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Comparison of Karyotypic Triats of *Thymus* species in Iran

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ABSTRACT

Twelve accessions of thyme medicinal plant belonging to Iranian species of thymus pubescens, T. carmanicus , T. fallax, T. kotschyanus and T. daenensis were studied in aspect of cytogenetical marker. Root tips were examined for karyological studies. Two ploidy levels (diploid and tetraploid) and two chromosome numbers (30, 60) were recognized.Type of chromosomes in all of populations were metacentric (m), and located in 1A except for T. kotschyanus-2(Qom-Karamjegan) with 6sm+24m karyotype formula and 2B stebbins classes. The size of mitotic chromosomes was very small and the mean length of total chromosome varied 0.849 µm in T daenensis-3(Isfahan) to 1.626 µm in T. kotschyanus-2(Qom-Karamjegan). In addition, T. kotschyanus-2 with the highest of A_1 and A_2 (interachromosomal and interchromosal asymmetry index) had karyotype heterogeneity. The results of principal components analysis (PCA) and cluster analysis (Ward) separated all of populations based on ploidy levels and karyotypic traits.

Key words: Chromosome, Karyology, ploidy ,Cluster,Thyme.

INTRODUCTION

Iran has a great deal of ecological diversity and has a rich herbal flora which is still much unstudied regarding inheritance studies [36]. Among these plants, *Thymus* is a polymorphic genus and belongs to the family Lamiaceae [4], usually well known as ‘‘Avishan” in Persian and also well known as a aromatic perennial herb originated from Mediterranean region [10], [32].The genus *Thymus* L. (Lamiaceae) consists of about 928 species of herbaceous perennials and sub shrubs or shrubs. Some botanists recognize 300-400 species in Africa, Europe, and temperate Asia; others have suggested that many of these species should be treated as intraspecific taxa of *Thymus serpyllum* Linnaeus. Klokov reported that fertile hybrids are common in areas of overlap between some species [19].

Eighteen species of *Thymus* are introduced in Iranian flora and among these, four species (*T. persicus, T. daenensis, T. lancifolius* and *T. marandensis*) are native of Iran [10] ,[32] , [13]. *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, and carminative as well as treating colds [1] , [37]. Recent studies imply that these species have strong antibacterial activities [35].

T. pubescens Boiss. & Kotschy ex Celak., Is a perennial plant widely spread out in Iran and Turkey. This plant has low shrubs with woody based stems and recumbent to upright [37]. The flower branch is 2 to 13 cm. The flowers are red or purple-blue and are 5 to 8 mm and flowering begins from spring until summer [13].

T. caramanicus Jalas, is an endemic species grown in Iran. Kermanian thyme has a wooden plant, perennial and Grey colored with C3 metabolism system that will be 25-50 cm tall depending the climate of growth region and soil

quality. At present time, this plant is cultivated in medium scale in Iran, showing antibacterial, antimycotic, antioxidative, natural food preservative, and mammalian age delaying properties [32]. In Iranian folk medicine, leaves of this plant are used in treatment of rheumatism, skin disorders and as an antibacterial agent [37].

T. fallax Fisch Mey, is an aromatic plant belonging to the Lamiaceae family,used for medicinal and spice purposes almost everywhere in the world. It is a pleasant smelling perennial shrub. It is generally distributed in Western Mediterranean (Iran, and Turkey) and Southern Italy [5], [3]. It is also distributed in Inland Anatolia. It grows in several regions of the world such as rocky slopes and grassy areas at 1400-2500 m.

T. kotschyanus Boiss. & Hohen, it is a perennial plant. It grows up to 20cm of height. On the small wooden branches, dark, green sharp and pointy leaves grow. The aromatic leaves are used as spice and medicine. The whit flowers are scented. This species grows in mountainous regions and although is dispersed almost all over the world, but actually accumulates in Mediterranean region. This species has the largest dispersion in Iran [12].

T. daenensis Celak, is an endemic species of Iran. A perennial dwarf shrub native plant to semi-arid zones of Iran is considered as an aromatic and medicinal plant. The aerial parts of *T. daenensis* are commonly used as spices, condiments and flavoring agents [28], [37], [27].Among the species grown in Iran, *T. daenensis* Celak. and *T. kotschyanus* Boiss. & Hohen. are more widely used for these purposes [37], [27]. This plant that grows in most temperate regions, it is recognized from other species by its narrow leaves [2].

Chromosomal information is an important key for taxonomy, phylogeny, evolution, genetics and breeding in Thyme plants. However, the identification of chromosomes has been difficult in thyme because of the small chromosome size and the similarity in chromosome morphology [38], [16], [14], so many of researcher reported of chromosome numbers and others explain methods of research without comparison of species for chromosome characteristics, for example, 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 [20]. In other work, *T. praecox* was considered as a species with various chromosome numbers of 24, 28, 50, 54, 56 and 58 [7]. The species of *T. herba-barona* Loisel.subsp. *herba-barona* showed 2n=2x=28, 2n=4x=56 and even 2n=6x=84 [26]. Data on chromosome numbers in the genus *Thymus* sect. *Serpyllum* from Carpathians and Pannonia, are presented 21 new data for 9 species at the same time. In two cases they represented the first datum for: *T. alternans* Klokov 2n=56 and *T. bihoriensis* Jalas, 2n=28 [21]. So, these variations in chromosome number actually show the most variations in *Thymus* species, that it is request more research about this genus.

Here, we present the first report of the chromosome numbers, ploidy levels and comparison of karyotypic triats of some populations of *T. daenensis*, *T. kotschyanus*, *T. pubescens*, *T. carmanicus*, and *T. fallax* collected in Iran. 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

***Plant materials*—**Seeds and clones of plants were collected from various locations (Tab.1). The plants were identified by Flora Iranica [28]. Vouchers are deposited in gene bank RIFR (Research Institute of Forest and Rangelands) from Iran.

Chromosome analysis— Mitotic chromosomes were studied in meristematic cells of root tips (1-2 cm in length) obtained from seeds and rooted cuttings at 20°C. Root tip meristems were pretreated with 0.5% saturated Alphabromo-Naphthalene [38] for 4 h at 4°C, fixed in Chromic acid 1%, Formaldehyde 10% (1:1) for 24 h at 25°C [8], then the root tips were rinsed in distilled water for 3h, and were hydrolyzed in 1 M NaOH at 60 °C for 5 min, and then rinsed in distilled water for 2-3 min. Finally, staining was carried out using Aceto-Iron-Hematoxylin for 5 h. at room temperature. After staining, the root tips washed in distilled water for 2 h. Then slides were prepared by squashing in a droplet of 45% acetic acid, metaphases were captured using an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of about 2000x.
The best metaphase plates were selected and used to prepare the karyotype by Adobe Photoshop 7.0 software, finally karyotypic characters measured by Micro Measure 3.3 software [29]. For each population five karyograms were drawn based on length of chromosome size (arranged large to small).

***Karyotype characterization*—**The following parameters were measured in each metaphase plate to characterize the karyotypes numerically: diploid number of chromosomes(2n), long arm(LA), short arm(SA), total length(TL=LA+SA), arm ratio(AR=LA/SA), centromeric index [CI=SA/(LA+SA)], difference of range relative length ($DRL=Max_{RL\%}-Min_{RL\%}$), value of relative chromatin(VRC=ΣTL/n), karyotype formula(KF) according to

Levan's method [17], contribution of each arm from each chromosome to the total length of the karyotype (LA%)=[(LA/Σ(LA+SA))×100, (SA%)=[(SA/Σ(LA+SA)×100], total form percentage(TF%)=[(ΣSA/ΣTL) ×100] [9], interachromosomal asymmetry index (A_1)=1-[Σ(SA/LA)/n], where SA and LA are the mean length of short and long arms of each pair of homologous, respectively and n is the number of homologous, interchromosomal asymmetry index(A_2)=s/x, where s and x are the average of standard deviation and mean of chromosome length respectively[30], percent of symmetry index(%SI)=[(length of smallest chromosome/length of longest chromosome)×100]; centromeric gradient value (CG)=[(length of median short arm/length of median chromosome)×100; dispersion index (DI)=[A_2×CG] [18]. Also karyotypic evolution has been determined using the symmetry classes of Stebbins (SC), [34].

In order to determine the variation between populations, unbalanced completely randomized design (CRD) was performed on normal data and parameter means were compared by Duncan's test. The principal components analysis (PCA) was performed to evaluate the contribution of each karyotypic parameter to the ordination of species. Clustering was performed using the Ward method after calculation of cophenetic correlation coefficient (*r*) to examine karyotype similarity among populations. Numerical analysis were performed using SAS ver. 6.12 [31], JMP ver. 3.1.2 [15], and STATISTIXL ver. 1.7 Softwares [33].

Table 1- populations of *Thymus* karyologically studied

population	Location	Gene bank code(RIFR)
T. daenensis(1)	Isfahan-Arabshah	10533
T. daenensis(2)	Gazvin-Gagazan	10529
T. daenensis(3)	Isfahan	10572
T. kotschyanus(1)	Gazvin-Tarom	10501
T. kotschyanus(2)	Qom-Karamjegan	10503
T. kotschyanus(3)	Qom- Kahak	10504
T. pubescens(1)	Uromeyeh	10577
T. pubescens(2)	Savojbolag	10549
T. pubescens(3)	Damavand- uzin	10553
T. carmanicus(1)	Isfahan-1	10572
T. carmanicus(2)	Isfahan-2	10573
T. fallax	Hamadan-Nahavand	10543

RESULTS

With relation to the plant material assayed, Karytype analyses of five different species (12 populations) of *Thymus* were determined. This study reveals a detailed picture of the chromosome features in some *thymus* species.

Chromosome numbers—The pictures of the mitotic metaphases and their karyograms of the populations were presented in figure1. The results showed that the basic chromosome number was x=15, and ploidy levels were different in populations (2x and 4x). The somatic chromosome numbers (2n) and karyotypic details for the studied populations were presented in Table 2.

Chromosome size and karyotype features— Size of chromosomes in all of populations were small, and type of all chromosomes usually were metacentric (m) and rarely sub-metacentric(sm)**.** Symmetry of Stebbin's (SC) and asymmetry indices of Romro-Zarco (A_1 and A_2) are given in Table 2, and represented graphically in figure 2. The relationship of asymmetry indices (A_1 and A_2) with respect to Stebbins' classification clarified in figure 2.

Analysis of karyotype characteristics— A statistical comparison based on unbalanced completely randomized design demonstrates that there are significant differences among the species for all the measured traits except, AR, CI, TF%, CG and A_1 ($P<0.01$ & $P<0.05$)(Tab.3). Therefore, these results indicate significant quantitative changes in amount of chromatin in *Thymus* species. Mean of chromosomes analysis of *Thymus* populations (TL, LA, SA, AR, CI, TF%, DRL A_1, A_2, LA%, SA%, SI, CG and DI), based on Duncan's test represented in Table 4. The mean value of chromosomes total length (TL) was varied from 1.625 µm in *T. kotschyanus* (2) to 0.849 in *T. daenensis*(3). The mean value of chromosomes long arm (LA) was varied from 0.957 µm in *T. kotschyanus* (2) to 0.479 in *T. daenensis*(3). The mean value of chromosomes short arm (SA) was varied from 0.669 µm in *T. kotschyanus*(2) to 0.370 in *T. daenensis*(3). The value of arm ratio (AR) was varied from 1.417 µm in *T. kotschyanus*(2) to 1.238 µm in *T. carmanicus*(2). The value of centromeric index(CI) was varied from 0.447 µm in *T. carmanicus*(2) to 0.415 µm in *T. kotschyanus*(2), so the highest and the lowest of AR and the lowest and the highest of CI were in *T. kotschyanus*(2) and *T. carmanicus*(2), respectively.

Using principal component analysis (PCA), of the karyotypic parameter shows that the first three principal components justify %99.28 of total variance. In the first component, A_2, SI and DI values which had the highest coefficients of eigen values, were the most important traits. In the second component, LA%, SA% and DRL and finally in third component, Total Length (TL), long arm (LA) and short arm(SA) had the most important role for total variation (Table 5). By cutting dendrogram resulted from cluster analysis (Ward) based on their karyotypic traits on Table 4, the populations were classified into four groups, in metric distance 1.79 (Fig. 4). The highest distance (6.97) were obtained between *T. daenensis*(1) and *T. kotschyanus*(2), that indicates the least affinity between them. The lowest metric distance (0.29) values were obtained between two populations, *T. daenensis (1) and T. pubescens* (2) that indicate the highest affinity between them.

DISCUSSION

Since *Thymus* is an out-crossing plant and have inter and intra species hybridization, so they show morphologically and genetically variations among themselves. One of the genetically variations in *Thymus* is the number of chromosomes that it is clearly detectable. We have to study genetically and cytogenetically traits before to begin of improvement of plants. In recent years karyological analysis have an important role in solving taxonomic problems. Cytological studies on *Thymus* genus are very limited. Some researchers contributed for a few species on these subjects, but just a few of them have explained about morphometry of chromosomes and the others just have given chromosome numbers [16].

The most frequent basic chromosome numbers described for *Thymus* genus are x=7, x=14 and x=15. Some researches performed on different species of *Thymus* and the results showed that the basic chromosome number was x=15 and ploidy level introduced diploid and tetraploid [14], [38], [16], but other researches showed the basic chromosome number was x=15 or x=14 with different ploidy level such as 2n=60, 2n=58, 2n=56 and 2n=54 [11], [25].

This study showed that the basic chromosome number was x=15 and the ploidy levels were different among populations (2n=2x=30, 2n=4x=60). The chromosome number of different populations of *T. daenensis* were different (2n=2x=30 and 2n=4x=60) but among populations of *T. kotschyanus*, *T. pubescens* and *T. carmanicus* were the same (2n=4x=60) and *T. fallax* with one population was diploid (2n=2x=30). Morales showed that a main basic chromosome number is x=7 that the other basic chromosome numbers(x=14 & x=15) probably originated from x=7 [25] , [26] . More studies indicated different ploidy levels in the same species. So these results show polyploidy occurs in this genus. Different populations of *T. daenensis* and *T. kotschyanus* showed two ploidy levels(2n=2x=30 & 2n=4x=60) [14] .Also Mehrpur and Morales showed that different populations of *T. pubescens* and *T. kotschyanus* with x=15 have two ploidy levels(diploid & tetraploid) [22],[23].In *T. kotschyanus*, some researchers showed x=15 with two ploidy levels(diploid & tetraploid), but other researchers showed that x=14 or 15 with 2n=60, 57, 56 and 54 [24], [6] ,[21]. [38].

In this research the chromosome number of *T. carmanicus* and *T. fallax* are being reported for the first time.

Type of chromosomes in all populations were metacentric and are located in stebbins classes(SC) 1A, except for *T. kotschyanus*(2) that with 6Sm+24m karyotype formula is located in 2B class. Also *T. kotschyanus*(2) with the highest value of arm ratio(AR) and the lowest value of centromeric index(CI) and *T. carmanicus*(2) with the highest values of CI &, TF% and the lowest value of AR have asymmetric and symmetric karyotype respectively.

In addition, asymmetric karyotype can be determined by using of Romero-Zarco asymmetry indexes (A_1, A_2), TF% and DRL values. *T. kotschyanus*(2) with symmetric classes(2B), have the highest A_1(0.272), A_2(0.230) and DRL(3.202) values, and the lowest value of TF%(41.511), was introduced as the most asymmetric karyotype among populations. On the contrary *T. carmanicus*(2) with symmetric classes(1A), had the lowest value of A_1(0.181) and the highest value of TF%(44.692), was introduced as the most symmetric karyotype (Table 2).

Other parameters for measuring of karyotype asymmetry are dispersion Index (DI), Symmetry Index percentage (SI) and Centromeric Gradient (CG) values. In order to refine the measure of karyotype asymmetry we used DI index that has the potential to decipher even the minor karyotipic variations. *T. kotschyanus*(2) had the lowest of SI(40.044) and the highest of DI(3.124), so *T. kotschyanus*(2) had the most asymmetric karyotype among in twelve populations. Karyotypes with high levels of symmetry have been considered to be primitive species [34].

The variance of different populations according to A_1 and A_2 values in addition to various symmetrical states by stebbins is presented in figure 2. This diagram shows that the *T. kotschyanus*(2) with stebbins classes(2B) and the

most of A_1 and A_2 values, separated from other populations and located in above of diagram. Other details of karyotype characteristics showed at Tabel 2.

The highest VRC among all populations was recorded for *T. kotschyanus*(2) (Qom-Karamjegan) (1.625) and the lowest was for *T. daenensis*(3)(0.849), this subject shows that the *T. kotschyanus*(2) have the long chromosomes and the *T. daenensis*(3) have the short chromosomes compared to the other populations.

Analysis of variance for karyotypic data based on unbalanced completely randomized design (CRD) demonstrates that there are significant differences among populations for all of the measured traits except AR, CI, TF%, A_1 and CG, so each of traits can be created variance among these populations of *Thymus* species (Table.3).

In principal components analysis(PCA), with regards to the importance of the first component in grouping the populations, it seems that the basic factor for separation the populations of *thymus* genus was interchromosomal asymmetry index, for example *T. kotschyanus*-2 had the highest A_2 and DI values with the lowest of SI value in comparison with other populations, and in second component the main traits that they have role in grouping of populations were LA% , SA% and DRL values, so *T. daenensis*-2 and *T. daenensis*-3 and *T. fallax* (2n=2x=30) had the highest LA% and SA% and almost the highest, DRL, so they are located in the same group, and in third component , TL, LA and SA values were the main traits in grouping of populations(Tab.5 and fig.2).

The results of Cluster analysis (Ward) revealed that *T. kotschyanus*-2 with had the highest of A_2; classified in a separate group and *T. daenensis*-2, *T. daenensis*-3 and *T. fallax* with the same chromosome number (2n=2x=30), grouped together and *T. kotschyanus*-1, *T. carmanicus*-1 and *T. carmanicus*-2 with had similar LA and SA values grouped together and other populations with had similar karyotypic traits (TA, LA, SA, AR, CI, SI) separated from other populations and located in the same group. Hence this grouping correctly separate populations based on PCA, and refer to ploidy levels and traits of chromosomes. So in grouping populations of *Thymus* spp. intrachromosomal asymmetry index and ploidy levels are the importance traits (fig. 4).

Finally previous reports and our recent findings 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. On the other hand the different species of *Thymus* (diploid and tetraploid) have symmetric and primitive karyotypes (have small and metacentric chromosomes), probably indicating inter or intra hybridization, such similarity in their karyotyes does not prevent their successful crosses and disturbance in reproduction.

In this sense, our results are useful in study of evolutionary species by symmetric and asymmetric karyotypes, breeding, plant taxonomy and phylogenetic analysis.

Table 2- Karyotype characteristics of twelve populations of *Thymus*.

Taxon	2n	SC	AR	CI	A_1	A_2	LA%	SA%	TF%	DRL	VRC	SI	CG	DI	KF
T. daenensis-1	60	1A	1.333	0.429	0.238	0.134	1.904	1.429	42.875	1.839	0.882	56.897	42.613	2.377	30m
T. daenensis-2	30	1A	1.276	0.439	0.208	0.131	3.737	2.929	43.942	2.965	0.916	62.599	43.394	2.377	15m
T. daenensis-3	30	1A	1.288	0.437	0.209	0.140	3.750	2.916	43.737	3.061	0.849	62.603	44.365	2.456	15m
T. kotschyanus-1	60	1A	1.306	0.434	0.221	0.191	1.888	1.446	43.371	2.768	1.026	43.155	43.157	2.872	30m
T. kotschyanus-2	60	2B	1.417	0.415	0.272	0.230	1.950	1.384	41.511	3.202	1.626	40.044	42.477	3.124	6sm+24m
T. kotschyanus-3	60	1A	1.302	0.434	0.220	0.139	1.885	1.448	43.450	2.157	0.926	55.804	42.372	2.397	30m
T. pubescens-1	60	1A	1.327	0.430	0.234	0.121	1.900	1.432	42.975	1.728	0.934	57.978	43.223	2.288	30m
T. pubescens-2	60	1A	1.280	0.439	0.207	0.139	1.871	1.462	43.870	1.745	0.876	58.555	43.152	2.444	30m
T. pubescens-3	60	1A	1.270	0.441	0.205	0.118	1.864	1.469	44.064	1.433	0.935	64.659	43.401	2.248	30m
T. carmanicus-1	60	1A	1.314	0.432	0.229	0.170	1.892	1.441	43.245	2.203	0.876	51.148	43.076	2.706	30m
T. carmanicus-2	60	1A	1.238	0.447	0.181	0.154	1.844	1.496	44.692	2.186	0.893	52.687	44.885	2.629	30m
T. fallax	30	1A	1.318	0.431	0.226	0.124	3.790	2.877	43.153	2.870	1.132	64.154	44.049	2.335	15m

2n-somatic chromosome number, SC-symmetry classes of Stebbins, AR-arm ratio, CI-centeromeric index, A_1-intrachromosome asymmetry index, A_2-interchromosome asymmetry index, LA%-relative length of long arm, SA%-relative length of short arm, TF%-total form percentage, DRL-difference of relative length, VRC- value of relative chromatin, SI-symmetry Index percentage, CG-centromeric gradient, DI-dispersion Index, KF-karyotype Formula(m: metacentric, sm: submetacentric).

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

Source of variation	Degrees of freedom	Mean of squares												
		TL	LA	SA	AR	CI	TF%	DRL	A_1	A_2	LA%	SA%	SI	CG	DI
Population	11	0.103**	0.039**	0.015**	0.005^{ns}	0.0001^{ns}	1.853^{ns}	0.140**	0.001^{ns}	0.004*	2.776**	1.681**	174.836*	2.153^{ns}	0.174*
Error	29	0.018	0.007	0.002	0.003	0.0001	1.265	0.035	0.0009	0.001	0.002	0.002	64.269	2.577	0.078
CV%		14.252	15.865	12.659	4.742	2.586	2.586	12.545	14.384	11.037	1.988	2.580	14.071	3.699	11.275

Table 4- Mean of chromosome analysis of *Thymus* populations. TL-total length, LA-long arm, SA-short arm, AR-arm ratio, CI-centromeric index, TF%- total form percentage, DRL-difference of relative length, A1-intrachromosome asymmetry index, A2-interchromosome asymmetry index, LA%-relative length of long arm, SA%-relative length of short arm, SI-symmetry Index percentage, CG-centromeric gradient, DI-dispersion Index.

Taxon	TL	LA	SA	AR	CI	TF%	DRL	A_1	A_2	LA%	SA%	SI	CG	DI
T. daenensis-1	0.882c	0.503bc	0.378c	1.333ab	0.429ab	42.875ab	1.839cd	0.238ab	0.134bc	1.904bc	1.429 bc	56.897a	42.613a	2.377bc
T. daenensis-2	0.916bc	0.513bc	0.402bc	1.276b	0.439a	43.942a	2.965ab	0.208b	0.131c	3.737a	2.929 a	62.599a	43.394a	2.377bc
T. daenensis-3	0.849c	0.479c	0.370c	1.288b	0.437a	43.737a	3.061ab	0.209b	0.140bc	3.750a	2.916 a	62.603a	44.365a	2.456bc
T. kotschyanus-1	1.025bc	0.581bc	0.445bc	1.306b	0.434ab	43.371ab	2.768abc	0.221ab	0.191ab	1.888bc	1.446 bc	43.155bc	43.157a	2.872ab
T. kotschyanus-2	1.625a	0.957a	0.669a	1.417a	0.415b	41.511b	3.202a	0.272a	0.230a	1.950b	1.384 c	40.044c	42.477a	3.124a
T. kotschyanus-3	0.925bc	0.524bc	0.402bc	1.302b	0.434ab	43.450ab	2.157bcd	0.220ab	0.139bc	1.885bc	1.448 bc	55.804ab	42.372a	2.397bc
T. pubescens-1	0.933bc	0.532bc	0.401bc	1.327ab	0.430ab	42.975ab	1.728d	0.234ab	0.121c	1.900bc	1.432 bc	57.978a	43.223a	2.288c
T. pubescens-2	0.876c	0.492bc	0.384c	1.280b	0.439a	43.870a	1.745d	0.207b	0.139bc	1.871bc	1.462 bc	58.555a	43.152a	2.444bc
T. pubescens-3	0.935bc	0.523bc	0.412bc	1.270b	0.441a	44.064a	1.433d	0.205b	0.118c	1.864c	1.469 b	64.659a	43.401a	2.248c
T. carmanicus-1	0.876c	0.497bc	0.379c	1.314ab	0.432ab	43.245ab	2.203abcd	0.229ab	0.170abc	1.892bc	1.441 bc	51.148abc	43.076a	2.706abc
T. carmanicus-2	0.892bc	0.494bc	0.399bc	1.238b	0.447a	44.692a	2.186abcd	0.181b	0.154bc	1.844c	1.496 b	52.687abc	44.885a	2.629bc
T. fallax	1.132 b	0.645 b	0.486 b	1.318 ab	0.431 ab	43.153 ab	2.870 ab	0.226 ab	0.124c	3.790 a	2.877 a	64.154a	44.049a	2.335c

***-Significant at 1% level of probability, *- Significant at 5% level of probability, ns- Non significant*

Table 5- Eigenvectors from the first three principal components for seven karyotype parameters to classify twelve populations of *Thymus*.

	TL	LA	SA	DRL	A_2	LA%	SA%	SI	DI	Eigen Value	Percent of Variance	Cum percentage of variance
First component	0.389	0.389	0.387	0.227	0.406	-0.109	-0.129	-0.373	0.403	5.373	59.704	59.704
Second component	0.133	0.129	0.139	0.469	-0.060	0.585	0.575	0.215	-0.049	2.71	30.145	89.850
Third component	0.404	0.401	0.407	-0.37	-0.33	-0.074	-0.103	0.351	-0.354	0.49	9.43	99.279

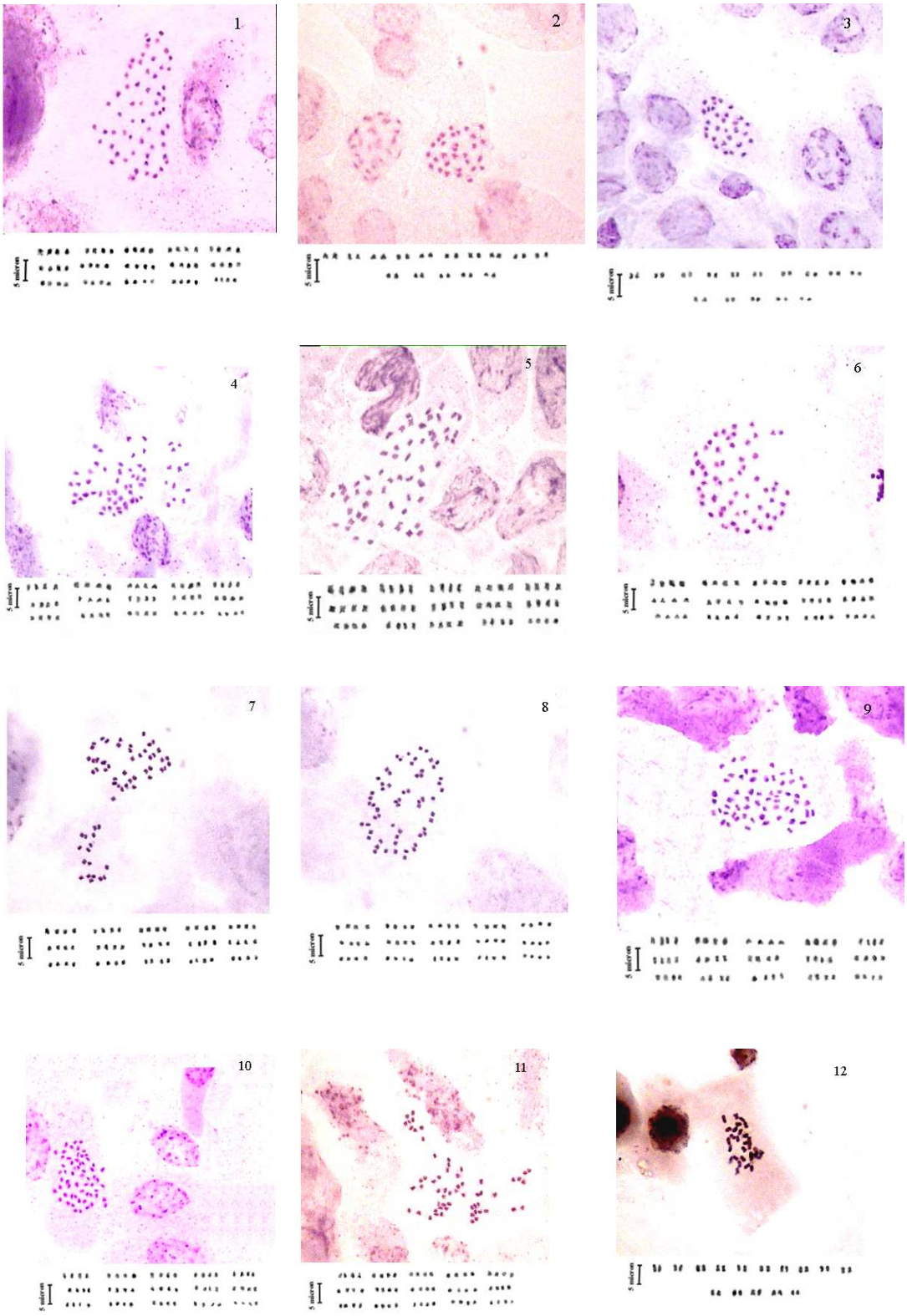

Fig.1- Mitotic metaphase of *Thymus* populations accompanied by karyograms.

1.*T.daenensis*-1(2n=4x=60);2.*T.daenensis*-2(2n=2x=30);3.*T.daenensis*-3(2n=2x=30); 4.*T.kotschyanus*-1(2n=2x=60);5.*T.kotschyanus*-2(2n=2x=60);6.*T.kotschyanus*-3(2n=2x=60); 7.*T.pubescens*-1(2n=2x=60);8.*T.pubescens*-2(2n=2x=60);9.*T.pubescens*-3(2n=2x=60); 10.*T.carmanicus*-1(2n=2x=60); 11.*T.carmanicus*-2(2n=2x=60); 12.*T.fallax*(2n=2x=30).

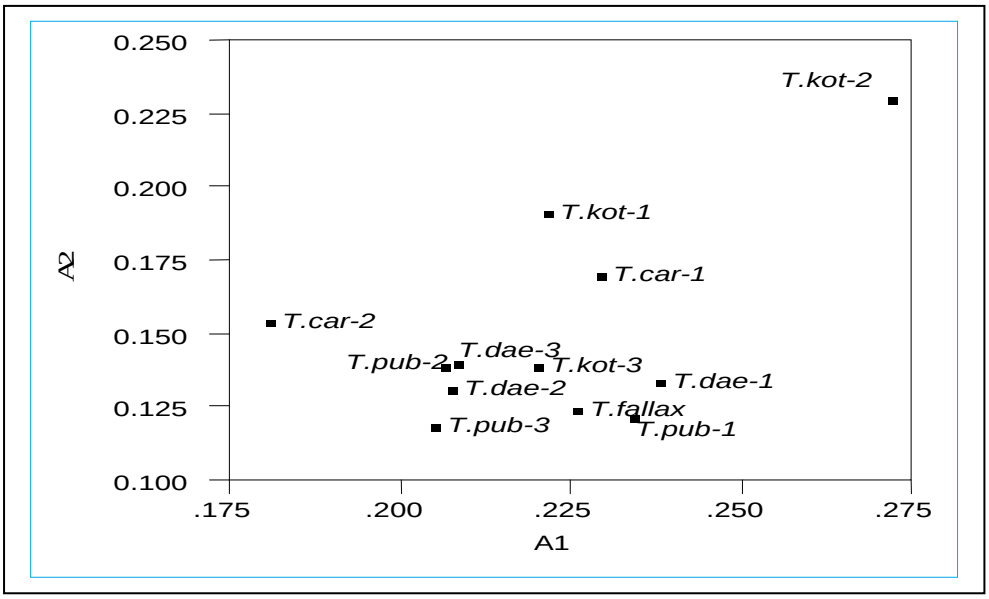

Fig 2- Scatter diagram of the Romero-Zarco asymmetry Indices with Stebbins, symmetry types. Values of A_1 and A_2 are summarized in Tab.2.

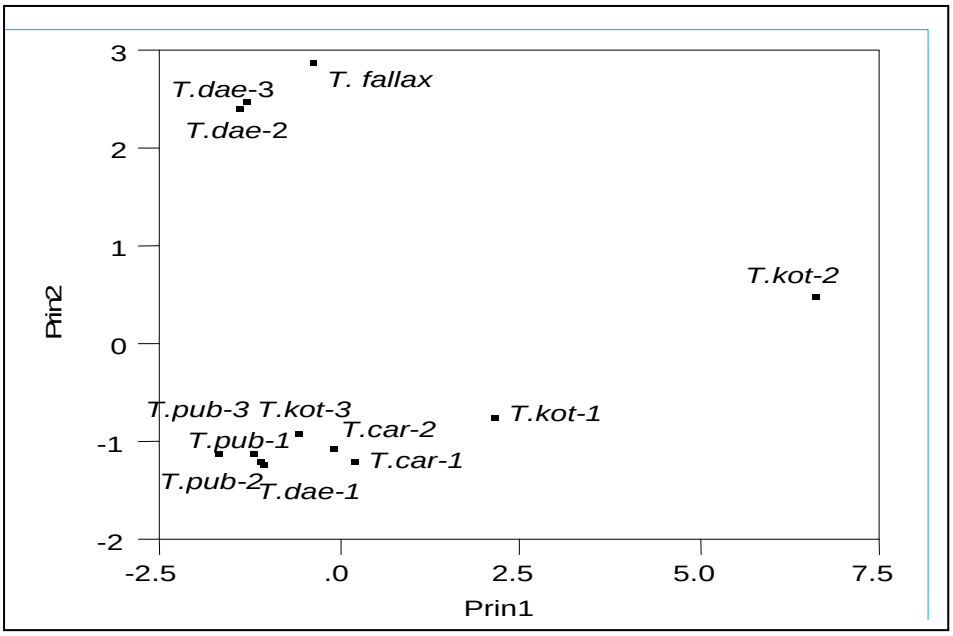

Fig. 3- Scatter plot of twelve populations for the first two principal components

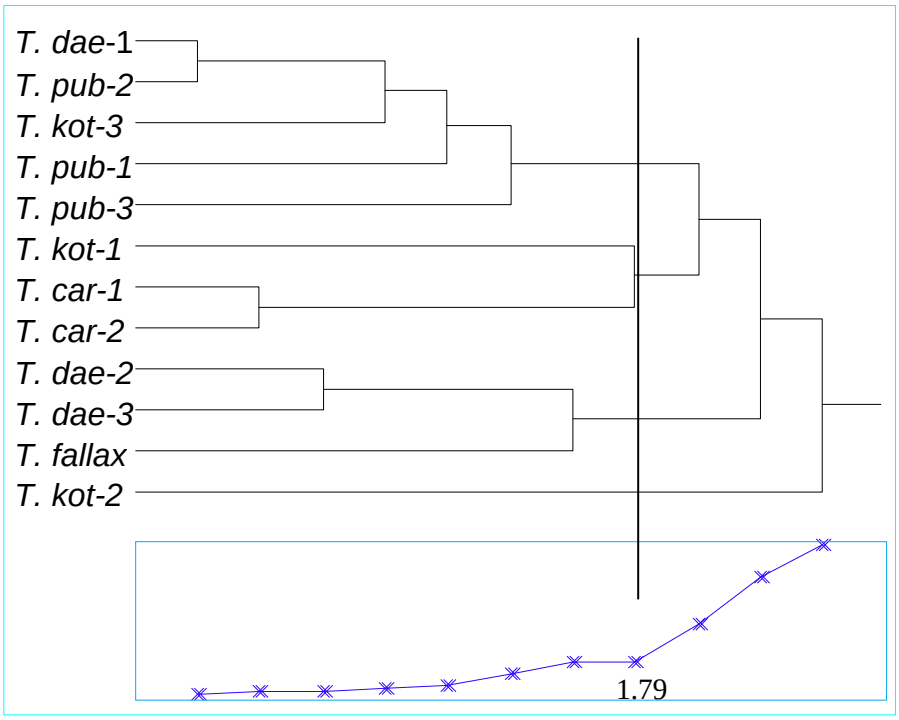

Fig. 4- Dendrogram of twelve populations of *Thymus* by analyzing 9 karyotypic parameters using Ward cluster analysis method. Cophenetic correlation γ=0.89.

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REFERENCES

[1] G. Amin**,** *Popular medicinal plants of Iran. Tehran, Iran: Tehran University of Medical Sciences Press*, **2005**, 1.
[2] B. Bahrini**,** *Final report in : Research Institute of Forests and Rangelands***. 2002**, 1.
[3] T. Baytop. *Ankara. Publication of the Turk Dil Kurumu (The Turkish Language Society)* **1997**, No: 578.
[4] M. Çelik, F. Ünal, D. Yüzbaşıoğlu, S. Yılmaz, H. Aksoy and Ş. Karaman, *Cytotechnology*, **2006**, vol.: 51(**2**), 99-104.
[5] P.H. Davis**,** *Edinburgh University Press: Edinburgh.* **1982**, Vol.**7**, Pp.349.
[6] J.A. Elena-Rossello**,**. *Anales Jard. Bot.* Madrid, **1981**,38(**1**):51-60.
[7] A. Fernandes and M.T. Leitao**,**. *Mem. Soc. Brot.*, **1984**, **27**: 27-75.
[8] S.M. Hesamzadeh Hejazi , M. Ziaei Nasab, *Caryologia*, **2010**, Vol.63 (1), 18-31.
[9] Y. Huziwara**,** *Amer. J. Bot.*, **1962**, 49: 116-119.
[10] J. Jalas**,** *Flora Iranica* **1982**, No. **150**, pp. 536–538. New York: Springer.
[11] J. Jalas, *Hereditas*, **2010**, Vol.**34**: 414-434.
[12] A. Jamshidi, M. Aminzadeh, H. Azarnivand, and M. Abedi, *Medicinal plant research network* (MPRN) **2006**.
[13] Z. Jamzad**,** *Research Institute of Forsts and Rangelands publication*, **2009**, Pp: 171
[14] H. Javadi, S.M. Hesamzadeh Hejazi, M. Babayev, *Caryologia*, **2009**.Vol.62 (**4**):316-325.
[15] Jump, *JMP/STAT for windows, version 3.1.2.SAS Institute Inc*, **1995**.
[16] R. Kalvandi, S. M. Hesamzadeh Hejazi, M. Atri, M. Mirza, Z. Jamzad, K. Safikhani**,** *Asian.J. of cell Biology*, **2012,** 3(8):3916-3925
[17] A. Levan, K. Fredge, and A. A. Sanberge. *Hereditas*, **1964**, **52**:201-220.
[18] U.C. Lavania, and S. Srivastava, *J.Biosci.*, **1992**, 17(2):179-182.
[19] H. W. Li, L.C. Hedge**,** *Flora of China*, **1994**, Vol.17.
[20] J. Lopez-Pujol, M. Bosch, J. Simon, and C. Blanche, *Ann. Bot.*, **2004**, **93**: 1-10.
[21] P. Mártonfi, L. Mártonfiová **,** *Thaiszia*, **1996, 6**: 25-38.
[22] S. Mehrpur, H. Mirzaie-Nodoushan, A. Majd, and F. Sefidkon, *Cytologia*, **2002**, **67**:343-346.
[23] R. Morales, *Anales Jard. Bot.* Madrid, **1980**, 36:339-348 (In Spanish).
[24] R. Morales**,** *Anales Jard. Bot.* Madrid, **1981**, 38(**1**):253-257.
[25] R. Morales, *J. Ruizia.*, **1986**, **2**: 321-324.

[26] R. Morales**,** *Taylor and Francis, London*, **2002**. Pp. 1- 43.
[27] B. Nickavar, F. Mojab, R. Dolat-Abadi**,** *Food Chem.J.* **2005**, **90**:609–611
[28] K.H. Rechinger**,** *Flora Iranica*. **1982**, No. **150**, Pp 540-546.
[29] A. Reeves**,** *Genome*, **2001**,**44**: 439-443.
[30] Z.C. Romero**,** *Taxon*, **1986**,**35**:526-530.
[31] SAS**,** *SAS Institute Inc, Cary, NC 27513, USA*, **1996.**
[32] E. Stahl-Biskup, F. Saez**,** *London: Taylor and Francis*, **2002**, 331Pp.
[33] Statisti XL, *University of Western Australia from http://www.statistixl.com.* **2007**.
[34] G.L. Stebbins**,** *London:E. Arnold*. **1971**.
[35] R. Vila**,** *London: Tyler and Francis*, **2002**, pp.144-175.
[36] M.A. Zaidi, S.A. Crow**,** *Pakistan. J. Ethnopharmacol*. **2005**, **96**: 331-334.
[37] A. Zargari**,** *Iran: Tehran University Press.* **1990**, Vol. **4**, 28–42.
[38] M. Ziaei Nasab, S. M. Hesamzadeh hejazi, M.R. Bihamta, M.Mirza, M.A.Naderi-Shahab, African Journal of Biotechnology, **2012**,Vol.11(5), pp.1028-1036.