A comparison of chromosome characters in three species of *Thymus* (Lamiaceae) collected in Russia and Mongolian Altai

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ABSTRACT. Characters of the resting, the mitotic prophase and metaphase chromosomes in three species of *Thymus* were studied. The species studied had commonly resting chromosomes of the complex chromocenter type and mitotic prophase chromosomes of the proximal type. *Thymus mongolicus* showed the chromosome numbers of $2n=26$ and 27 and *T. przewalskii* and *T. roseus* showed the chromosome number of $2n=26$. The chromosome number of $2n=26$ for *T. przewalskii* and that of $2n=27$ for *T. mongolicus* were reported here for the first time and that of $2n=26$ for *T. roseus* and *T. mongolicus* verified the previous reports. The chromosome number of $2n=27$ for *T. mongolicus* seemed to have one B-chromosome as $2n=26+1B$ with respect to the existence of a peculiar heteropycnotic body at resting stage and high pollen stainability. Three species were commonly placed in the diploid level if the basic chromosome numbers of $x=13$ was accepted. Their karyotypes at mitotic metaphase were quite similar to each other regarding chromosome sizes, chromosome types according to the centromeric position and mono-modality of chromosome lengths from the longest to the shortest chromosomes except for satellite sizes.

KEYWORDS: Chromosome character, Lamiaceae, Mongolia, Russia, *Thymus*

Thymus L., the Lamiaceae (Labiatae) is small subshrub plants and consists of approximately 400 species (Willis 1982). They are distributed in the temperate zone of the Eurasian continent, mainly in the Mediterranean region (Willis 1982) and grow in diabase, serpentine and calcareous rocks, limestone, in cracks rocks, on a bank, steppe, on sandy soils, xerophilous grassland, on loessic slopes and along road sides. *Thymus* has always been taxonomically problematic group and views concerning the taxonomic definition of the forms are extremely varied. It produces some natural hybrids. Plants of the genus is often cultivated and propagate to extract the aromatic ethereal oil and flavouring.

The first cytological observation in *Thymus* was counting and documenting the chromosome number: $2n=24$ in *T. serpyllum* reported by Löve and Löve (1942). Then, many more researchers have found at least 50 different chromosome numbers from $2n=14$ of *T. seravschanicus* to $2n=90$ of *T. zygioides* var. *lycaonicus*. They also have found aneuploidy in *T. longedentatus* as well as autopolyploidy in about 170 taxa included 139 species, 17 subspecies, 12 varieties and two hybrids by Löve and Löve (1942), Saaremaa (1947), Jalas (1948), Jalas and

Kaleva (1966, 1967), Trela-Sawicka (1968, 1970, 1972), Gogina and Svetozarova (1972), Gurzenkov (1972), Lee (1972), Jalas and Uotila (1976), Krogulevich (1978), Arrigonia *et al.* (1980), Bir and Saggoo (1980), Elena-Roselló (1980), Morales Valverde (1980, 1986, 1986a, 1990), Astanova (1981), Hruška and Bellomaria (1982), Fernandes and Leitao (1984), Funamoto *et al.* (1993), Markova and Goranova (1994), Mártonfi and Mártonfi (1996), Boscaiu *et al.* (1998), Tahiri *et al.* (1998), Constantinidis *et al.* (2002). Jalas (1948), Jalas and Kalev (1967) and Mártonfi and Mártonfi (1996) and Mehrpure *et al.* (2002) have discussed chromosomal evolution of *Thymus* regarding their basic chromosome numbers.

This paper reports on chromosome characters of the resting, mitotic prophase and metaphase chromosomes in three species of *Thymus* collected in Russia and Mongolia by our field expedition.

MATERIALS AND METHODS

Plant materials Eleven plants of *Thymus mongolicus* (Ronn.) Ronn., *T. przewalskii* (Kom.) Nakai and *T. roseus* Schipcz. (= *T. serpyllum* L.) were collected in three sites in Russia and Mongolian Altai (Table 1). They were

Table 1. Collection sites, sample and chromosome numbers in three species of *Thymus* observed

Species	Collection site	Sample number	Chromosome number (2n)
<i>T. mongolicus</i> (Ronn.) Ronn.	Mongolia, Bayan-Ulgii Province, Bayan-Ulgii-Aimak, Lake side of Eli-Hur, 48°35' 52" N, 090°00' 24" E, alt. 2,070 m.	1	26
		2	27
<i>T. przewalskii</i> (Kom.) Nakai	Russia, Primorye Territory, Kankaishiy District, west shore of Khanka Lake, 4 km from Turiy Rog City, 45°11' 87" N, 131°59' 85" E, alt. 76 m.	3	26
<i>T. roseus</i> Schipcz. (= <i>T. serpyllum</i> L.)	Russia, Altai Territory, Altai Republic, Onguday District, the Chuya river joins the Katun river, 50°23' 75" N, 088°40' 39" E, alt. 780 m.	5	26

brought back to Japan and cultivated in unglazed flowerpots in shade place in the experimental garden of Showa Pharmaceutical University.

Taxonomic treatments followed Czerepanov (1995) and Doron'kin (2006). The voucher specimens were deposited in Funamoto's personal herbarium in Showa Pharmaceutical University.

Observation of chromosomes Freshly growing root-tips were collected from the above plants. They were cut off 5-10 mm long and pretreated in 2mM 8-hydroxyquinoline at about 20°C for 4 h, and then, fixed in 45% acetic acid for 10 min at ca 2°C. They were macerated in a mixture of 1N hydrochloric acid and 45% acetic acid (1:1) for 20-23 sec at about 60°C, and stained with 2% aceto-orcin for about 30 min at room temperature in a moist chamber with 45% acetic acid. Slide preparations were made with the conventional squash methods. The slides were faintly heated under alcohol frame for 1-2 sec before observation. Pollen grains were stained with 2% aceto-orcin to count at least first 1,000 grains for pollen stainability.

Crassification for the chromosome types at resting and mitotic prophase stages followed Tanaka (1971, 1977). Crassification for the chromosome types by the centromeric positions at mitotic metaphase stage followed Levan *et al.* (1964). Measurements of chromosome lengths made in good mitotic metaphase cells.

RESULTS AND DISCUSSION

Thymus mongolicus (Ronn.) Ronn., *T. przewalskii* (Kom.) Nakai and *T. roseus* Schipcz. (synonym *T. serpyllum* L.) had common chromosomal characters such as the complex chromocenter type of the resting chromosomes, which had numerous chromomeric granules, fibrous threads and chromatin blocks and the small chromatin blocks were irregular in shape and varied in number (Fig. 1a, e, h and k), and the proximal type of mitotic prophase chromosomes, which had the early condensed segments confined to the proximal regions of both short and long arms or only the short arm, showing clear condensation transition from the proximal to the distal regions (Fig. 1b, f, i and l). The mitotic metaphase chromosomes of the three species studied had commonly small sized chromosomes and

mono-modality of the chromosome lengths from the longest to the shortest chromosomes, and thus, formed similar karyotypes to each other (Table 2; Fig. 2).

Thymus mongolicus is distributed in Kazakhstan, Siberia in Russia, Mongolia and North Central and North East in China. Three plants of this species were collected on a cracks rocks in Bayan-Ulgii Province, Mongolia (Table 1). One of them had the chromosome number of 2n=26 (Fig. 1c and d) and the other two plants had 2n=27 (Fig. 1g). The first report on the chromosome number of 2n=26 in *Thymus* was made in *T. pseudo-allicus* by Jalas and Kaleva (1966). The chromosome number of 2n=26 in *T. mongolicus* in the present study verified the previous report by Doron'kin (2006). Thus, the chromosome number of 2n=27 documented here was very first report for this species. In the resting nuclei in the two plants which showed the chromosome number of 2n=27, peculiarly a large condensed heteropycnotic block (Fig. 1e) was found. However, such a large heteropycnotic block has never been seen in the resting nucleus in the plant with the chromosome number of 2n=26 (Fig. 1a). The plants with the chromosome numbers of 2n=26 and 27 had a high pollen stainabilities with 93.46% and 90.27%, respectively. Following these characters in the resting chromosomes and the pollen stainability the chromosome number of 2n=27 could be constituted by 2n=26+1B. *Thymus mongolicus* must be a diploid if the basic chromosome number of x=13 was accepted.

The plants with 2n=26 showed the chromosome sizes ranged from 1.5 to 1.0 µm long and total 30.6 µm long averaged 1.2 µm long, and the karyotype of median-centromeric and submedian-centromeric chromosomes (Fig. 2a and b; Table 2). In contrast, the plants with 2n=27 showed the chromosome sizes ranged from 1.5 to 0.9 µm long and total 31.7 µm long averaged 1.17µm long, and the karyotype of median-centromeric and submedian-centromeric chromosomes (Fig. 2c and d; Table 2). A small dot-shaped satellite was rarely observed on medium or small sized chromosome.

Thymus przewalskii is distributed in Far East Russia and North Central, North East and South East in China. Three plants of this species were collected in a bank on the shore of Lake Hanka in Primorye Territory, Russia (Table 1), and showed commonly the chromosome

Fig. 1. Karyomorphological comparisons of three species of *Thymus*. a-d. *T. mongolicus* ($2n=26$). e-g. *T. mongolicus* ($2n=27$). h-j. *T. przewalskii* ($2n=26$). k-m. *T. roseus* ($2n=26$). a, e, h and k. Resting chromosomes. b, f, i and l. Somatic prophase chromosomes. c, d, g, j and m. Mitotic metaphase chromosomes. Arrows indicate satellites and arrows head represent B-chromosome. d is the drawing of the photograph c. Bar=3 μ m.

number of $2n=26$ (Fig. 1j) that was reported here for the first time. This species could be diploid if the basic chromosome number of $x=13$ was accepted. The chromosome sizes of this species ranged from 1.5 to 0.9 μ m long and total 31.3 μ m long averaged 1.2 μ m long, and the karyotype of median-centromeric and submedian-centromeric chromosomes (Fig. 2e and f;

Table 2). The small dot-shaped satellite was rarely observed in medium or small sized chromosomes.

Thymus roseus (= *Th. serpyllum*) is widely distributed in Europe, Mediterranean and Asian continent. Five plants of this species were collected in a locality, on calcareous rocks in Altai Republic in Altai Territory, Russia (Table 1), and showed commonly the chromosome

Table 2. Karyotype comparisons of three species of *Thymus* observed

Species	Chromosome number	Cell No.	Chromosome length (μm)				Form
			The longest Mean±SD (Ranges)	The shortest Mean±SD (Ranges)	Total Mean±SD (Ranges)	Average Mean±SD (Ranges)	
<i>T. mongolicus</i>	2n=26	4	1.5±0.21 (1.6-1.3)	1.0±0.07 (1.0-0.9)	30.6±3.52 (33.7-27.5)	1.2±0.14 (1.3-1.06)	m+sm
<i>T. mongolicus</i>	2n=27	11	1.5±0.13 (1.6-1.2)	0.9±0.09 (1.1-0.8)	31.7±2.87 (36.2-27.7)	1.17±0.11 (1.34-1.03)	m+sm
<i>T. przewalskii</i>	2n=26	7	1.5±0.08 (1.6-1.4)	1.0±0.05 (1.0-0.9)	31.3±0.78 (32.0-29.7)	1.2±0.03 (1.23-1.14)	m+sm
<i>T. roseus</i> (= <i>T. serpyllum</i>)	2n=26	12	1.4±0.11 (1.6-1.3)	0.9±0.05 (1.0-0.9)	30.0±1.72 (32.9-28.2)	1.15±0.07 (1.27-1.08)	m+sm

SD: standard deviation. m: median-centromeric chromosome, sm: submedian-centromeric chromosome

Fig. 2. Karyotypes of three species of *Thymus*. a-d. *T. mongolicus*. e-f. *T. przewalskii*. g-h. *T. roseus*. b, d, f and h are the drawings of the photographs a, c, e and g, respectively. Arrows point satellites. Bar=3 μm.

number of $2n=26$ (Fig. 1m). This chromosome numbers of this species were previously reported as $2n=24$ and 50 by Löve and Löve (1942), Jalas (1948), Krogulevich (1978), Mártonfi and Mártonfiova (1996), Lövkvist and Hultgård (1999), and $n=12$ and 13 by Bir and Saggoo (1980) and Gill (1984). This species could be a diploid if the basic chromosome number of $x=13$ was accepted. The chromosome sizes of this species ranged from 1.4 to 0.9 μm long and total 30 μm long averaged 1.15 μm long, and the karyotype of median-centromeric and submedian centromeric chromosomes (Fig. 2g and h; Table 2). A little large dot-shaped satellite was rarely observed in medium or small sized chromosomes.

Three species of *Thymus* observed had very similar chromosomal characters in resting and mitotic prophase and metaphase chromosomes and basic chromosome number except for satellite sizes.

The lowest chromosome number of $2n=14$ for *Thymus* was reported in *T. seravschanicus* by Astanova (1981) and thus, the basic chromosome number of $x=13$ shown in the present three species might be secondary basic number if we accepted chromosomal evolutions proposed by Gill (1981).

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