

## CHROMOSOME REPORTS ON TWO SPECIES OF THYMUS (LAMIACEAE)

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*Thymus* (Thyme) is one of the most important medicinal plants which is highly variable within and between the species. This variation is in morphology, components of essential oil and number of chromosomes. So, in this study, two *Thymus* (Thyme) species including *Th. trautvetteri* Klokov & Desj-Shost and *Th. fedtschenkoi* Ronniger which are distributed in south of Azerbaijan country, were investigated. Examined species belong to the sect. *Serpyllum* and subsection *Kotschyani*. Some species of this section are taxonomically very difficult and show the widest chromosomal variation. In this research, the somatic chromosome numbers of examined species were counted at least in five mitotic cells. The basic chromosome number was  $x=15$  with two ploidy levels. *Th. trautvetteri* with  $2n=4x=60$  and *Th. fedtschenkoi* with  $2n=2x=30$  were tetraploid and diploid respectively. Chromosome counts for these species are new reports.

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**Key words.** *Thymus*, Chromosome number, Ploidy.

### اطلاعات کروموزومی دو گونه از جنس آویشن (*Thymus*)

حمیده جوادی، عضو هیات علمی موسسه تحقیقات جنگلها و مراتع کشور و دانشجوی دکترای دانشگاه دولتی باکو، آذربایجان. سید محسن حسامزاده حجازی، استادیار گروه زیست فناوری، موسسه تحقیقات جنگلها و مراتع کشور. مجنون شیخبابا بابایف، استاد دانشگاه دولتی باکو، آذربایجان.

آویشن (*Thymus*) یکی از گیاهان مهم دارویی است که تنوع زیادی را در درون و بین گونه‌ها نشان می‌دهد. این تنوع در مورفولوژی، ترکیبات روغن‌های ضروری و تعداد کروموزومی دیده می‌شود. در این تحقیق دو گونه از جنس *Thymus* (*Th. trautvetteri* Klokov & Desj-Shost و *Th. fedtschenkoi* Ronniger) که از منطقه جنوب کشور آذربایجان جمع‌آوری شده بودند از لحاظ تعداد کروموزومی و سطح پلوئیدی مورد مطالعه قرار گرفتند. گونه‌های مورد مطالعه به بخش *Serpyllum* و زیربخش *Kotschyani* تعلق دارند. برخی از گونه‌های این بخش از لحاظ تاکسونومی مشکل بوده و تنوع کروموزومی زیادی را نشان می‌دهند. در این تحقیق تعداد کروموزوم‌های سوماتیک برای گونه‌ها حداقل در 5 سلول متافازی تعیین گردید. در هر دو گونه پایه کروموزومی  $x=15$  بوده و دو سطح پلوئیدی را نشان می‌دهند. گونه *Th. trautvetteri* با  $2n=4x=60$  و گونه *Th. fedtschenkoi* با  $2n=2x=30$  به ترتیب تتراپلوئید و دیپلوئید بودند. گزارش تعداد کروموزومی برای این دو گونه برای اولین بار می‌باشد.

### Introduction

Within the *Labiata* family, with about 220 genera, the genus *Thymus* is one of the eight most important genera with regard to the number of species included. The common English word 'thyme' has traditionally been used to name both the genus and its most commercially used species (Morales, 2002).

*Thymus* belongs to the tribe *Menthae*, subfamily *Nepetoideae* and includes 300-400 species and subspecies and over 300 cultivars of aromatic perennial herbaceous plants and subshrubs (Evans, 1989; Morales, 1997; Pedersen, 2000). This genus is widely distributed in the Old World (in temperate zones) and on the coasts of Greenland, from the Macaronesian

Tab. 1. Materials used for karyological study of *Thymus* species.

Species	Location	Altitude (m)	Herbarium code
<i>Thymus trautvetteri</i>	Azerbaijan ,Arasbaran-Gardiz	2400	95797
<i>Thymus fedtschenkoi</i>	Azerbaijan , Astara	1400	95798

Region, Northern Africa and the Sinai Peninsula, through the West and East Asia. However, the central area of this genus surrounds the Mediterranean Sea (Morales, 1997; Pedersen, 2000; Demissew, 1993), the Mediterranean countries, including North West Africa, North of the Sahara, Turkey, Russia, Mongolia and Japan and as far South as the Himalaya. The Mediterranean region can be described as the centre of the genus - strictly speaking the West Mediterranean region (Javadi et al. 2009). Republic of Azerbaijan is the largest country in the Caucasus region of Eurasia. The Phrygana of Nakhchivan is very diverse at 1,000 to 1,500 (M) altitude, with more than 300 species represented. The species of thyme (*Thymus*) are very typical of those arid habitats (<http://azerbaijan24.com>).

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), which makes taxonomic studies in this genus very difficult, especially in some taxonomical groups e.g. in the section *Hyphodrami* and particularly in the section *Serpyllm*, where the concept of species is more difficult to apply (Morales, 2002). So the first step to solve the problems in taxonomic studies is to use chromosomal studies (karyology).

In recent years there have been numerous papers reporting chromosome counts for species of *Thymus*. The following chromosome numbers are known:  $2n=24, 26, 28, 30, 32, 42, 48, 50, 52, 54, 56, 58, 60, 84$  and  $90$ , corresponding to the diploid, tetraploid and hexaploid levels (Lopez-Pujol et al. 2004). The most frequent numbers are  $2n=28, 30, 56$  and  $60$ . The basic number of  $x=15$  with different ploidy levels is present in the majority of species (Jalas, 2010; Morales, 1986). Therefore, *Thymus* is a complex genus with a various range of chromosome number that it is request more research about this genus.

In this paper, two species of *Thymus* are investigated in view of chromosome number and ploidy levels, which belong to Azerbaijan. These two species have not been studied before.

## Materials and Methods

In this study, we used root tip meristems from seedling obtained by the germination of ripe seeds collected from various locations (2 species) on wet filter paper in Petri dishes and left at 25°C temperature. The species studied are listed in table 1.

Root tip meristems obtained from seedlings were pretreated with % 0.05 (w/v) 8-hydroxyquinoline for 4 to 5 h at 16°C. Pretreated root tips were fixed in a 3:1 (v/v) mixture of 95 % (v/v) ethanol and propionic acid for 24 h. Root-tips were hydrolyzed in 1M HCl for 5 to 7 min at 60°C and stained in Schiff's reagent for 2 h at room temperature. Feulgen stain was removed and the root tips were rinsed with cold double-distilled water and stained with Carbol fuchsin stain overnight at 0 to 4°C in a refrigerator. After staining, the root tips were washed three to four times with cold double distilled water and stored in cold double- distilled water in a refrigerator. Root tips were squashed in a droplet of %45 (v/v) acetic acid and lactic acid (10:1). The preparations were observed with an optical microscope (BH2 Olympus supplemented Digital color video camera) at a magnification of about 2000 X.

The best metaphasical plates were selected and captured. For each population prepared 5 mitotic metaphase.

## Results

The results showed that the basic chromosome number was  $x=15$  and the species showed two ploidy levels (diploid and tetraploid) (Fig.1).

1. *Thymus trautvetteri* Klovov & Desi-Shost (Fig.1),  $2n=4x=60$ .

This species grows in Iran and Talesh. Chromosome count of  $2n=4x=60$  for this species is the first report.

2. *Thymus fedtschenkoi* Ronniger (Fig.1),  $2n=2x=30$ .

This species grows in Iran, Turkey and Caucasus, also exists in regions of Caspian and Irano-Turanian at 250-3750m altitude (Jamzad, 2009). Chromosome count of  $2n=4x=30$  for this species is the first report.

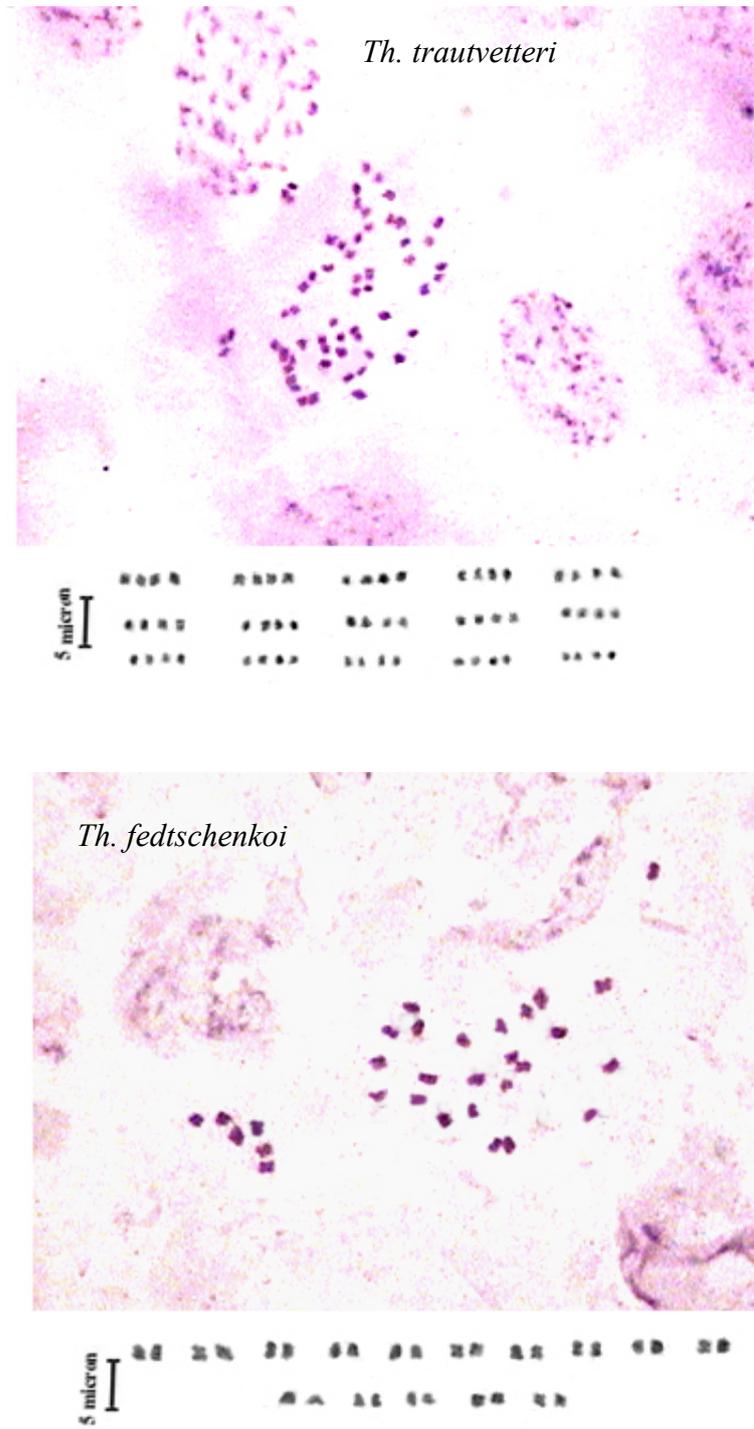


Fig. 1. Root tip metaphase plate of *Thymus* species (X2000).

## Discussion

The results of this research allow us to compare the chromosome number and ploidy levels in two species of *Thymus* (*Th. trautvetteri* and *Th. fedtschenkoi*), that located in section *Serpyllum* and subsection *Kotschyani*, for the first time.

Based on Morales survey, many Asian species, located in section *Serpyllum* subsection *Kotschyani*, that are difficult taxonomically and have widest chromosomal variation. Many researchers found in the species of *Serpyllum* different chromosome numbers  $2n=28, 32, 35, 52, 56, 58$  and  $90$  (Morales, 1997).

Based on research, eight accessions of Thyme medicinal plant, belonging to Iranian endemic *Th. daenensis*, *Th. eriocalyx*, *Th. migricus* and an unknown *Thymus* species were tested for karyotypic analysis. Six accessions were distinguished as diploids and two as tetraploids (Mahdavi & Karimzadeh, 2010).

The basic chromosome number for *Thymus* is mostly  $x=7, 14$  and  $15$ . Some of researchers have shown that basic chromosome number for *Th. kotschyanus*  $x=15$  with diploid and tetraploid ploidy levels (Morales, 1980 & 1989). The others have shown that it could be  $x=15$  or  $14$  with different ploidy levels ( $2n=60, 57, 56, 54$ ). The basic chromosome numbers  $x=14$  and  $x=15$  have probably originated from a basic number  $x=7$  (Morales, 1981; Elena-Rossello, 1981; Martonfi & Martonfi, 1996).

Karyotypic study of two populations of *Th. kotschyanus* from Ghazvin and Zanjan region also showed  $2n=30$  &  $60$  with two ploidy levels (diploid & tetraploid) (Javadi et al. 2009).

Lopez (2004) showed two ploidy levels (diploid & tetraploid) and five different chromosome number in *Thymus* species ( $2n=2x=28, 30$  &  $2n=4x=54, 56, 58$ ).

Morales and Mehrpur showed ploidy variation in different species of *Thymus*, for example, *Thymus pubescence* and *Thymus kotschyanus* with  $x=15$  and two ploidy level, diploid and tetraploid (Morales, 1980; Mehrpour et al. 2002).

Based on this survey reveals that in two species the basic chromosome number  $x=15$  with different ploidy levels (diploid  $2n=2x=30$  & tetraploid  $2n=4x=60$ ).

Hence, according to previous and present reports, it can be pointed out that remarkable variation in *Thymus* chromosome number can reflect the nuclear DNA variation and show the most variation in *Thymus* species, so it requests more research on this genus.

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