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Full Length Research Paper

Assessment of karyotypical variation among 16 populations of *Thymus daenensis* Celak and *Thymus kotschyanus* Boiss. species in Iran

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In order to study the cytological variation between Iranian native *Thymus*, karyotypes of two taxa (16 populations) from different geographic origins were presented. The ploidy level was different in populations belonging to *T. kotschyanus* (2x and 4x), but the same level was found (2x) in *T. daenensis* populations. According to intrachromosomal asymmetry, *T. daenensis* (10534) had the most asymmetrical and evolutionary karyotype and *T. kotschyanus* (10513) had the most symmetrical karyotype in all of the populations. While based on interchromosomal asymmetry, among diploid populations, *T. daenensis* (10535) and among tetraploid populations, only *T. kotschyanus* (10515) had the most asymmetrical karyotype. In terms of the Stebbins' system, the karyotype were of populations seizes 1A classes. The results of analysis of variance revealed significant differences between the populations based on all karyotypic characteristics (P<%1). The results of cluster analysis showed that populations of each species *T. daenensis* and *T. kotschyanus* have been grouped in separate cluster. The results seemed to provide enough genetic evidence to identify the species and useful data to clarify the interspecific relationships. Detailed karyotype analysis allows us to group the different populations and to specify their relationships.

Key words: Chromosome, karyotype, ploidy, principal components analysis, *Thyme*.

INTRODUCTION

The genus *Thymus* L. (Lamiaceae) with English and Persian common names "Thyme" and ''Azorbe/Avishan'', respectively consists of about 928 species of herbaceous, perennial and sub shrubs or shrubs distributed mainly over Mediterranean countries, northern part of Africa and Southern Greenland (Sunar et al., 2009). This genus is represented in Iranian flora by 17 species, and four of these species namely *Thymus carmanicus*, *T. daenensis*, *T. persicus* and *T. trautvetteri* are endemic for Iran (Nickavar et al., 2004). The aromatic and medicinal properties of *Thymus* have made it one of the most popular medicinal plants. Its oil is among the world's top ten essential oils (Rahimmalek et al., 2009). *Thymus* species are commonly used as spices, herbal tea, insecticide and flavoring agents. Also, *Thymus* species have been most frequently used in traditional herbal medicine due to its antiseptic, carminative, expectrant, antispasmodic and anti-inflammatory properties. Infusion and decoction of aerial parts of *Thymus* species are used to produce a tonic, digestive, antitussive and for the treatment of colds in Iranian traditional medicine. Moreover, recent studies have showed that this genus have strong antifungal, antiviral, antibacterial, anti-parasitic, spasmolytic and antioxidant activities (Sunar et al., 2009). Among the species grow in Iran, *T. daenensis* and *T. kotschyanus* are vast spread and more widely used for these purposes (Rahimmalek et al., 2009). Both species are perennial herbs and according to Flora Iranica

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Table 1. The examined populations of *Thymus*, Gene bank code and location.

S/N	Population	Gene bank code (RIFR)	Location
1	*T. kotschyanus*	10500	Qazvin- Gagazan
2	*T. kotschyanus*	10502	Qom- kahak
3	*T. kotschyanus*	10505	Qazvin- Alamut
4	*T. kotschyanus*	10507	Zanjan-zanjan rood
5	*T. kotschyanus*	10508	Qazvin
6	*T. kotschyanus*	10509	Tehran- savojbolagh
7	*T. kotschyanus*	10510	Azerbaijan-baku1
8	*T. kotschyanus*	10512	Azerbaijan-baku2
9	*T. kotschyanus*	10513	Damavand-Lar
10	*T. kotschyanus*	10515	Alborz-Sirachal
11	*T. daenensis*	10531	Zanjan- Kinevars
12	*T. daenensis*	10534	Qazvin
13	*T. daenensis*	10535	Isfahan
14	*T. daenensis*	10536	Damavand-Homand
15	*T. daenensis*	10537	Lorestan-Khorramabad
16	*T. daenensis*	10538	Isfahan- fereydoon shahr

belong to the *Serphyllum* section and kotschiani sub-section (Rechinger, 1984).

Thyme is one of the most important plants that is highly variable within and between the species and it has been a taxonomically problematic group and views concerning the taxonomic definition of the forms are extremely varied. It has also produced some natural hybrids. Taxonomic complexity of the genus *Thymus* and great difference among the authors as far as their conception of species or other taxa in this genus is concerned make even the complex evaluation of karyological situation difficult (Martonfi and Martonfiova, 1996). In literature, many a times, single data are found and the mistakes in taxa determination cannot be excluded, especially when herbarium specimens are difficult to obtain. In addition, an attitude of particular authors to the interpretation of taxa is not sufficiently clear (Martonfi and Martonfiova, 1996). Above all, these authors contributed to more complex view of chromosome numbers in the genus or, at least, in certain region (Martonfi and Martonfiova, 1996).

Chromosomal information is an important key for taxonomy, phylogeny, evolution, genetics and breeding in thyme plants. However, because of the small chromosome size and the similarity in chromosome morphology, the identification of chromosomes has been difficult in thyme (Javadi et al., 2009; Yavari et al., 2010). The first cytological observation in *Thymus* genus was counting and documenting the chromosome number 2n = 24 in *T. serpyllum* reported by Löve and Löve (1942). Since then, many more researchers have found at least 50 different chromosome number from 2n = 14 of *T. seravschanicus* to 2n = 90 of *T. zygioides* var. lycaonicus (Funamoto et al., 2008). The chromosome numbers in genus *Thymus* known as: 2n = 24, 26, 28, 30, 32, 42, 48, 50, 52, 54, 56, 58, 60, 84 and 90, corresponding to the diploid, tetraploid and hexaploid levels and the secondary basic numbers x = 14 and x = 15 probably originated from a basic number x =7 and the most frequent chromosome numbers are 2n = 28, 30, 56 and 60 (Javadi et al., 2009).

Aneuploidy has been an important phenomenon during the evolution of this genus and it is responsible for the other numbers. There are a lot of interesting cases of different ploidy levels within the same species (Stahl-Biskup and Saez, 2002). Mehrpur et al. (2002) studied two species of the *Thymus* genus karyologically and taxonomically and found two ploidy levels diploid (2n=2x=30) and tetraploid (2n=4x=60) in *T. kotschyanus* species. The researches showed that the morphology and different components of essential oils in different species of *Thymus* are variable due to hybridization and polyploidization (Lopez-Pujol et al., 2004). The main purpose of this study was to investigate the ploidy levels and chromosome number of *T. daenensis* and *T. kotschyanus* from different regions and provinces (16 regions and six provinces) of Iran and to compare the results with previous research (Javadi et al., 2009) so as to obtain a firm and clear results in terms of haploidy levels and karyological characteristics for these species in Iran. Also, the results of this research would be useful for a better understanding of its taxonomy and breeding purposes such as intraspecific hybridization and genetic variation induction.

MATERIALS AND METHODS

In this study, we examined 16 populations representing *T. daenensis* and *T. kotschyanus* species. The names of the populations, gene bank code and location are listed in Table 1.

Mitotic chromosomes were studied in meristematic cells of root tips obtained from rooted cuttings at 20°C. Root tip meristems (1 cm) were pretreated with 0.5% saturated α-bromonaphthalene at 4°C for 4 h, fixed in 10% formaldehyde and 1% chromic acid (1:1) for at least 16 h at room temperature. The root tips were then rinsed for 3 h in distilled water and were hydrolyzed in 1 M NaOH at 60°C for 7 min and stained with hematoxiline for 3 to 4 h at room temperature. The roots were then gently squashed in mixture of 45% acetic acid: lactic acid (10:1).

Cytological investigation

For cytological investigation, images were captured with a BH_2 Olympus supplemented digital color video camera at a magnification of about 2000X. The best metaphasical plates were selected (at least 3 plates) and measured by Micromeasure 3.3 software (Reeves and Tear, 1997 to 2000). In each mitotic metaphase, the arm's length of each chromosome was measured according to previous studies (Hesamzadeh Hejazi and Rasuli, 2006; Hesamzadeh Hejazi and Ziaei Nasab, 2009). The following parameters were estimated in each metaphase plate to characterize the karyotypes numerically: Long arm (LA), short arm (SA), total length (TL) [LA+SA], arm ratio (AR) [LA/SA] and centromeric index (CI) [SA/ (LA+SA)]. Karyotype asymmetry was estimated using the total form percentage (TF%) [(ΣSA/ΣTL)*100] (Huziwara, 1962), difference of range relative length (DRL) [$Max_{RL\%}$-$Min_{RL\%}$], percent of symmetry index (%SI), intrachromosomal asymmetry index (A_1) $\left[1-\sum(\overline{SA}/\overline{LA})/n\right]$ and interchromosomal asymmetry index (A_2) $[S\bar{d}/\overline{X}]$ where *n* is the number of homologues, $\bar{Sd}$ is the average of standard deviation, and $\overline{X}$ is the mean chromosome length (Romero Zarco, 1986). Karyotypic evolution was determined using the symmetry classes of Stebbins (SC) (Stebbins, 1971). Karyotype formula was determined from chromosome morphology based on centromere position in accordance with the classification of Levan et al. (1964). For each population, karyograms were drawn based on length of chro-mosome size (arranged large to small).

Statistical analysis

In order to determine the variation between populations, one-way ANOVA was performed on normal data and mean of parameters were compared by Duncan's multiple range test. The principal components analysis (PCA) was performed to determine the most important variables on the variation between populations. A cluster analysis of the karyotype data performed using the Ward (1963) method to examine karyotype similarity among populations. Numerical analyses were performed using SAS and JMP.

RESULTS AND DISCUSSION

This study reveals a detailed picture of the chromosome features in some *Thymus* species. The pictures of the mitotic metaphases and their karyograms of the popu-lations are presented in Figure 1. The somatic chromo-some numbers (2n), ploidy levels, ranges of chromosome length, symmetry index percentage, intra- and inter-asymmetry indices, difference of range relative length, total form percentage, symmetry classes, total karyotype length (TKL) and karyotype formula (KF) of the taxa and populations investigated are summarized in Table 2. The somatic chromosome number and details of the karyotypes of the studied populations, revealed that *T. daenensis* populations were diploid (2n=2x=30), while *T. kotschyanus* populations possessed two ploidy levels, diploid (2n=2x=30) and tetraploid (2n=4x=60). Similar researches performed in different populations of *T. kotschyanus* also showed that the basic chromosome number was x = 15 and ploidy levels are introduced as diploid and tetraploid (Morales, 1980, 1989; Mehrpur, 2002; Javadi, 2009). This object indicates that the stability level of ploidy of *T. kotschyanus* is unstable and the taxonomic position of this species makes it difficult. Moreover, Mahdavi and Karimzadeh, (2010) reported that four populations of *T. daenensis* from different provinces in Iran contain chromosome numbers with this detail, *T. daenensis* Celak. (Esfahan- Fereydunshahr) 2n=2x=30, *T. daenensis* Celak. (Markazi –Varcheh) 2n=2x=30, *T. daenensis* Celak. (Lorestan –Zagheh) 2n=4x=56 and *T. daenensis* Celak. (Lorestan –Chaghalvandi) 2n=4x=60. However, our study indicated that the chromosome number in the population of the Lorestan province was 2n=2x=30.

Furthermore, total karyotype length, roughly indicative of the DNA content, ranged from 54.39 to 72.51 µm in diploid taxa and from 95.52 to 118.20 µm in tetraploid taxa. Also, size of the chromosomes among the populations and species varied 1.31 in *T. kotschyanus* (10515) to 3.08 µm in *T. daenensis* (10531). All of the populations had mainly 'm' type chromosomes (centro-mers at median region). However, two populations 10537 and 10538 of *T. daenensis* each possessed two 'sm' type chromosomes (Submetacentric). In addition, among the studied populations, the highest TF% value (46.92) and the highest SI% value (72.02) was estimated on *T. kotschyanus* (10513) and the lowest TF% value (43.08) was estimated on *T. daenensis* (10534); thus, the asymmetric karyotype. In view of this fact that fewer DRL value illustrated more symmetry of karyotype, *T. kotschyanus* (10513) and *T. daenensis* (10535) respectively with DRL 1.13 and 3.55 values had the most symmetric and asymmetric karyotypes. Intrachromo-somal asymmetry index (A_1) showed sharp differences between the chromosome arms in the different taxa. In general, based on intrachromosomal asymmetry (A1 and %TF), *T. daenensis* (10534) had the most asymmetric and evolutionary karyotype and *T. kotschyanus* (10513) had the most symmetrical karyotype in all of the populations. According to interchromosomal asymmetry (A2 and DRL), among diploid populations, *T. daenensis* (10535) and among tetraploid populations only *T. kotschyanus* (10515) had the most asymmetrical karyotype. Similarly, high DRL value leads to more changes in the construction of chromosomes, but it is mentioned that the DRL is the dependent to ploidy

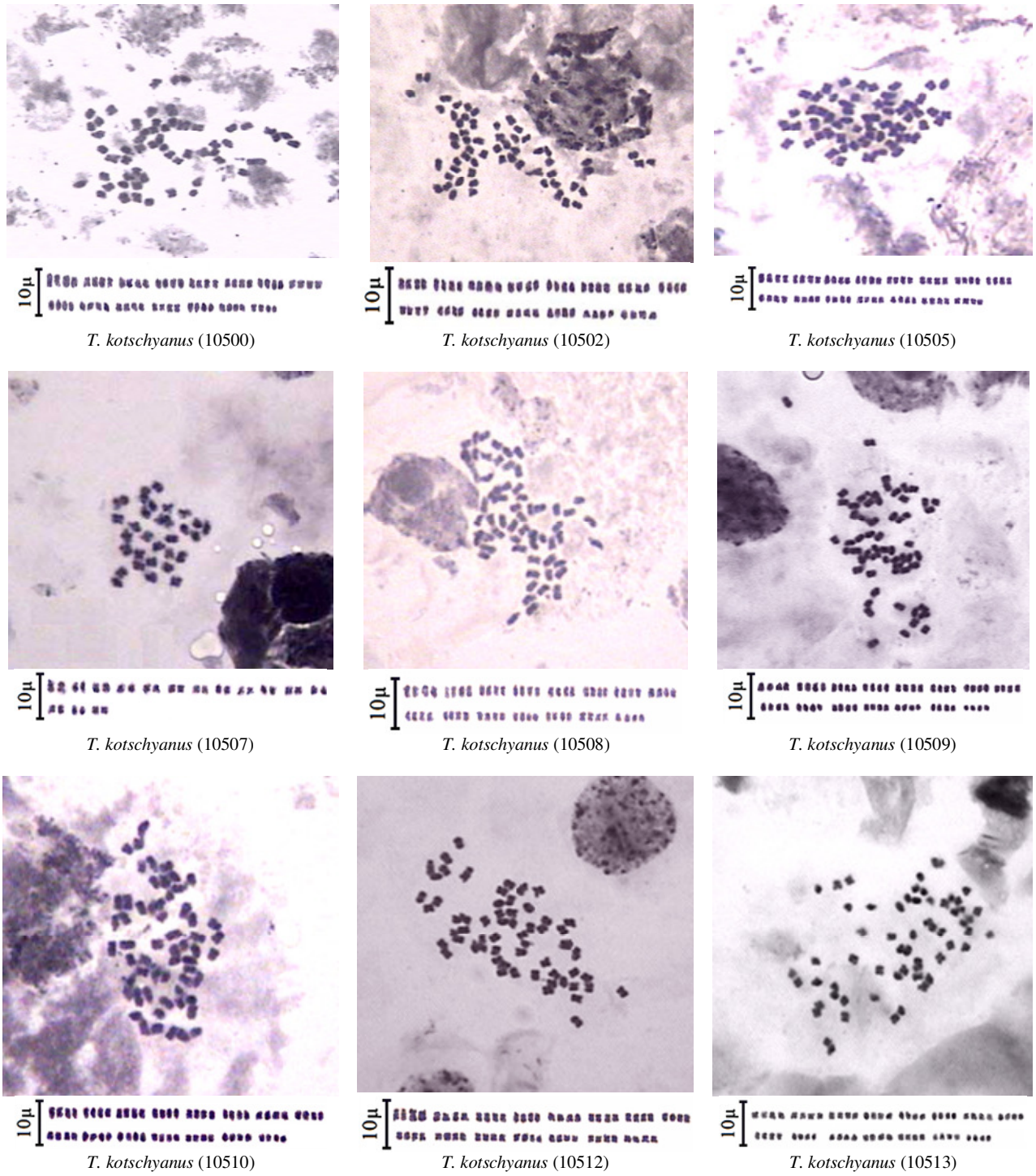
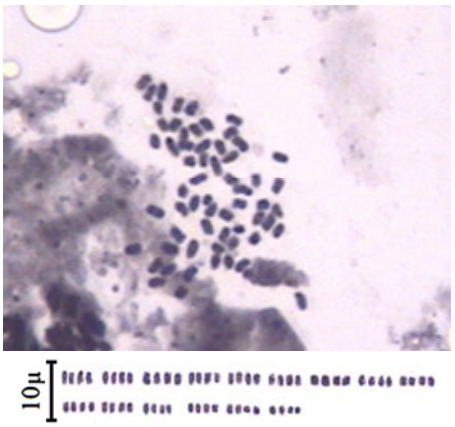


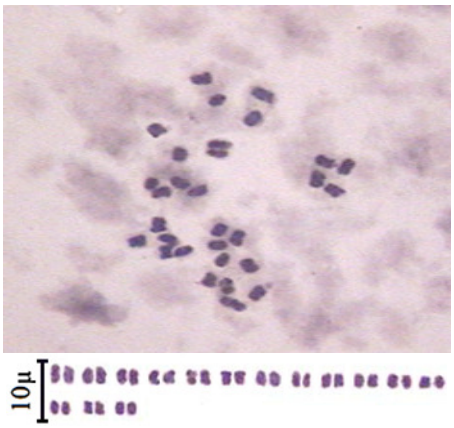
Figure 1. Mitotic metaphase of *Thymus* populations accompanied by karyograms.

levels and chromosome numbers. Therefore, it is not a good criterion for comparing various species with different ploidy levels, because the DRL values are lesser at upper ploidy levels than lower ploidy levels. So, this parameter will be useful for comparison of species with the same ploidy levels.

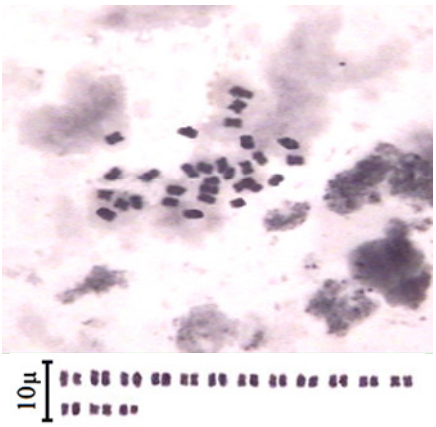
In terms of the Stebbins' system, the karyotype of populations seizes 1A classes were considered majorly primitive classes in this system. By using A_1 and A_2 parameters, we can determine the more asymmetric karyotype among the populations which have the similar Stebbins classes of symmetry. The populations which are



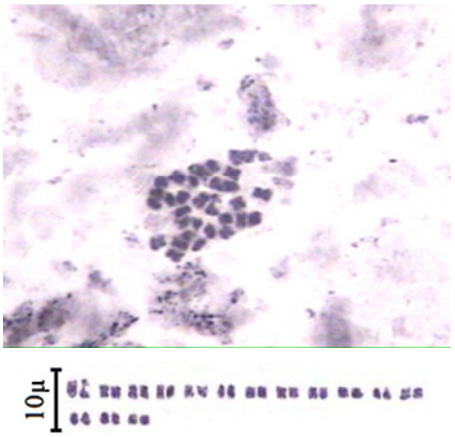
T. kotschyanus (10515)



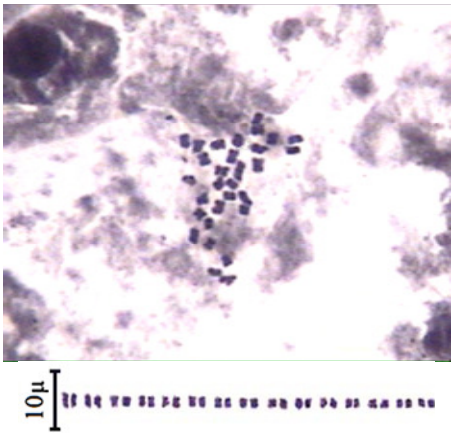
T. daenensis (10531)



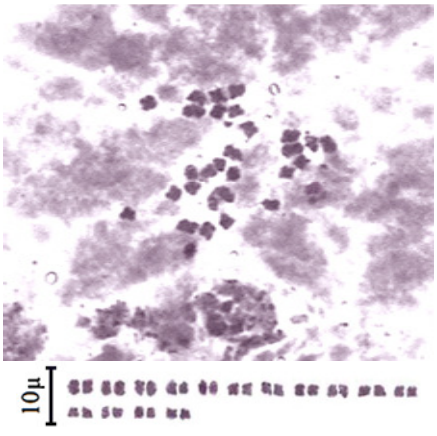
T. daenensis (10534)



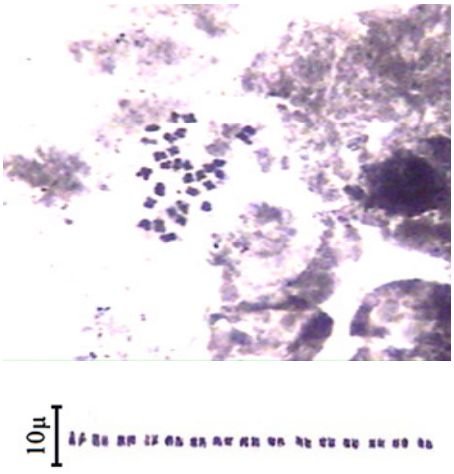
T. daenensis (10535)



T. daenensis (10536)



T. daenensis (10537)



T. daenensis (10538)

Figure 1. contd.

classified as 1A group also showed the lowest value of A_2 in range of 0.078- 0.142 and the highest value of TF% ranged from 43.08 to 46.92. Furthermore, to analyze the variability of the karyotypes among populations, the length of chromosome, the long and short arms of chromosome, the arm ratio values, the difference of range relative length, the total form percentage and asymmetry indexes (A_1, A_2) were compared by one-way analysis of variance (CRD). Also, Duncan test was carried out to test differences between each pair of means. The results of variance analysis revealed significant differences between the populations based on

Table 2. Karyotypic characters of different *Thymus* taxa and populations.

Population	2n	Ploidy level	Chromosome length range	SI (%)	A_1	A_2	DRL	TF %	SC	TKL (µm)	K.F
T. kotschyanus (10500)	60	4x	1.60 - 2.73	58.61	0.205	0.124	1.91	43.99	1A	118.20	60m
T. kotschyanus (10502)	60	4x	1.48 - 2.19	67.58	0.135	0.101	1.29	46.28	1A	110.10	60m
T. kotschyanus (10505)	60	4x	1.55 - 2.30	67.39	0.171	0.102	1.33	45.18	1A	113.16	60m
T. kotschyanus (10507)	30	2x	1.64 - 2.59	63.32	0.141	0.127	3.11	46.06	1A	60.84	30m
T. kotschyanus (10508)	60	4x	1.50 - 2.30	65.22	0.136	0.108	1.43	46.27	1A	112.26	60m
T. kotschyanus (10509)	60	4x	1.33 - 2.25	59.11	0.121	0.120	1.74	46.55	1A	106.14	60m
T. kotschyanus (10510)	60	4x	1.48 - 2.39	61.92	0.200	0.110	1.59	44.11	1A	114.24	60m
T. kotschyanus (10512)	60	4x	1.51 - 2.36	63.98	0.162	0.114	1.55	45.26	1A	110.10	60m
T. kotschyanus (10513)	60	4x	1.39 - 1.93	72.02	0.115	0.078	1.13	46.92	1A	95.52	60m
T. kotschyanus (10515)	60	4x	1.31 - 2.43	53.91	0.220	0.142	2.02	43.41	1A	111.18	60m
T. daenensis (10531)	30	2x	2.06 - 3.08	66.88	0.182	0.104	2.84	44.80	1A	72.51	30m
T. daenensis (10534)	30	2x	1.84 - 2.81	65.48	0.228	0.116	2.91	43.08	1A	66.48	30m
T. daenensis (10535)	30	2x	1.76 - 2.93	60.07	0.172	0.133	3.55	45.15	1A	66.12	30m
T. daenensis (10536)	30	2x	1.68 - 2.73	61.54	0.180	0.131	3.44	44.81	1A	61.62	30m
T. daenensis (10537)	30	2x	1.71 - 2.74	62.41	0.207	0.117	3.11	43.69	1A	66.48	28m + 2 sm
T. daenensis (10538)	30	2x	1.54 - 2.39	64.44	0.143	0.126	3.12	45.69	1A	54.39	28m + 2 sm

SI, Symmetry index; A_1, intrachromosomal asymmetry index; A_2, interchromosomal asymmetry index; DRL, difference of range relative length; TF%, total form percentage; SC, symmetry classes; TKL, total karyotype length; KF, karyotype formula.

all karyotypic characteristics ($P<0.01$). This indicated occurrences of quantitative changes in chromosome size of the studied populations (Table 3). Significant effect of chromosomal traits proved karyotypic variations between populations, thus indicating the importance of chromosome study to distinguish the state of evolution and affinity between different species.

The Duncan's test applied to the chromosome morphometric traits showed a highly significant difference among the all examined populations (Table 4). So, mean value of chromosomes total length was varied from 1.59 in *T. kotschyanus* (10513) to 2.42 µm in *T. daenensis* (10531). The results showed that the average length of chromosomes in the *T. daenensis* is much greater than *T. kotschyanus.* However, chromosomal data in this study reflect the small size of chromosomes in these species. This has been caused many problems associated with karyotype studies. In addition, the mean value of chromosomes long arm was varied from 0.85 in *T. kotschyanus* (10513) to 1.33 µm in *T. daenensis* (10531). Also the mean value of chromosomes short arm was different from 0.75 µm in *T. kotschyanus* (10513) to 1.08 µm in *T. daenensis* (10531). Moreover, using principal components analysis (PCA), the first three independent components accounted about 98% of total variation. The first component indicated that long arm length, arm ratio, total form percentage and intrachromosomal asymmetry index were important characters for classification of populations with about 67% of total variation. Total length of chromosome and short arm length of chromosome were important traits in the second component (20.34%), while third component (10.65%) accentuates A_2 and DRL values (Table 5).

Grouping of studied populations was based on their karyotypic traits (Figure 3). By cutting the dendrogram resulting from cluster analysis by Ward method with cophenetic correlation coefficient (r = 0.86) in metric distance 2.97, the populations classified under four groups which certainly the first and the second components had the most significant role in separated classes. The results show that populations of each species *T. daenensis* and *T. kotschyanus* was grouped in separate cluster. This indicated that each species have specific features of chromosomes. Thus, these studies could greatly help us in the

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

S.O.V	D.F	Mean of square							
		TL	LA	SA	AR	DRL	TF%	A1	A2
Population	15	0.133**	0.050**	0.021**	0.010**	2.233**	4.201**	0.004**	0.001*
Error	32	0.010	0.005	0.002	0.003	0.200	1.2391	0.0014	0.001
C.V. %	-	5.077	6.218	4.586	4.580	19.879	2.469	20.151	16.150

** and * : Significant at 1 and 5%, respectively. DF, Degree of freedom; TL, total length; LA, long arm; SA, short arm; AR, arm ratio; DRL, difference of range relative length; TF%, total form of percentage; A1, intrachromosomal asymmetry index; A2, interchromosomal asymmetry index.

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

Populations	TL	LA	SA	AR	DRL	TF%	A1	A2
T. kotschyanus (10500)	1.97^{bcd}	1.10^{bcdef}	0.87^{fcde}	1.273^{abcd}	1.91^{cd}	43.99^{bcd}	0.20^{ab}	0.12^{ab}
T. kotschyanus (10502)	1.84^{cde}	0.99^{efg}	0.85^{def}	1.160^{bcd}	1.29^{d}	46.30^{abc}	0.13^{bc}	0.10^{ab}
T. kotschyanus (10505)	1.89^{cd}	1.03^{ef}	0.85^{def}	1.211^{abcd}	1.32^{d}	45.27^{abcd}	0.16^{abc}	0.10^{ab}
T. kotschyanus (10507)	2.03^{bc}	1.09^{cdef}	0.93^{bcd}	1.171^{bcd}	3.11^{ab}	46.06^{abc}	0.14^{abc}	0.13^{a}
T. kotschyanus (10508)	1.87^{cd}	1.01^{efg}	0.87^{cdef}	1.160^{bcd}	1.42^{d}	46.31^{abc}	0.13^{bc}	0.11^{ab}
T. kotschyanus (10509)	1.77^{de}	0.95^{fg}	0.82^{efg}	1.148^{cd}	1.73^{d}	46.62^{ab}	0.11^{c}	0.12^{ab}
T. kotschyanus (10510)	1.90^{cd}	1.06^{def}	0.84^{defg}	1.265^{abcd}	1.60^{d}	44.22^{abcd}	0.19^{abc}	0.11^{ab}
T. kotschyanus (10512)	1.84^{cde}	1.00^{efg}	0.83^{efg}	1.210^{abcd}	1.55^{d}	45.25^{abcd}	0.16^{abc}	0.12^{ab}
T. kotschyanus (10513)	1.59^{e}	0.85^{g}	0.75^{g}	1.131^{d}	1.13^{d}	46.92^{a}	0.11^{c}	0.08^{b}
T. kotschyanus (10515)	1.85^{cd}	1.05^{def}	0.81^{fg}	1.305^{ab}	2.02^{bcd}	43.41^{cd}	0.22^{ab}	0.14^{a}
T. daenensis (10531)	2.42^{a}	1.33^{a}	1.08^{a}	1.233^{abcd}	2.82^{abc}	44.80^{abcd}	0.18^{abc}	0.11^{ab}
T. daenensis (10534)	2.22^{ab}	1.26^{ab}	0.95^{bc}	1.323^{a}	2.91^{abc}	43.08^{d}	0.22^{a}	0.12^{ab}
T. daenensis (10535)	2.20^{ab}	1.21^{abcd}	1.00^{ab}	1.214^{abcd}	3.55^{a}	45.16^{abcd}	0.17^{abc}	0.14^{a}
T. daenensis (10536)	2.05^{bc}	1.13^{bcde}	0.92^{bcde}	1.232^{abcd}	3.45^{a}	44.81^{abcd}	0.17^{abc}	0.13^{a}
T. daenensis (10537)	2.22^{ab}	1.25^{abc}	0.97^{b}	1.292^{abc}	3.09^{ab}	43.66^{cd}	0.21^{ab}	0.12^{ab}
T. daenensis (10538)	1.81^{cde}	0.99^{efg}	0.83^{efg}	1.189^{abcd}	3.12^{ab}	45.70^{abcd}	0.14^{abc}	0.13^{a}

TL, Total length of chromosome; LA, long arm; SA, short arm; AR, arm ratio; DRL, difference of relative length; TF%, total form percentage; A_1, intra-chromosome asymmetry index; A_2, interchromosomal asymmetry index.

Table 5. Eigen vectors from the first three principal components for eight karyotype parameters to classify 16 populations of *Thymus*.

Parameter	First component	Second component	Third component
TL	0.344	**0.356**	-0.222
LA	**0.371**	0.260	-0.215
SA	0.290	**0.488**	-0.223
AR	**0.363**	-0.331	-0.049
DRL	0.271	0.393	**0.421**
TF%	**-0.366**	0.321	0.044
A1	**0.367**	-0.312	-0.081
A2	0.228	0.011	**0.815**
Eigen Value	6.029	1.831	0.959
Percentage of Variance	66.993	20.348	10.655
Cum Percentage of variance	66.993	87.342	97.997

TL, total length; LA, long arm; SA, short arm; AR, arm ratio; DRL, difference of range relative length; TF%, total form of percentage; A1, intrachromosomal asymmetry index; A2, interchromosomal asymmetry index.

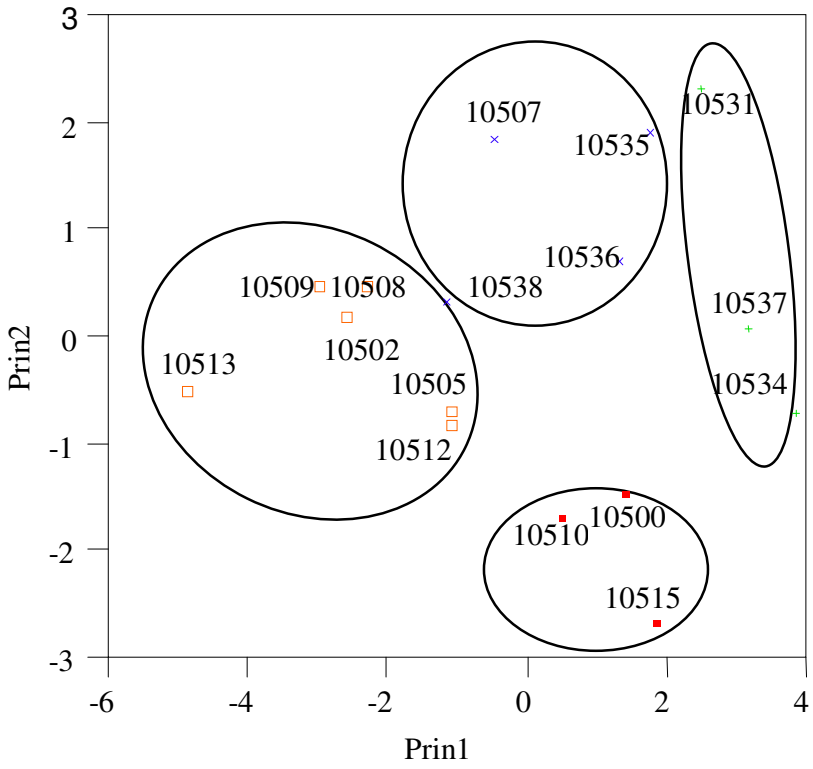


Figure 2. Scatter plot of 16 populations for the first two principals.

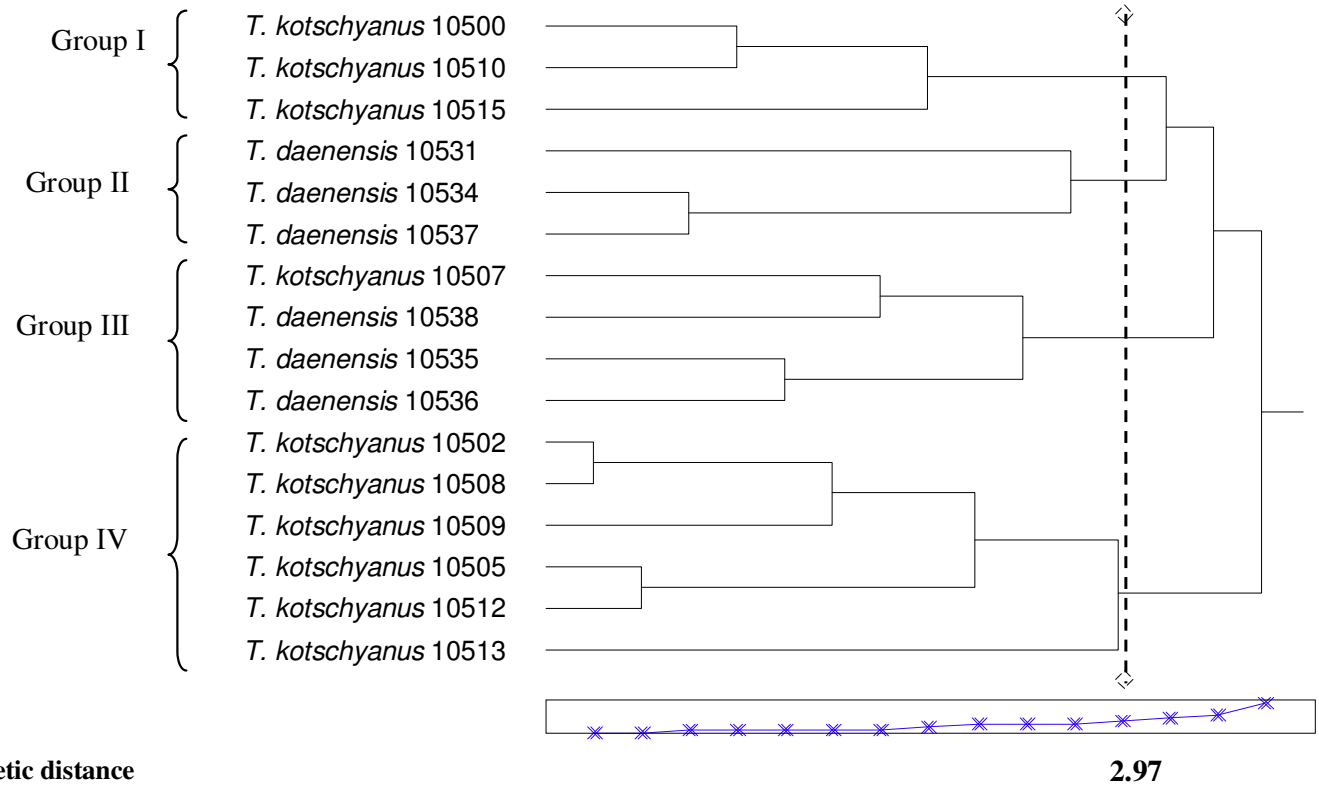


Figure 3. Dendrogram of 16 populations of *Thymus* by analyzing eight karyotypic parameters using Ward cluster analysis method. Cophenetic correlation $r = 0.86$.

classification and taxonomic studies. The first cluster includes the populations of *T. kotschyanus* (10500, 10510 and 10515 with 2n=4x). *T. daenensis* (10531, 10534 and 10537 with 2n=2x=30) through the difference of traits such as LA value and most asymmetrical karyotype, was separately classified as another group (second cluster). *T. kotschyanus* (10507) and populations of *T. daenensis* (10535, 10536 and 10538 with 2n=2x=30) grouped together in same cluster (third cluster) that it seems the factors of similarity were A2 and DRL values (Table 4 and Figure 3). Therefore, the population of *T. kotschyanus* (10507) can be used in hybridization program with *T. daenensis* species. The other populations of *T. kotschyanus* (10502, 10505, 10508, 10509, 10512 and 10513) with the same chromosome number (2n=4x=60) and with similar karyotypic traits (LA, TF% and A1) classified as a fourth group.

The highest metric distance (7.79) was obtained between *T. kotschyanus* (10502) and *T. kotschyanus* (10500) which implies the least affinity between them. The lowest metric distance (0.427) was obtained between two populations of *T. kotschyanus* (10502) and *T. kotschyanus* (10508) which also indicate the least karyotypic difference between them (Figure 3). The diagram of populations' dispersion based on two first components, showed that the populations separated in four groups, which completely fits with results obtained through the grouping analysis by Ward method (Figure 2).

Conclusion

The present study showed the change in the chromosomal traits as one of the mechanism of inter and intra-species diversification in the *Thymus* genus as well as the earlier cytological reports. As a rule, the variation of climate and soil in Iran provides a suitable field for plant variations. One of the genetic variations in *Thymus*, which was clearly detectable, was the chromosome numbers and structural changes of chromosomes. These genomic differences could be used for breeding purposes.

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