chromosome number of *L. cardiaca* is $x = 9$ and one chromosome number, corresponding to ploidy levels in the studied populations of this species (diploid). The results of the present study are important for breeding programs and for understanding the taxonomic position of this species.

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