A NEW CHROMOSOME NUMBER REPORT AND KARYOTYPE VARIATION IN THYMUS TRANSCASPICUS KLOKOV

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Karyotypes of five populations of *Thymus transcaspicus* from different geographic sites in Iran are presented. The basic chromosome number of the studied populations was obtained as x=15. Presence of 30 chromosomes in Tiwan, Hikooh, Pakotel and Golmakharan populations and 60 chromosomes in Reiin population corresponds to diploid (2n = 2x = 30) and tetraploid levels (2n = 4x = 60), respectively. Chromosome number of 2n= 30 is reported for the first time in the species. Metacentric chromosomes are the most common, but sub-metacentric chromosomes are rare. Based on intrachromosomal asymmetry (A_1 and TF %), population Reiin had the most asymmetric karyotype and population Golmakharan had the most symmetrical karyotype among the populations. According to interchromosomal asymmetry (A_2 and DRL), among diploid populations, population Pakotal had the asymmetrical karyotype. The results indicate that at least in Iran *T. transcaspicus* is not a uniform species in regard to its chromosome number and karyotype. Meanwhile poloidy has played a role in the evolution within species.

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Key words: *Thymus*; Lamiaceae; symetric karyotype; diversity; cytology; Iran

گزارش یک عدد کروموزومی جدید و تنوع کاریوتیپ در *Thymus transcaspicus* Klokov.

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کاریوتیپ ۵ جمعیت از گونه Thymus transcaspicus از مناطق مختلف ایران ارایه می شود. عدد پایه کروموزومی x=15 برای جمعیت های مورد مطالعه بدست آمد. وجود ۳۰ کروموزوم در جمعیت های تیوان، هیکوه، پاکتل و گلمخاران و ۶۰ کروموزوم در جمعیت رئین نشان دهنده دو سطح دیپلوئید و تتراپلوئید در گونه است. عدد کروموزومی 2n=30 برای اولین بار در گونه گزارش می شود. کروموزوم های متا سانتریک نوع غالب و ساب متاسانتریک بندرت دیده می شود. بر اساس شاخص عدم تقارن کروموزومی A1 و TF جمعیت رئین دارای متقارن ترین کاریوتیپ و جمعیت گلمخاران دارای نامتقارن ترین کاریوتیپ بود. بر اساس شاخص عدم تقارن کروموزومی A2 و DRL در بین جمعیت های دیپلوئید پاکتل دارای کاریوتیپ نامتقاران بود. نتایج نشان می دهد که حداقل در ایران این گونه یک گونه یکنواخت نیست . همچنین پلوئیدی در تکامل گونه نقش موثری دارد.

INTRODUCTION

Genus *Thymus* is one of the eight most important genera in Lamiaceae family with regard to the number of species included. Taxonomically *Thymus* belongs to the tribe Menteae within subfamily Nepetoideae. Available data report 215 species for the genus. Most species live on rocks or stones and well drained soils. *Thymus* is widely distributed in the old world. Mediterranean region has been considered as the center of diversity of the genus. Hybridization is known to be

a common phenomenon in the genus, even between species of taxonomically remote groups (Morales, 2002, Jalas, 1982). Species of *Thymus* are very variable in chromosome number and chromosomal morphology. Chromosomes are very small (1-2 micrometer) and different levels of polyploidy have been reported in the genus, with the most frequent chromosome numbers 2n= 28, 30, 56 and 60. Secondary basic chromosome number of 14 and 15 originated from basic number x=7. During the evolution of genus aneuploidy also is an important phenomena and responsible for numbers such as 24, 26, 32, 42, 48, 50, 52, 54, 58 and 90. Different levels of ploidy also observed in the same species (Girón et al., 2012; Kalvandi *et al*., 2012; Mahdavi and Karimzadeh, 2010; Javadi et al., 2009; Javadi et al., 2012; Funamoto *et al*., 2008). *Thymus transcaspicus* (Lamiaceae) is distributed in Iran and Turkmenistan. The species belongs to sections *Serpyllum* (Miller) Bentham and subsection *Kotschyani* Kolkov. in which the chromosome numbers differ from 2n=30 to 60 corresponding to the diploid and tetraploid levels. Previous reports on *T. transcaspicus* belong to Jalas and Pohjo (1965) and Jalas and Kaleva (1967), who reported 2n= 60 from the specimens originated from Asia Media but there is no report related on *T. Transcaspicus* from Iran. The objective of this study is to investigate karyotypes of 5 populations of *T. transcaspicus* in Iran, which could be useful in phylogenetic studied of genus and also breeding investigations.

MATERIALS AND METHODS

Seeds were collected from 5 wild populations in Iran and germinated on wet filter paper at 20 ^{o}C. One cm long roots were pretreated with 8-hydroxyquinolin and then washed in distilled water and fixed in carnoy solution 1 for 24 hours. Hydrochloric acid (1N) was applied for 7 min to hydrolyze the roots. Staining was done by orcein for 24 h at room temperature. Stained roots squashed in one drop of 45% acetic acid and examined by a ZEISS Axiophot compound microscope. Cells in metaphase stage were photographed with a D450; Canon Inc. Japan digital camera. Sampling locations and their geographic coordinates are shown in table 1.

Ten well prepared metaphasic cells were selected and some chromosomal characteristics such as total chromosomal length (TL), long arm length (L), short arm length (S), the arm ratio (AR) [LA/SA], and centromeric index (CI), were measured using Micro Measure ver. 3.3 software. The following karyological parameters were determined: ratio between the largest and the smallest chromosome, total chromosome form (TF%) (Huziwara, 1962), coefficient variation of chromosome length (CV_{CL}), coefficient variation in centromeric index (CV_{CI}), difference of range relative length (DRL), intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) (Romero Zarco, 1986), Dispersion Index (DI) where CV represents the coefficient of variation for chromosome length and CG represents the centromeric gradient value (Lavania and Srivastava 1992). Karyotypic characteristics have been 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. In order to determine the variation between populations, one-way ANOVA was performed on normal data and mean comparison of data was carried out by Donken test. Cluster analysis was done based on all examined characters using ward method and by SPSS version 12.0.

Table 1. Geographic location and climatic conditions of studied *Thymus transcaspicus* Klokov populations.

Province	Location	Altitude (m)	Longitude (E)	Latitude (N)	Average annual temperature (C^{o})	Average annual rainfall (mm)	Land use
Razavi khorasan	Tiwan	2300	E58º 35´ 18"	N37º 27´ 13"	13.9	310.0	Grassland
Razavi khorasan	Gomakharan	2454	E58º 23´ 14"	N36º 57´ 17"	11.9	337.9	Farming
North khorasan	Reiin	2150	E57º 03´ 10"	N37º 23´ 53"	13.5	270.0	Pasture
North khorasan	Pakotal	2049	E57º 27´ 47"	N37º 16´ 18"	12.7	253.0	Farming
Semnan	Hiqu	2026	E53º 27´ 48"	N36º 01´ 5"	11.2	267.9	Pasture

RESULTS AND DISCUSSION

The somatic chromosome numbers (2n) and karyotypic details for the studied populations were presented in tab.3. Photographs of the mitotic metaphases and their related ideogram of the populations were presented in fig 2. Chromosome numbers in Tiwan, Golmakharan, Pakotal and Hiqu were diploid 2n=30, this is the first report of diploid level from the species, while in Raein was tertraploid and 2n= 60 which is in agreement with the reports from Asia media specimens (Jalas and Pohjo (1965) and Jalas and Kaleva). The same numbers have been reported in other members of subsection *kotschyanii* (Javadi *et al.*, 2009; Mahdavi *et al.*, 2010; Ziaei Nasab *et al*., 2012). Similar to finding of current study, the other researchers observed various ploidy levels among different populations of one species within genus *Thymus* (Kalvandi, et al. 2012. Javadi, *et al*., 2009). The average length of chromosomes varied from 1.12 to 1.42 µm. The shortest chromosome was observed in Tiwan and the longest in Hiqu. The average length of long and short arms changed from 0.62 to 0.76 µm and 0.49 to 0.62 µm, respectively (table 2). The highest and lowest amounts of both parameters were observed in Tiwan and Hiqu populations, respectively. Metacentric chromosomes were dominant in almost all the populations, exception observed in Reiin, which contained both meta and sub-metacentric chromosomes. Based on Stebbins classification of karyotypes all the populations were placed in class 1A indicating primitive karyotype, while Reiin population placed in class 2A. It can be concluded that Reiin had the most asymmetric karyotpe compare to other populations. Ziaei Nasab et al. (2012) reported the same class for *T. kotschyanus* and *T. daenensis*. The other reasons, which confirmed the asymmetric karyotype of Reiin, were the lowest percentage of TF (43.17%) and highest amount of A1 (0.23). The most symmetric karyotype belonged to Golmakharan population with A1= 0.18 and TF= 44.87%. The highest amounts of A2 and CV_{CL} were observed in Pakotal population, indicated the asymmetry between chromosomes and variation in chromosomal length. CV_{CI} which shows the heterogeny within the chromosomes based on centromer position revealed that Reiin with the amount of 7.97 has the highest and Golmakharan with the amount of 6.08 has the lowest CV_{CI}. The lowest amount of DRL (2.68) was observed in Reiin. Although the high amount of DRL indicates the high variation in chromosome structure which observed in Reiin population but this parameter is reliable only in populations with same poloidy level. Our results is in agreement with Kalvandi et al. (2012) which showed that in *Thymus eriocalys* the amount of DRL in tetraploid populations is lower than diploids. Among the diploid populations DRL of Pakotal with largest amount (3.51) had the most asymmetric karyotype. Based on DI, Reiin and Golmakharan had the most asymmetric and symmetric karyotypes, respectively.

Table2. Karyotype characteristics and means comparison of chromosomal traits in five populations of *T. transcaspicus* (abbreviations: same superscript letters indicate non-significant differences.*code number of specimen deposited to herbarium of faculty of agriculture, University of Tehran, Karaj. Iran).

population	2n	x	SC	A1	A2	CV_{CL}	CV_{CI}	TF%	CG	DI	%DRL	KF
Tiwan- 6464*	30	15	1A	0.21^{ab}	0.13^{a}	13.33^{a}	7.50^{ab}	$43.86b^{c}$	45.58^{a}	6.07^{a}	3.14^{ab}	15m
Reiin- 6461*	60	15	2A	0.23^{a}	0.14^{a}	14.11^{a}	7.97^{a}	43.17^{c}	44.54^{a}	5.85^{a}	2.68^{b}	28s + 2sm
Pakotal- 6463*	30	15	1A	0.20^{ab}	0.14^{a}	14.72^{a}	6.81^{abc}	$43.97b^{c}$	43.58^{a}	6.11^{a}	3.51^{a}	15m
Hiqu-6465*	30	15	1A	0.19^{b}	0.12^{a}	12.93^{a}	6.55^{bc}	44.68^{ab}	45.14^{a}	6.21^{a}	3.35^{ab}	15m
Golmakharan-6463*	30	15	1A	0.18^{b}	0.13^{a}	13.45^{a}	6.08^{c}	44.87^{a}	46.01^{a}	6.57^{a}	3.11^{ab}	15m

Table 2. (Continued).

population	TL (µm)	L (µm)	S (µm)	AR	CI
tiwan	1.12d	0.62c	0.49e	1.309ab	0.438ab
reiin	1.24c	0.70b	0.53d	1.357a	0.431b
pakotal	1.35b	0.75a	0.59b	1.293b	0.441a
Hiqu	1.42a	0.76a	0.62a	1.262b	0.445a
Golmakharan	1.22c	0.68b	0.55c	1,255b	0.446a

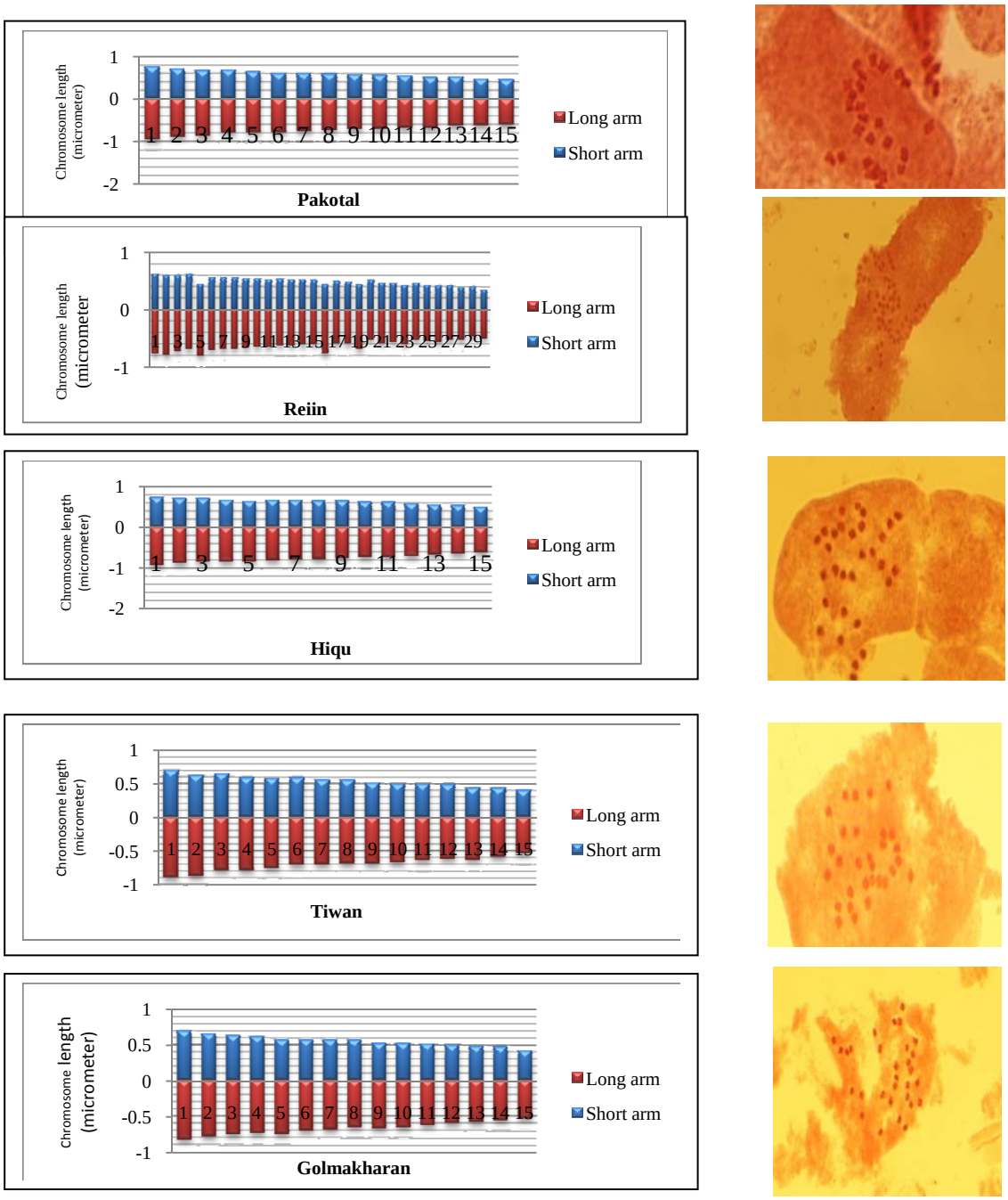

Fig. 2. Mitotic metaphase of *Thymus transcaspicus* populations and related ideograms.

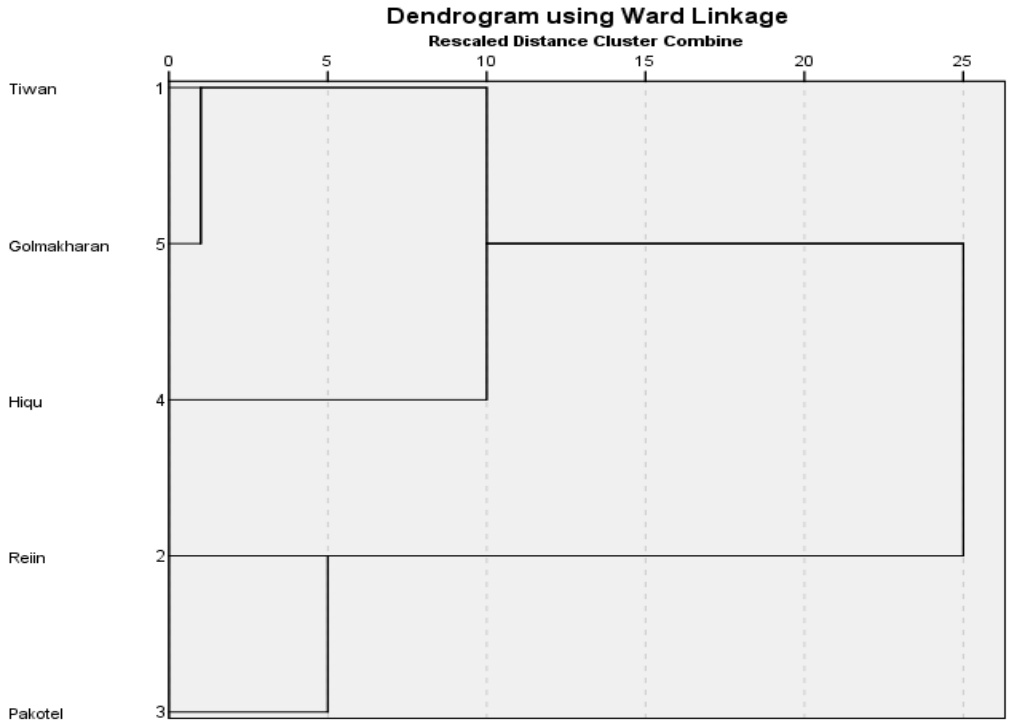

Fig.1— Dendrogram of five populations of *Thymus transcaspicus* by analyzing karyotypic parameters using ward cluster analysis method.

Cluster analysis based on all chromosomal characteristics classified the populations in three groups at the distance of 10 (fig.1). The first group consisted of Tiwan and Golmakharan, the second group of Hiqu and the third group of Reiin and Pakotal. The highest similarity observed in Tiwan and Golmakharan. The tertraploid population Rein was grouped with diploid population Pakotal because of similarities in several other morphological traits and indices of chromosomes (tab.2). Variations in chromosomal morphology of *T. transcaspicus* could be a good reason to describe the morphological variations between and within the populations. Our previous work on morphological traits of the same populations of *T. transcaspicus* studied here revealed high variation in morphological traits and populations classified in three groups. Comparison of two classification showed that only two populations Tiwan and Golmakharan grouped together in both classifications (Moradi et al., 2014). The above results indicate that at least in Iran *T. transcaspicus* is not a uniform species in regard to its chromosome number and karyotype.

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