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Karyological and Nuclear DNA Content Variation in Some Iranian Endemic *Thymus* Species (Lamiaceae)

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ABSTRACT

Thymus **is a medicinal plant which contains one of the world's top ten essential oils, exhibiting antibacterial, antioxidative, food preservative and mammalian age-delaying properties. This work was aimed at identifying between-species variations requiring for selecting appropriate parents for hybridization. Six Iranian endemic *Thymus* accessions belonging to *Thymus daënensis*, *T. eriocalyx* and *T. migricus* were studied. Root tips were examined for karyological studies and fresh young leaves of the standard reference (Parsley, *Petroselinum crispum*, 2C DNA= 4.45 pg) and the *Thymus* samples stained with propidium iodide (PI) for flow cytometric (FCM) measurements. Two ploidy levels (diploid and tetraploid) and 3 chromosome numbers (30, 56, 60) were recognized. The latter chromosome number is being reported for the first time on *T. daënensis* accession. FCM measurements showed that 2C DNA contents varied from 1.02 to 2.42 pg, verifying more than 2-fold variations and showing a genome size range of 499 to 1182 Mbp, correspondingly. The mean amount of 2C DNA/chromosome and mean of monoploid genome size were not proportional to ploidy. 2C-values were correlated with, and linearly regressed upon somatic metaphase, considering either total chromosome volume (TCV) or total chromatin length (X).**

Keywords: Chromosome, DNA C-value, Genome size, Medicinal plant, *Thymus daënensis*, *T. eriocalyx*, *T. migricus.*

INTRODUCTION

Thyme (*Thymus*, Lamiaceae) is one of the most important medicinal plants. Its oil is among the world's top ten essential oils, exhibiting antibacterial, antimycotic, antioxidative, food preservative and mammalian age-delaying properties (Brown, 2002; Omidbaigi, 2009). The morphology and different components of essential oils in different species of *Thymus* are variable due to hybridization and polyploidization, despite its rare self-pollination (Lopez-Pujol *et al*., 2004). In general, intraspecific hybrids of the genus *Thymus* seem to possess intermediate morphological characteristics and composition of essential oil in comparison with the relevant characteristics of the parent plants (Loziene *et al*., 2002). Inheritance studies would also be difficult due to low germination rates (Lopez-Pujol *et al*., 2004) and cytological analyses are technically extremely difficult due to the very small size of its chromosomes (Morales, 1998). However, it has been reported that *Thymus* genus represents two ploidy levels (diploid and tetraploid) and five different chromosome numbers: 2n= 2x= 28, 30 and 2n= 4x= 54, 56, 58 (Lopez-Pujol *et al*., 2004). In other work, *T. praecox* was considered as a species with various chromosome numbers of 24, 28, 50, 54, 56 and 58 (Fernandes and Leitao, 1984). The species *T. herba-barona* Loisel displayed 2n= 2x= 28, 2n= 4x= 56 and even 2n= 6x= 84 (Morales, 2002).

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Table 1. Local information of collected Iranian endemic *Thymus* accessions studied.

Species	Iranian local collection sites	Latitude and Longitude	Accession code
T. daënensis Celak.[a]	Fereydunshahr, Esfahan, Iran	32° 55′ N 50° 07′ E	FRS
T. daënensis Celak.	Varcheh, Markazi, Iran	34° 06′ N 50° 40′ E	ARV
T. eriocalyx (Ronniger) Jalas	Malayer, Hamadan, Iran	34° 17′ N 48° 49′ E	MAL
T. migricus Klokov & Desj.-Shost.	Salmas, Azarbayjan-e Gharbi, Iran	38° 10′ N 44° 44′ E	SAL
T. daënensis Celak.	Zagheh, Lorestan, Iran	33° 28′ N 48° 41′ E	KHZ
T. daënensis Celak.	Chaghalvandi, Lorestan, Iran	33° 38′ N 48° 33′ E	KHC

[a] Celakovsky.

Hence, according to previous reports, it can be pointed out that there are remarkable variations in *Thymus* chromosome number which can reflect the nuclear DNA amount variations. This amount has been estimated in diploid *T. vulgaris* (2C DNA= 1.54 pg) using FCM (Marie and Brown, 1993). Nuclear DNA content is an important characteristic which is useful in many aspects, e.g. taxonomical, ecological, physiological, cell and molecular biology, plant breeding and genome evolution studies (Bennett and Leitch, 2000; Greilhuber *et al*., 2005; Knight *et al*., 2005; Doležel *et al*., 2007a). Therefore, intra- or inter-specific variation in genome size is real and expected, e.g. when it reflects karyotypic variation in the number and size of chromosomes (Bennett *et al*., 2008). Many plants (e.g. *Euphorbia pulcherrima*) have some composition such as anthocyanin which can affect the estimation of DNA amount, resulting in artifacts (Bennett *et al*., 2008), but no anthocyanin has been reported in *Thymus* (Loziene *et al*., 2002; Fraternale *et al*., 2003; Mirza and Bahr, 2003; Sajjadi and Khatamsaz, 2003; Seidler-Lozykowska *et al*., 2008; Omidbaigi, 2009). Monoploid genome size (the amount of DNA of one chromosome set, 1 C*x*-value, with chromosome base number x) and holoploid genome size (the amount of DNA of the whole chromosome complement, 1 C-value, with chromosome number n, irrespective of the degree of generative polyploidy, aneuploidies, etc.) are described by Greilhuber *et al*. (2005). In *Thymus* genus, variation in chromosome number can suggest intra- and inter- specific variations in genomic DNA amounts.

In the present work, six accessions of *Thymus*, Iranian endemic taxon, were studied. Four of which were *T. daënensis* Celak. and the other two accessions were *T. eriocalyx* (Ronniger) Jalas and *T. migricus* Klokov and Desj.-Shost. These six *Thymus* accessions were investigated according to the karyological squash technique, nuclear DNA content and genome size using FCM.

MATERIALS AND METHODS

Plants

Seeds of six Iranian endemic accessions of *Thymus* were obtained from the germplasm collection of the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran. Their origins, geographical descriptions, latitudes and longitudes, and codes in this study are listed in Table 1 and illustrated in Iran's map using GIS Microsoft (Figure 1). For karyotypic analysis of somatic chromosomes, the seeds were germinated on wet filter paper in Petri

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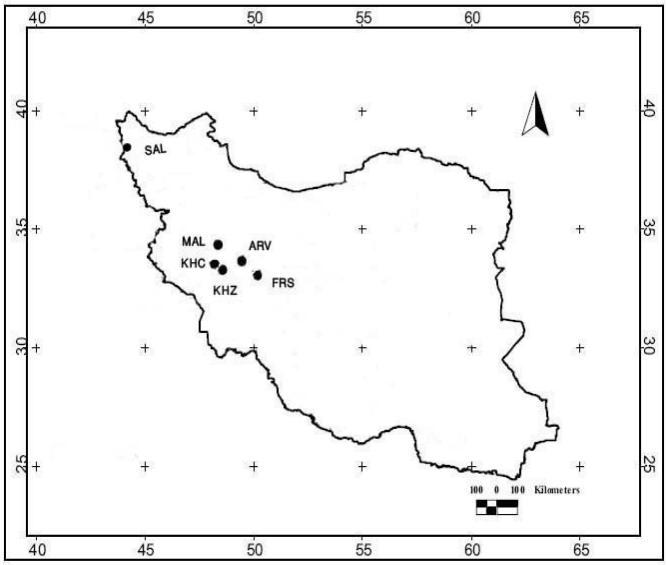

Figure 1. Collection sites of accessions of *Thymus* genus on the map of Iran designed using GIS Microsoft.

dishes at room temperature (RT). For nuclear DNA amount estimation, the seeds were grown in the field conditions at the experimental location in Alborz Research Institute, Karaj (35° 49′ N, 50° 58′ E) in March 2005.

Chromosome Preparation

To induce and synchronize cell division, the 0.5 cm-long root tips of *Thymus* accessions were first physically cold pre-treated at 4°C for 12 hours and then were maintained at RT for 45 minutes. They were then chemically pre-treated in saturated aqueous α-bromonaphthalene at 4°C for 3 hours in darkness. The physico-chemical pre-treated root tips were fixed in freshly-prepared cold 3:1 (v/v) absolute ethanol:glacial acetic acid for 20 hours and then stored in 70% (v/v) aqueous absolute ethanol at 4°C until required. This was followed by hydrolysis in 1M HCl at 60°C for 15 minutes in a water bath and stained by 2% (w/v) aceto-orcein at RT for 2 hours in darkness. The stained root tips were thereafter squashed in a drop of 45 % (v/v) acetic acid. Five well-spread monolayer metaphase plates from different individuals were analyzed per *Thymus* accession. Super high quality microscopic photographs were taken using a DP12 digital camera interfaced to a BX50 Olympus microscope. The total length of each chromosome (TL) was measured and then total chromatin length ($X= 2\sum TL$) and total chromosome volume (TCV) were calculated for each accession, using the formula $2\pi r^2 \times TL$, where "*r*" is the average radius of chromosome cross section. The 5-cell replicate mean data of TL, X and TCV for each *Thymus* accession are shown in Table 2.

Flow Cytometric Analysis

The internal standard employed was Parsley (*Petroselinum crispum*, 2C DNA= 4.45 pg, Yokoya *et al*., 2000). Healthy fresh young leaves (total 0.6 g) of the standard reference (Parsley) and of the sample (*Thymus*) were chopped with a sharp razor blade in ice-cold nucleic extraction buffer; Partec, CyStain UV precise P, Germany.

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Table 2. Mean (n= 5) chromosomal information for *Thymus* accessions.

Accession code	Ploidy level	2n	TL [a]±SE [b] (μm)	TCV [c]±SE (μm^3)	X [d] (μm)
FRS	2x	30	0.987 ± 0.015	0.511 ± 0.022	29.600
ARV	2x	30	0.922 ± 0.021	0.347 ± 0.010	27.660
MAL	2x	30	1.060 ± 0.016	0.503 ± 0.015	31.900
SAL	2x	30	1.073 ± 0.011	0.554 ± 0.019	32.195
KHZ	4x	56	1.067 ± 0.012	0.665 ± 0.020	59.730
KHC	4x	60	1.066 ± 0.013	0.707 ± 0.020	63.960

[a] Total length of each chromosome, [b] Standard error, [c] Total chromosome volume ($2\pi r^2 \times TL$), [d] Total chromatin length ($_2\sum TL$).

The chopped leaves were filtered through 50 μm, followed by 30 μm nylon mesh filters. After adding 2,000 mm^3 PBS (Phosphate-buffered saline); consisting of, per liter, 8 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, pH 7.0-7.2, centrifuged at 1000×g at 4°C for 6-7 minutes. The pellet was re-suspended in 500 mm^3 PBS, 1.5 mm^3 Ribonucleas A (34 mg cm^{-3} solution) and 5 mm^3 propidium iodide (PI; 10 mg cm^{-3} solution) incubated in the dark for 5-6 min on ice. The samples were re-centrifuged, then re-suspended under the same above mentioned conditions, storing in the dark for 45 minutes on ice. For each accession, at least three different fresh leaf samples were examined. Fluorescence intensity was measured, using a PAS III Flow Cytometer (Partec, Germany) equipped with an Argon ion laser (488 nm). Measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale and, typically, at least 5,000 nuclei were analyzed for each sample. The absolute DNA amount of a sample was calculated based on the values of the G_1 peak means (Doležel *et al*., 2003 and 2007b; Doležel and Bartoš, 2005) as follows:

Sample 2C DNA (pg) content= [(Sample G_1 peak mean)/(Standard G_1 peak mean)]×Standard 2C DNA amount (pg)

Statistical Analyses

For either diploids or tetraploids, analysis of variance was carried out using Balanced Model in Minitab Statistical Software (Fry, 1993; Ryan and Joiner, 2001). Identification of between-*Thymus* accessions differences in the 2C-values are based on the outcome of these tests. Tukey's test was used for mean comparisons between four diploid accessions (Forni-Martins and Calligaris, 2002; Seijo and Fernandez, 2003; Suda *et al*., 2003) and the t-test was used for comparison of the two tetraploid accessions. 1C-value was calculated based on a converting formula proposed by Doležel *et al*. (2003), when 1 pg of DNA represents 978 mega basepairs (Mbp). The relationship between mean (n= 3) 2C-values of leaf samples with mean (n= 5) metaphasic root tips chromosomal parameters was examined, considering that a cell at metaphase of mitotic division has 4C DNA amount.

RESULTS

Karyological Analysis

Among six *Thymus* accessions studied, four had chromosome numbers of 2n= 2x= 30 and the other two accessions had chromosome numbers of 2n= 4x= 56 and 60, respectively, in all the cells analyzed (Table 2 and Figure 2). In general, the very small chromosomes in all *Thymus* accessions examined, had a mean total length range from 0.92 to 1.07 μm in SAL and ARV (Table 1) diploid accessions, respectively (TL; Table 2). The diploid MAL accession

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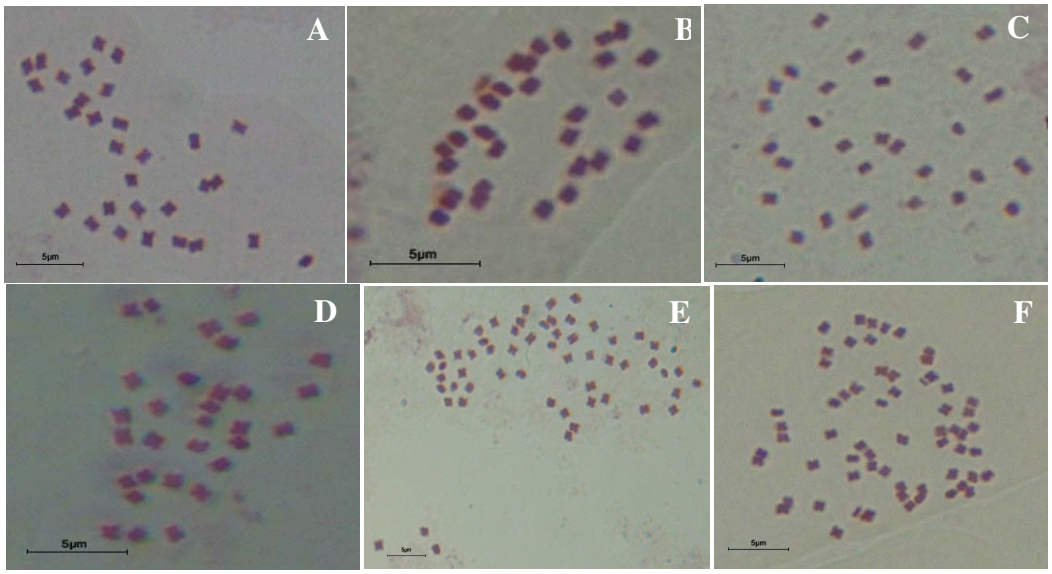

Figure 2. Karyotypes for *Thymus* accessions: (A) FRS, 2n= 2x= 30, *T. daënensis* Celak., (B) ARV (2n= 2x= 30, *T daënensis* Celak., (C) MAL, 2n= 2x= 30, *T. eriocalyx* (Ronniger) Jalas, (D) SAL, 2n= 2x= 30, *T. migricus* Klokov & Desj.-Shost., (E) KHZ, 2n= 4x= 56, *T. daënensis* Celak., (F) KHC, 2n= 4x= 60, *T. daënensis* Celak.. Bar= 5 µm.

had the same chromosome length (1.06 µm) as the tetraploid (*T. daënensis*) KHC or KHZ (1.07 µm). The total chromosome volume (TCV) ranged from 0.347 to 0.707 μm^3 (ARV and KHC accessions; *T. daënensis*, respectively). Table 2 also shows the range of 27.66 to 63.96 µm in total chromatin length (X) belonging to ARV and either KHZ or KHC accessions, correspondingly. On the whole, such chromosomal data exhibited that the KHC tetraploid *Thymus* accession appeared to have the largest values for all chromosomal indices examined while the ARV diploid showed the smallest values. On the other hand, these data confirmed that the values of X were almost doubled in both tetraploid (KHZ and KHC) *Thymus* accessions compared to those in the diploids.

Flow Cytometric Analysis

Six accessions of *Thymus* genus were analyzed using Partec FloMax software Ver. 2.4 d for the estimation of nuclear DNA content. The histograms obtained for analyzing the amount of nuclear DNA in leaves contained two peaks (Figure 3): the left peaks refer to the unknown *Thymus* samples and the right peaks to the known Parsley reference standard. The accessions 2C-value mean comparisons are shown in Table 3. This table shows DNA contents (pg) of *Thymus* accessions and their related genome sizes (Mbp). The *2C*-value ranged from 1.02 to 2.42 pg (referring to a diploid ARV, *T. daënensis*, and a tetraploid KHC, *T. daënensis*, accessions, respectively). Among four diploids examined, a difference of 0.39 pg in 2C-value (range 1.02-1.41) was distinguished in spite of having the same chromosome numbers of 30 (Table 3). In other words, within the diploids, the lowest and the highest amounts of nuclear DNA were identified in ARV and SAL (*T. migricus*), respectively. Between two tetraploids with a difference of four chromosomes (56 *vs.* 60), a very small difference of 0.14 pg in 2C-value (range 2.28-2.42) was recognized (Table 3). On the other hand, the mean 2C-value of a KHC tetraploid

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Table 3. Mean (n= 3) 2C-value and genome size of *Thymus* accessions.

Accession code	Ploidy level	2n	2C-value (pg±SE)	1C-value (pg)	Holoploid genome size (Mbp)	Monoploid genome size (Mbp)
FRS	2x	30	1.095 ± 0.021**c***	0.547	535	535
ARV	2x	30	1.020 ± 0.078bc	0.510	499	499
MAL	2x	30	1.285 ± 0.023ab	0.642	628	628
SAL	2x	30	1.413 ± 0.033a	0.706	690	690
KHZ	4x	56	2.280 ± 0.053x	1.140	1115	558
KHC	4x	60	2.418 ± 0.082x	1.209	1182	591

* Means with the same symbol letter (within each group of 2x or 4x) are not statistically different (P> 0.05).

(2n= 60, 2.42 pg) was estimated to be exactly twice the value of the four diploids (2n= 30, 1.2 pg). No significant difference of 2C-value between the two tetraploid accessions (KHZ, KHC) was recognized by Tukey's test, despite the 4-chromosome difference. This was because of the very small size of chromosomes resulting in no marked difference in 2C-value between these two tetraploids. Alternatively, the holoploid genome size (Table 3) ranged from 499 Mbp (diploid ARV) to 690 Mbp (diploid SAL) showing the difference of 191 Mbp, and that in two tetraploids ranged from 1,115 Mbp (KHZ) to 1,182 Mbp (KHC) a difference of 67 Mbp. Among chromosomal parameters studied, TCV and X were significantly correlated (r= 0.913** and 0.991***, respectively, in Figuress 4a, b) with 2C-values showing positive linear relationships (b= 4.323** and 0.037***, respectively, in Figures 4a, b).

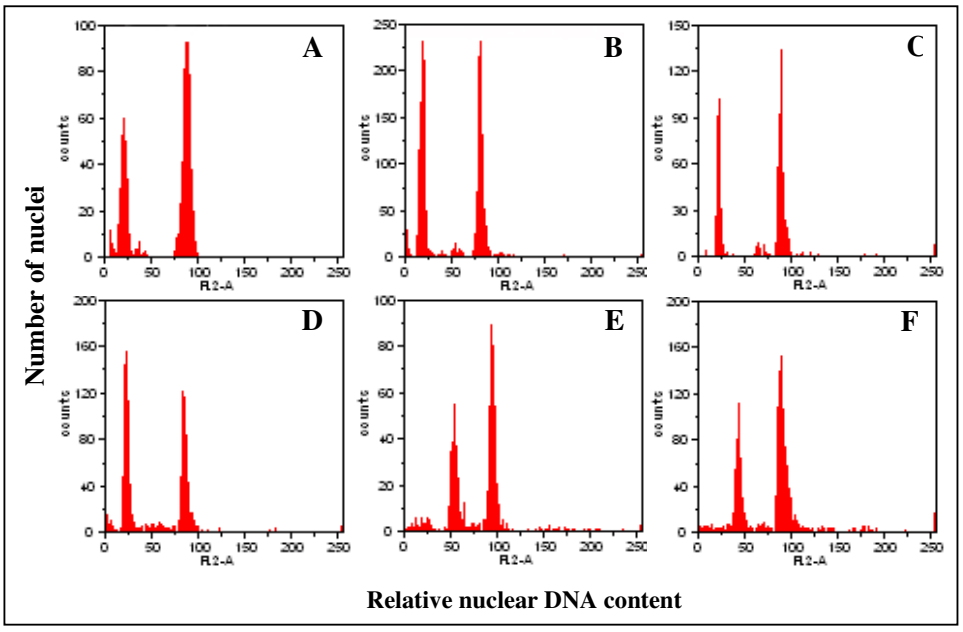

Figure 3. Flow cytometric histograms showing the difference in 2C DNA content for *Thymus* accessions: (A) FRS, 2n= 2x= 30, *T. daënensis* Celak., (B) ARV (2n= 2x= 30, *T daënensis* Celak., (C) MAL, 2n= 2x= 30, *T. eriocalyx* (Ronniger) Jalas, (D) SAL, 2n= 2x= 30, *T. migricus* Klokov & Desj.-Shost., (E) KHZ, 2n= 4x= 56, *T. daënensis* Celak., (F) KHC, 2n= 4x= 60, *T. daënensis* Celak.. The left peaks refer to the *Thymus* samples and the right peaks to the Parsley (*Petroselinum crispum*, 2C DNA= 4.45 pg) reference standard.

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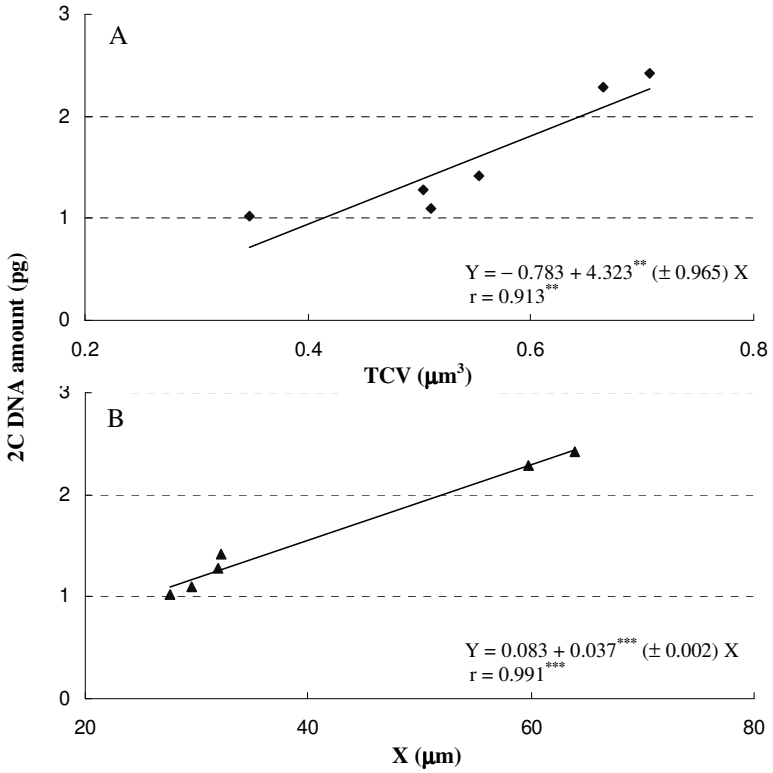

Figure 4. Mean (n= 3) leaf 2C DNA amounts (pg) of six *Thymus* accessions plotted against mean (n = 5) root-tip total chromosome volumes (TCV, μm^3) (A) and chromatin lengths (X, µm) at mitotic metaphase (B).

DISCUSSION

Karyological Analysis

Considerable inter- and intra-specific variations were recognized in either the chromosomal indices, DNA C-values or genome size in the *Thymus* genus accessions examined. In general, very small-sized chromosomes were identified in all *Thymus* accessions examined, ranging from 0.92 to 1.07 µm. This is in support of Morales (1998) who reported that cytological studies were extremely difficult to conduct in *Thymus* species because of their very small-sized chromosomes. The resultant data in the present report verified two ploidy levels of diploids and tetraploids and three different chromosome numbers of 30, 56 and 60. In previous works, such ploidy levels and chromosome numbers of 30 (Murin, 1997) and 56 (Martonfi and Martonfiova, 1996, Lopez-Pujol *et al*., 2004) were also reported for other species of *Thymus* genus. However, *T. praecox* was reported to have various chromosome numbers of 24, 28, 50, 54, 56 and 58 (Fernandes and Leitao, 1984), *T. herba-barona* Loisel had 28, 56 and 84 (Morales, 2002), *T. bihorieisis* (A. KERN) Jalas had 28 and *T. alternans* Klokov showed 56 (Martonfi and Martonfiova, 1996), *T. vulgaris* displayed 30 (Murin, 1997), and *T. zygis* subsp. Zygis and subsp. Gracilis showed 2n= 28 and *T. zygis* subsp. Sylvestris showed 2n= 56 (Lopez-Pujol *et al.*, 2004). A new finding in the present *Thymus* work was the first report of the chromosome number of 60 in an Iranian endemic tetraploid *T. daënensis* accession (KHC; Chaghalvandi, Lorestan, Iran): this

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chromosome number has not been reported so far for any other *Thymus* species. More interestingly, this *Thymus* accession (KHC) displayed four chromosomes more than an other Iranian endemic tetraploid *T. daënensis* accession (KHZ; Zagheh, Lorestan, Iran) which was collected from the same province of Lorestan (Northwest) with only small differences in latitude and longitude (10′ Northern and 8′ Western, see Table 1 and Figure 1). Previous reports and our recent findings may allow us to deduce the instability in either ploidy level or chromosome number in different *Thymus* species, probably due to natural and/or interspecific hybridization and polyploidization. This makes difficulty in recognizing and determining the original of taxonomic situations of *Thymus* species. Morales (2002) also emphasized that different ploidy levels of some *Thymus* species reported by several studies indicated that polyploidization probably occur frequently in this genus. One of the most interesting cases was reported on *T. herba-barona* Loisel, with 2n= 28, 56 and 84 (Morales, 2002). According to Morales (1986) studies on the meiosis and demonstrating quadrivalents, the tetraploid numbers (54, 56 and 58) had different origins: 2n= 56 (*T. carnosus* Boiss.) was probably derived from a duplication of a 2n= 28 genome (*i.e.* autopolyploidy), 2n= 58 might originate from a hybridization of two taxa with n= 14, 15 and a subsequent doubling of chromosome number (*i. e.* autopolyploidy followed by tetrasomic, 2n= 4x+2= 4×14+2), and 2n= 54 was probably derived from a 2n= 56 plant which had lost two chromosomes (i. e. autopolyploidy followed by nulisomic, 2n= 4x–2). On the other hand, allozyme studies supported the hypothesis that *T. loscosii* is an autotetraploid (Lopez-pujol *et al*., 2004). Considering the chromosomal studies in the present report, the same four 30-chromosome diploid *Thymus* accessions showed small inter- and intra-specific variations in TL, TCV and X. Considering the latter chromosome index, *T. migricus* (SAL) which was collected geographically furthest from other accessions (Table 1 and Figure 1) appeared to display the largest chromatin length among other *Thymus* accessions, either diploid or tetraploid. This may indicate that geographical adaptation influences the chromosome length. On the other hand, the different species of *Thymus* (diploids and tetraploids) have symmetric and primitive karyotypes (Mahdavi *et al*., 2009), probably indicating inter/intra hybridization. Such similarity in their karyotypes does not prevent their successful crosses and disturbance in reproduction.

Flow Cytometric Analysis

FCM has successfully been used to recognize the stability ploidy level (Wyman *et al*., 1992). In the present work, different Iranian *Thymus* accessions segregated according to their amount of nuclear DNA, show inter/intra species variations verifying the karyological results. *Thymus vulgaris* is the species whose 2C-value has been reported (2C DNA= 1.54 pg by Marie and Brown, 1993). The surprising finding is that the two tetraploid *Thymus* accessions (KHZ and KHC) with two different chromosome numbers of 56 and 60 and with two different 2C-values were collected from the same province (Lorestan) with only a small difference in latitude and longitude (Table 1). The question remains unanswered as to why the two tetraploid *Thymus* accessions from the same province differed in 4 chromosomes? The 2C DNA amount/chromosome mean comparison between 4-diploids (0.0401 pg) and 2-tetraploids (0.0405 pg) was not statistically different (*t*-value= –0.09; P> 0.05). The same was true for monoploid genome size (588 Mbp for diploids, 574.5 Mbp for tetraploids; *t*-value= 0.2; P> 0.05). In other words, these data showed that the mean 2C-value and mean monoploid genome size were not proportional to ploidy in the studied *Thymus* accessions. Leitch and Bennett (2004) have shown that in angiosperms the mean genome size of

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polyploids was significantly lower than that of diploids. Studying nine genera of New Zealand grasses with different ploidy levels, Murray *et al*. (2005) reported smaller genome sizes in polyploids compared with diploids. In some cases the differences were not great, emphasizing that this reflected the recent nature of speciation/polyploidization. Tuna *et al*. (2001) reported a slight reduction of DNA content of bromegrass germplasm accessions as the ploidy level increased. Such reduction happening during allopolyploidization in the wheat could be a necessary adaptation for the establishment and stabilization of polyploid genomes (Ozkan *et al*., 2003). Thus, polyploidy is clearly a possible contributor to C-value variation, but the relationship between these is not straightforward (Murray *et al*., 2005). In our present report, we found no relationship between 2C-value of *Thymus* diploids and tetraploids. Possibly, if more *Thymus* species could be found, studied and analyzed the result may or may not be changed.

Our other karyotypic unpublished data on *Thymus* accessions (Mahdavi *et al*., 2009) clearly showed a karyotype formula of 56m for tetraploid KHZ and 58m+2sm for KHC. The first one (KHZ) illustrated similar chromosome types as in the diploids "*m*; metacentric", but the second one (KHC) differed in the 2 chromosome types of "*sm*; sub metacentric". This may help us to deduce that the new reported 60 chromosome tetraploid *Thymus* tends to have a different evolutionary karyotype classification from the "1A" Stebbins karyotype category (complete symmetric karyotype) for all either diploids or a tetraploid (KHZ) to "2A" relatively symmetric karyotype (Mahdavi *et al*., 2009).

In the species of *T. daënensis*, 2C-value (from diploid to tetraploid) showed a positive correlation with either total chromosome volume or total chromatin length. In agreement with our data, such a relationship between nuclear DNA content and chromosomal parameters has been reported in different plants (e.g. Bennett *et al*., 1983 cited in Leitch and Bennett, 2007). On the other hand, interestingly, the monoploid genome size of *T. daënensis*, diploid accessions (FRS and ARV; 2n= 30, Mean= 517 Mbp) was significantly less (21.5%; P< 0.001) than that of other diploid accessions (MAL and SAL; 2n= 30, Mean= 659 Mbp). The average monoploid genome size of *T. eriocalyx* (MAL) and *T. migricus* was more than that of either diploids or tetraploids of *T. daënensis* (FRS, ARV, KHZ, KHC), giving us a clear picture of variation between these two-typed *Thymus* accessions. Such variation could be interesting for either induction of polyploids or producing hybrids between types of examined *Thymus* genus accessions. Hence, the most promising use of genome size could be used as a useful marker for detection of hybrids (Ellul *et al*., 2002).

In *Thymus* taxonomic study, it would be better to do morphologic study, chromosome count and ploidy level determination accompanied by the estimation of nuclear DNA amounts and to study the extensive complementary assessment of karyology and cytology (mitosis and meiosis). Our study allows us to recommend the study of more *Thymus* accessions collected from various different world-wide geographical locations in order to recognize their origins and production processes through the different ploidy levels and chromosome numbers. Accessions from different altitudes would be required to assess of inter- and intra-species variation in chromosome number and nuclear DNA amount. In general, knowledge and information on genome size could be useful for illustrating any relationship between DNA amount and plant ecophysiology (Thiem and Sliwinska, 2003; Greilhuber *et al*., 2005; Knight *et al*., 2005;Doležel *et al*., 2007a; Bennett *et al*., 2008).

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تنوع کاریولوژیکی و مقدار DNA هسته‌ای در تعدادی از گونه‌های *Thymus* (Lamiaceae) بومی ایران

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چکیده

تیموس (*Thymus*) یک گیاه دارویی است که دارای یکی از ده ماده مؤثره برتر جهان، با خواص ضد باکتریایی، آنتی اکسیدانی، خواص نگه دارنده غذایی و به تأخیر اندازنده پیری پستانداران است. هدف از این مطالعه تشخیص تنوع بین گونه‌ای است جهت انتخاب والدین مناسب که برای هرگونه تلاقی مورد نیاز است. تعداد شش نمونه تیموس بومی ایران متعلق به سه گونه *Thymus daënensis*، *T. eriocalyx* و *T. migricus* مورد مطالعه قرار گرفت. برای بررسی‌های کاریولوژیکی از مریستم ریشه و برای مطالعات فلوسایتومتری از برگ‌های جوان تازه گیاه رفرنس استاندارد جعفری (۲C DNA= ۴/۴۵ pg *Petroselinum crispum*,) و نمونه‌های گیاه تیموس رنگ آمیزی شده با PI استفاده گردید. دو سطح پلوئیدی (دیپلوئید و تتراپلوئید) و سه شمارش کروموزومی (۳۰، ۵۶ و ۶۰) تشخیص داده شد: تعداد کروموزومی آخر برای اولین بار در گونه *T. daënensis* گزارش می‌شود. اندازه‌گیری‌های فلوسایتومتریک نشان داد که مقدار ۲C DNA نمونه‌های تیموس بین pg ۲/۴۲ – ۱/۰۲ تغییر می‌کرد که نمایانگر دو برابر تنوع است و اندازه ژنوم آنها در دامنه Mbp ۱۱۸۲-۴۹۹ تخمین زده شد. میانگین نسبت مقدار ۲C DNA به کروموزوم و میانگین اندازه ژنوم مونوپلوئید با سطح پلوئیدی متناسب نبود. مقدار C ۲ هم با حجم کل کروموزوم (TCV) متافاری سماتیکی و هم با طول کل کروماتین (X) همبستگی و رابطه خطی نشان داد.

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