*J Genet Resour2020;6(1): 34-40* *Homepage: http://sc.journals.umz.ac.ir/*

**RESEARCH ARTICLE** *DOI: 10.22080/jgr.2020.2541*

# The Natural Variation in Six Populations of *Calendula officinalis* L.: A Karyotype Study

Maryam Fallahi[1], Abdollah Mohammadi[2] and Seied Mahdi Miri[1*]

[1] Department of Horticulture, Karaj Branch, Islamic Azad University, Karaj, Iran
[2] Department of Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran

**ARTICLE INFO**

***Article history:***
Received 13 December 2019
Accepted 15 January 2020
Available online 27 January 2020

***Keywords:***
Chromosomeal numbers
Cluster analysis
Genetic diversity
Karyology
Marigold

***Corresponding author:***
✉ S.M. Miri
smmiri@kiau.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

**ABSTRACT**

In the current investigation, karyotype analysis and chromosome characteristics of six populations of *Calendula officinalis* L. (pot marigold) from Iran are studied. Results showed that all populations were diploid (2n= 2x= 32), and had symmetrical karyotypes composing mainly of metacentric and submetacentric chromosomes. The mean chromosome length ranged from 1.05 in Karaj to 1.50 µm in Masjed Soleyman 1. Haploid genome length was in the range of 16.89 to 24.07 µm and mean centromeric index (CI) of complements varied from 0.38 to 0.44, indicating the role of the quantitative genomic changes in the diversification of *C. officinalis* populations. Cluster analysis using chromosomal parameters and based on the UPGMA method classified studied populations into three major groups. In addition, the principal component analysis revealed the first two components account for 99.8% of the total variance. The results of the present study revealed the natural variation in six populations of *C. officinalis*, which can further serve conservation and breeding planning.



Please cite this paper as: Fallahi M, Mohammadi A, Miri SM. 2020. The natural variation in six populations of *Calendula officinalis* L.: A karyotype study. *J Genet Resour* 6(1): 34-40. doi: 10.22080/jgr.2020.2541

## Introduction

Iran is one of the most important and unique countries in terms of topography, climate and geographical conditions in the Middle East (Ebrahimi *et al*., 2018; Jafari *et al*., 2014; Miri and Shamsolshoara, 2019; Roughani and Miri, 2018). The genus *Calendula* related to family Asteraceae (Compositae) includes about fifteen species in the Mediterranean, Irano-Turanian and Saharo-Arabian regions, which nine species including *C. officinalis*, *C. aurantiaca*, *C. tripterocarpa*, *C. arvensis*, *C. karakalensis*, *C. persica*, *C. sancta*, *C. alata*, and *C. palestina* are found in different regions of Iran (Jafari *et al*., 2014). *Calendula officinalis*, commonly known as marigold, is an annual herb growing about 80 cm tall, having corymbosely branched stem; a long taproot with numerous secondary roots; hispid, acute, oblanceolate, alternate and sessile leaves; flower head inflorescence (surrounded by two rows of hairy bracts). The plant has yellow to orange flowers with female ray florets and hermaphrodite, tridentate, tubular, disc florets; and curved, sickle-shaped and ringed achene (Khan *et al*., 2011; Jan *et al*., 2017).

A few species of *Calendula* have commercial value, among these *C. officinalis*, is used for medicinal or culinary as well as ornamental purposes (Baciu *et al*., 2013; Arora *et al*., 2013; Khalid and de Silva, 2012). It has been traditionally used to treat various skin tumors, dermatological lesions, ulcers, swellings and nervous disorders (Arora *et al*., 2013). The plant species has been reported to contain several classes of phytochemicals, including carbohydrates, amino acids, phenolic compounds, lipids, steroids, tocopherols, terpenoids, coumarins, quinones, volatile oils, and carotenoids. The major active constituents of the plant include triterpendiol esters, saponins, and flavonoids including rutin and hyperoside. The orange flower contains a high content of carotenoids including auroxanthin and

flavoxanthin (Honório *et al*., 2016; Jan *et al*., 2017; Khalid and de Silva, 2012; Muley *et al*., 2009; Verma *et al*., 2018).

A large and increasing number of cytological characters, such as the number, morphology, and meiotic behavior of chromosomes, as well as the nuclear DNA content, have been used to circumscribe *Calendula* taxa and infer their relationships, being of pivotal importance to the systematic community, especially for resolving the taxonomy of complex groups (Nora *et al*., 2013). Nevertheless, currently available cytogenetic information on *C. officinalis* is rather scarce (Samatadze *et al*., 2019). The basic chromosome numbers considered for this genus are 7, 8, 9, 11 and 15 (Dalgaard, 1986; Nora *et al*., 2013). The analysis of genome size variation in the *Calendula* homoploid taxa (i.e. taxa with 2n = 32) revealed that *C. officinalis* presented lower genome size than the remaining taxa (Nora *et al*., 2013). The karyotype of *C. officinalis* is composed of metacentric and submetacentric chromosomes that were small in size. This is, probably, why different karyotype formulas and chromosome numbers have been described for this species (Samatadze *et al*., 2019). This research was aimed to determine chromosome numbers, ploidy level and karyotype characteristics of six populations of *C. officinalis* from different geographic regions in Iran.

## Materials and Methods

### Plant materials

Karyotype analysis was performed using seeds of six *Calendula officinalis* populations that were collected from natural vegetation of different regions of Iran by the Research Institute of Forests and Rangelands (Table 1).

### Chromosome preparation

The seeds were soaked in 100 mg/l $GA_3$ for 24 h at 4˚C and germinated on damp filter paper in Petri dishes at 25˚C. Different protocols for pretreatment were tested and the best result was obtained from 8-hydroxyquinoline 0.005 M at 4˚C for 12 h. Sample roots were fixed in Carnoy I (ethanol: glacial acetic acid, 3:1) for 17-24 h at 4˚C, then root tips were rinsed three times with distilled water (5 min each). Hydrolysis was conducted with 5 N HCl at 60˚C for 13 min and was stained with 0.2% (w/v) Aceto-orcein for 5 days at 4˚C, then hand squashed in a droplet of 45% (v/v) acetic acid.

**Table 1.** Geographical information of the investigated *Calendula officinalis* populations.

| Population | Latitude | Longitude | Altitude* |
|---|---|---|---|
| Karaj | N 35° 49′ 57″ | E 50° 59′ 29″ | 1341 |
| Masjed Soleyman1 | N 31° 56′ 11″ | E 49° 18′ 14″ | 240 |
| Masjed Soleyman2 | N 31° 55′ 59″ | E 49° 17′ 60″ | 269 |
| Khuzestan 1 | N 31º 31' 83" | E 48º 67' 06" | 20 |
| Khuzestan 2 | N 31º 31' 90" | E 48º 68' 42" | 16 |
| Khuzestan 3 | N 31º 19' 84" | E 48º 41' 31" | 23 |

*=m

The slides were observed with an optical microscope (CX52 Olympus supplemented Panasonic digital camera (DMC-FX55)). At least ten well-spread metaphase plates from different individuals were selected and the value of the arm's length of each chromosome measured by Micro measure 3.3 software.

### Karyotype analysis

For numerical characterization of karyotypes, total length (TL), long arm (LA), short arm (SA), arm ratio (AR), genome size and centromeric index (CI) were calculated. According to the classification of Levan, chromosome morphology based on centromere position was described (Levan *et al.,* 1964). Idiograms were drawn for each population based on the length of the chromosome using Excel software.

To calculate the variation between populations, one-way ANOVA according to a completely randomized design was performed on normal data by using SPSS software and parameter means were compared by Duncan's multiple range test at $P<0.05$. Karyotypic data were subjected to Pearson's correlation analysis. Principal Component Analysis (PCA) was carried out to evaluate the contribution of each karyotypic parameter to the ordination of species. Cluster analysis was carried out using the UPGMA (Unweight Pair Group Method Arithmetic Mean) method by Minitab statistical package (ver. 16.0).

## Results

### Chromosome number and karyomprphology

Karyotype formula and parameters, as well as mitotic metaphases, idiograms, and karyograms for 6 Iranian local *C. officinalis* populations are presented in table 2 and fig. 1.

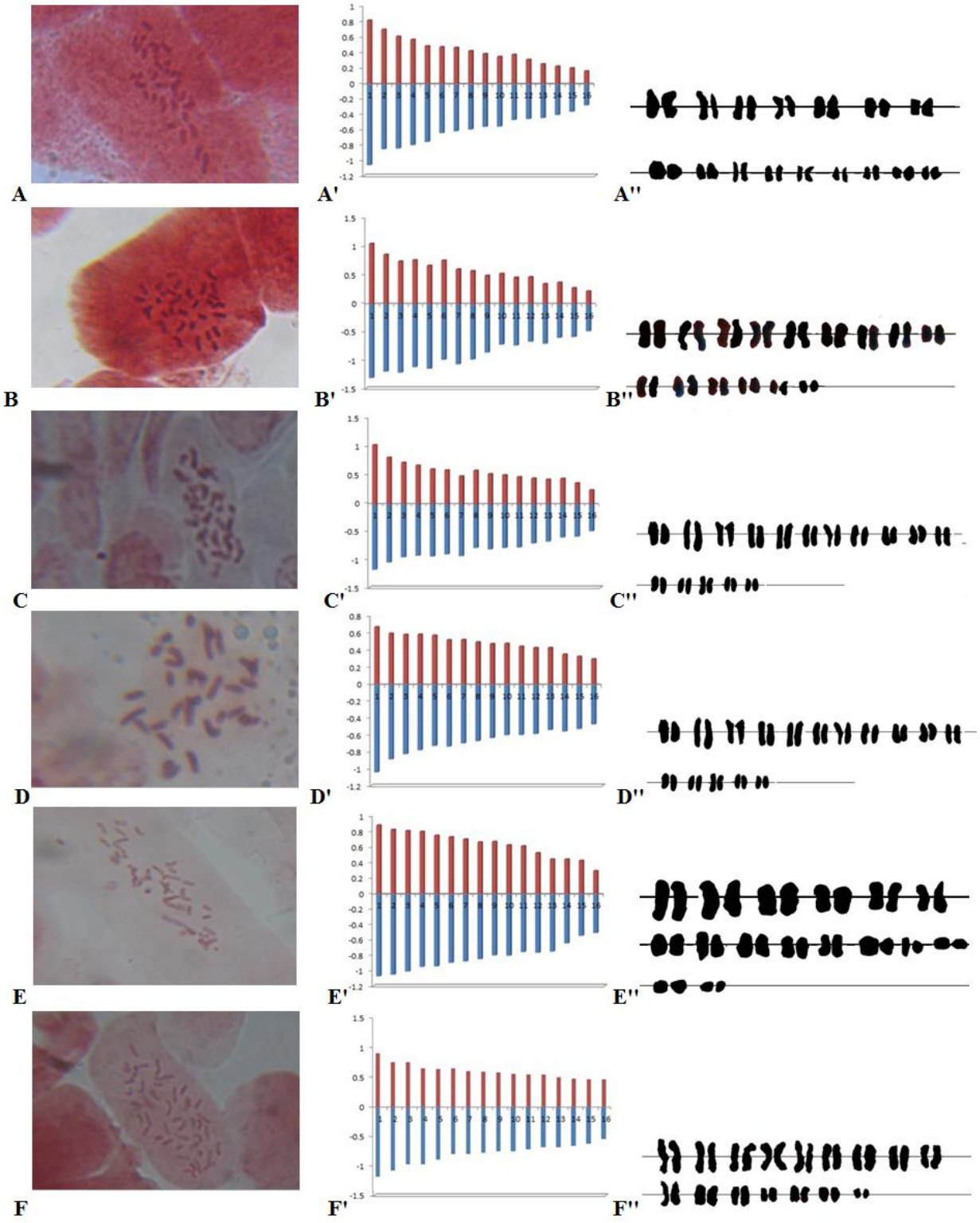


**Fig. 1.** Karyotypes, idiograms and karyograms of 6 populations of *C. officinalis*: A) Karaj; B) Masjed Soleyman 1; C) Masjed Soleyman 2; D) Khuzestan 1; E) Khuzestan 2; F) Khuzestan 3; A') Karaj; B') Masjed Soleyman 1; C') Masjed Soleyman 2; D') Khuzestan 1; E') Khuzestan 2; F') Khuzestan 3; A'') Karaj; B'') Masjed Soleyman 1; C'') Masjed Soleyman 2; D'') Khuzestan 1; E'') Khuzestan 2; F'') Khuzestan 3.

**Table 2.** Average of measured chromosomal traits in the studied populations

| Population | Ploidy | TCL* | CL* | LA* | SA* | AR | CI | KF |
|---|---|---|---|---|---|---|---|---|
| Karaj | 2n=2x=32 | 16.89 d | 1.05 d | 0.61 e | 0.44 e | 1.50 b | 0.41 c | 10m+4sm |
| Masjed Soleyman 1 | 2n=2x=32 | 24.07 a | 1.50 a | 0.91 a | 0.59 b | 1.68 a | 0.39 e | 7m+9sm |
| Masjed Soleyman 2 | 2n=2x=32 | 22.67 b | 1.41 b | 0.83 b | 0.58 c | 1.54 b | 0.40 d | 14m+2sm |
| Khuzestan 1 | 2n=2x=32 | 18.98 c | 1.18 c | 0.68 d | 0.50 d | 1.41 c | 0.42 b | 16m |
| Khuzestan 2 | 2n=2x=32 | 23.83 a | 1.49 a | 0.83 b | 0.66 a | 1.32 d | 0.44 a | 14m+2sm |
| Khuzestan 3 | 2n=2x=32 | 22.61 b | 1.41 b | 0.80 c | 0.60 b | 1.35 d | 0.43 a | 16m |

*=µm; TCL= total chromosome length; CL= chromosome length; LA= long arm; SA= short arm; AR= arm ratio; CI= centromeric index; KF= karyotype formula, Means with similar letter(s) in each column for each factor show insignificant differences (DNMT, $p \leq 0.05$).

All populations were diploid with 2n=2x=32 chromosomes. The chromosomes in all populations were mostly metacentric (m) or submetacentric (sm) (Table 2).

Analysis of variance indicated significant differences among populations for all of the karyological parameters. Means are shown in Table 2. Among the populations studied, the highest total chromosome length and chromosomes length were observed in Masjed Soleyman 1 (24.07 and 1.50 µm, respectively) and Khuzestan 2 (23.83 and 1.49 µm, respectively) populations. The longest long arm and short arm were found in Masjed Soleyman 1 (0.91 µm) and Khuzestan 2 (0.66 µm), respectively. The lowest values were detected in Karaj (16.89, 1.05, 0.61 and 0.44 µm, respectively).

The most common karyotypes formula among Iranian local *C. officinalis* populations was 16m and 14m+2sm, (every two populations) (Table 2). Karyotype formula showed a higher degree of symmetry; the karyotypes of Khuzestan 1 and 2 populations were the most symmetric (16 m), while karyotype of Masjed Soleyman 1 (7m + 9sm) with the highest arm ration (1.68) and the lowest centromere index (0.39) were the most asymmetric.

The magnitude of the correlation coefficients was in the range from -0.87 to 0.82. The characters' chromosome length (0.82), long arm (0.68) and short arm (0.61) showed a positive and significant association with the total chromosome length (Table 3).

On the other hand, the arm ratio showed a significant and negative correlation with the centromeric index (-0.87).

## UPGMA dendrogram

According to cluster analysis, the populations classified into three distinct groups. Khuzestan 2 and 3 were classified in the first group while the second group consisted of Masjed Soleyman 1 and 2 and the third group contained Karaj and Khuzestan 1.

Grouping of the populations was assessed based on their relative karyotypic characteristics (Fig. 2).

**Table 3.** Correlation coefficients between the karyotypic parameters of six *C. officinalis* populations

| Trait | TCL[1] | CL | LA | SA | AR |
|---|---|---|---|---|---|
| CL | 0.82** | | | | |
| LA | 0.68* | 0.79* | | | |
| SA | 0.61* | 0.80* | 0.08 | | |
| AR | -0.10 | -0.04 | 0.12 | 0.25 | |
| CI | 0.19 | 0.07 | -0.08 | 0.31 | -0.87** |

1: Symbols as in Table 2; *: P<0.05; **: P<0.01

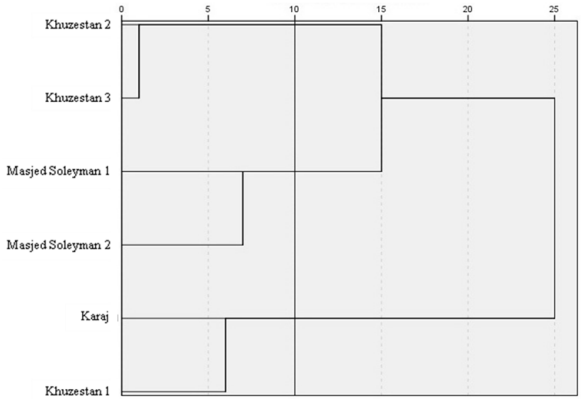


**Fig. 2.** UPGMA cluster dendrogram based on all studied chromosomal traits of 6 populations of *C. officinalis*

## Principal components analysis

The principal component analysis (PCA) based on karyotypic parameters revealed the first two principal components account for 99.8% of the total variations (Table 4).

The first component (57.7%) positively correlated with total chromosome length, long arm, and short arm while the second component

(42.0%) accentuated variation in arm ratio and centromeric index. The principal components were projected in a two-dimensional graphic for displaying genotypes distribution (Fig. 3).

**Table 4.** Eigenvalues and cumulative variance for two principal components for six *C. officinalis* populations

| Trait | PC1 | PC2 |
|---|---|---|
| chromosome length | -0.995 | 0.095 |
| long arm | -0.992 | 0.111 |
| short arm | -0.925 | 0.376 |
| arm ratio | 0.174 | 0.984 |
| centromeric index | 0.163 | 0.986 |
| Eigenvalue | 5.99 | 3.80 |
| Variance (%) | 57.76 | 42.05 |
| Cumulative (%) | 57.76 | 99.82 |

The arrangement of populations based on PCA was mostly agreed with the result of cluster analysis.

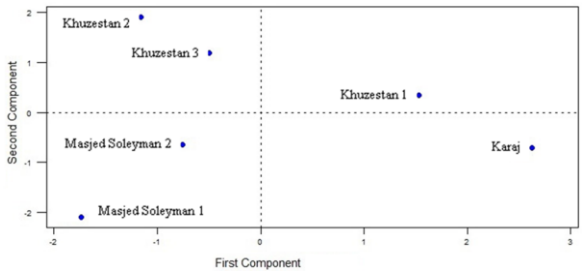


**Fig. 3.** Genotypes distribution biplot resulted in two first components of PCA.

## Discussion

In this study, chromosome numbers of six Iranian populations of *C. officinalis* (2n=2x=32) were identified for the first time in Iran. The results in the present study were in agreement with the chromosome number reported by Gill *et al*. (1985), Căpraru *et al*. (2004) and Samatadze *et al*. (2019). However, 2n = 14, 28 was reported as well (Nora *et al*., 2013). Based on the results of other *Calendula* species, researchers found 2n = 54 in *C. tripterocarpa* Rupr. (Dalgaard, 1986), 2n = 36, 44 in *C. arvensis* L. (Malallah and Brown, 1999; Vogt and Aparicio, 1999), 2n = 44 in *C. persica* C.A. Mey. (Janaki Ammal and Sobti, 1962) and *C. micrantha* Tineo and Guss. (Soliman, 2003). The results of this study reveal a detailed picture of the chromosome features in this species. The knowledge of chromosome numbers, karyotype evolution, ploidy level, and genome size can prepare additional information that has notable predictive powers (Ghasemi and Hesamzadeh Hejazi, 2018).

The karyotypes of the populations examined had a predominance of either metacentric or sub-metacentric chromosome types that agree with those published previously (Căpraru *et al*., 2004; Samatadze *et al*., 2019). Samatadze *et al*. (2019) mentioned that the presence of two satellite chromosomes pairs, but no satellite was observed among the populations studied. In the present study, the number of "m" chromosomes are more than "sm" chromosomes. This means that all populations in Iran have karyotypes in the early stage of evolution. Differences in karyotype formula found among *C. officinalis* populations suggest that chromosomes structural changes like translocations in metaphase I may have contributed to the diversification of the studied populations. Duncan's multiple range test applied to the chromosome morphometric traits showed a significant difference among examined populations. It was shown that *C. officinalis* have small chromosomes ranged from 1.30 (Gill *et al*., 1985) to 5.00 μm (Samatadze *et al*., 2019), while in our study, the chromosome length was detected slightly smaller than those (1.05 to 1.50 μm). Also, differences in climate including region, latitude, altitude, temperature, and precipitation have been correlated with different genome sizes (Du *et al*., 2017; Savaş Tuna *et al*., 2019). The population of Karaj, which grows in a region with higher altitude compared to Khuzestan province, has smaller chromosomes. Similarly, a significant negative correlation between genome size and altitude was found in *Dactylis glomerata* (Reeves *et al*., 1998), *Lilium* (Du *et al*., 2017) and two *Brachypodium* species (Savaş Tuna *et al*., 2019). However, Hoffmann *et al*. (2010) reported a positive correlation between altitude and 1C-value in *Olimarabidopsis pumila*, *Arabis montbretiana*, and *Arabis auriculata*. These relationships suggest that adaptation to habitat strongly influences chromosome length diversity, probably because different populations grow in different environments and at different latitudes and altitudes in a wide range of biotic and abiotic conditions that may shape natural genetic variation (Roughani *et al*., 2018a; Roughani *et al*., 2018b; Savaş Tuna *et al*., 2019).

Our results present the flexibility in the size of the *Calendula* genome and provide evidence supporting an adaptive hypothesis of genome size evolution in *Calendula*.

In conclusion, the main purpose of the present study was to choose *C. officinalis* populations with the most homology in chromosomal variations to cross in plant breeding programs. Significant karyotypic difference demonstrated between the *C. officinalis* populations. Crosses of Khuzestan 1 and 2, therefore suggested obtaining the higher genetic variation. Moreover, geographical region and altitude affected genome size. This type of analysis can help breeders select a variety of parents for heterosis breeding programs aimed at improving varieties.

## Conflicts of interest

The authors have no conflict of interest to declare.

## References


Arora D, Rani A, Sharma A. 2013. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula*. *Pharmacogn Rev* 7(14): 179-187.

Baciu AD, Pamfil D, Mihalte L, Sestras AF, Sestras RE. 2013. Phenotypic variation and genetic diversity of *Calendula officinalis* (L.). *Bulg J Agri Sci* 19(1): 143-151.

Căpraru G, Băra CI, Băra II, Cîmpeanu MM, Maxim E. 2004. The characteristics of mitotic chromosomes at *Calendula officinalis* L. *Gen Biol Mol* 2004: 223-225.

Dalgaard V. 1986. Chromosome studies in flowering plants from Macaronesia. *Anales Jard Bot Madrid* 43(1): 83-111.

Du YP, Bi Y, Zhang MF, Yang FP, Jia GX, Zhang XH. 2017. Genome size diversity in *Lilium* (Liliaceae) is correlated with karyotype and environmental traits. *Front Plant Sci* 8: 1303. doi: 10.3389/fpls.2017.01303.

Ebrahimi F, Ghorbani Nohooji M, Miri SM. 2018. Agronomic and pharmacological aspects of *Nerium oleander*: an important medicinal plant. The First National Congress and International Fair of Medicinal Plants and Strategies for Persian Medicine that Affect Diabetes, 9-11 October 2018, Mashhad, Iran.

Ghasemi E, Hesamzadeh Hejazi SM. 2018. Karyological studies in different populations of *Buxus hyrcana* (Buxaceae) in Iran. *Iran J Bot* 24(2): 156-162.

Gill BS, Gupta RC, Bedi YS. 1985. Karyological studies on some cultivated species of Compositae. *J Indian Bot Soc* 64: 219-225.

Hoffmann MH, Schmuths H, Koch C, Meister A, Fritsch RM. 2010. Comparative analysis of growth, genome size, chromosome numbers and phylogeny of *Arabidopsis thaliana* and three co-occurring species of the Brassicaceae from Uzbekistan. *J Bot* 2010: 504613. doi:10.1155/2010/504613.

Honório ICG, Bonfim FPG, Montoya SG, Casali VWD, João Paulo Viana Leite JPV, Cecon PR. 2016. Growth, development and content of flavonoids in calendula (*Calendula officinalis* L.). *Acta Sci Agron* 38(1): 69-75.

Jafari E, Assadi M, Ghanbarian GA. 2014. Notes on taxonomic situation of *Calenduleae* tribe in Iran. *IUFS J Biol* 73(2): 1-7.

Jan N, Andrabi KI, John R. 2017. *Calendula officinalis* - an important medicinal plant with potential biological properties. *Proc Indian Natn Sci Acad* 83: 769-787.

Janaki Ammal EK, Sobti SN 1962. Chromosome relationship in *Calendula* species. *Proc Indian Acad Sci* 55(3): 128-130.

Khalid KA, de Silva JAT. 2012. Biology of *Calendula officinalis* Linn.: focus on pharmacology, biological activities and agronomic practices. *Med Aromatic Plant Sci. Biotechnol* 6(1): 12-27.

Khan MU, Rohilla A, Bhatt D, Afrin S, Rohilla S, Ansari SH. 2011. Diverse belongings of *Calendula officinalis*: an overview. *Int J Pharm Sci Drug Res* 3(3): 173-177.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.

Malallah GA, Brown G. 1999. Determination of chromosome number of Kuwaiti Flora I. *Cytologia* 64: 181-196.

Miri SM, Shamsolshoara Y. 2019. Phytochemical and pharmacological properties of *Astragalus* species. 2nd National Conference Medicinal Plants and Species Affecting the Fatty Liver, 13 June 2019, Tehran, Iran.

Muley BP, Khadabadi SS, Banarase NB. 2009. Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): A Review. *Trop J Pharm Res* 8(5): 455-465.

Nora S, Castro S, Loureiro J, Gonçalves AC, Oliveira H, Santos C, Silveira P. 2013. Flow cytometric and karyological analyses of *Calendula* species from Iberian Peninsula. *Plant Syst Evol* 299: 853-864.

Reeves G, Francist D, Davies MS, Rogers HJ, Hodkinson TR. 1998. Genome size is negatively correlated with altitude in natural populations of *Dactylis glomerata*. *Ann Bot* 82: 99-105.

Roughani A, Miri SM. 2018. *Lepidium* species as antidiabetic herbal medicines. The First National Congress and International Fair of Medicinal Plants and Strategies for Persian Medicine that Affect Diabetes, 9-11 October 2018, Mashhad, Iran.

Roughani A, Miri SM, Hassandokht MR, Moradi P, Abdossi V. 2018a. Agro-morphological study on several accessions of garden cress (*Lepidium sativum*-Brassiaceae) in Iran. *Pak J Bot* 50(2): 655-660.

Roughani A, Miri SM, Hassandokht MR, Moradi P, Abdossi V. 2018b. Morphological variation of some *Lepidium draba* and *L. latifolium* populations. *Taiwania* 63(1): 41-48.

Samatadze TE, Zoshchuk SA, Hazieva FM, Yurkevich OY, Svistunova NY, Morozov AI, Amosova AV, Muravenko OV. 2019. Phenotypic and molecular cytogenetic variability in calendula (*Calendula officinalis* L.) cultivars and mutant lines obtained via chemical mutagenesis. *Sci Rep* 9(9155). doi:10.1038/s41598-019-45738-3.

Savaş Tuna GS, Başer İ, Tuna T. 2019. Genome size variation among natural populations of *Brachypodium distachyon* and *B. hybridum* collected from different regions of Turkey. *Turk J Bot* 43: 196-207.

Soliman MI. 2003. Genetic diversity of achene heteromorphism in Egyptian *Calendula micrantha* Tineo et Guss. *Asian J Plant Sci* 2(10): 782-789.

Verma PK, Raina R, Agarwal S, Kour H. 2018. Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn. *Pharm Biomed Res* 4(2): 1-17.

Vogt R, Aparicio A. 1999. Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus. *Bocconea* 1: 117-169.