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RESEARCH ARTICLE

Karyological analysis of Larch (*Larix sibirica* Ledeb.) from Mongolia

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Abstract

This paper deals with the karyotype study on *Larix sibirica* from Mongolia. Diploid chromosome number was established (2*n* = 24). Karyotype included 6 pairs (I-VI) of metacentric chromosomes and 6 pairs (VII-XII) of sub-metacentric chromosomes. Secondary constrictions were observed in eight chromosomes; interphase nuclei included from 1 to 8 nucleoli. Mean size of chromosomes of the first group is 12.8 μm, mean size of the chromosomes of the second group is 8.5 μm. Karyotype formula of *L. sibirica* from Mongolia is 2Msc+2M+2Msc+1Smsc+1Sm+4Smsc.Chromosomes of this species were analyzed using fluorescence *in situ* hybridization (FISH) with 45S and 5S ribosomal RNA gene probes. Mixoploidy and chromosome irregularities such as ring chromosomeswere also observed.

Keywords: *Larix sibirica,* conifers, karyotype, chromosomes, secondary constrictions, nucleoli, genome, chromosome mutations

Introduction

The genus *Larix* Mill. is one of the most widely spread and prominent genera among conifers of the world. Larch species are dominant over large areas of temperate and boreal zones of the Northern Hemisphere, grow in the mountains of East Asia and North America, and often form the northern timberline. The genus includes many economically valuable species. On the territory of Mongolia, two *Larix* species i.e., *L. sibirica* Ledeb., *L. gmelinii* (Rupr.) Rupr. and their hybrid *L. czekanowskii* Szaf. are found (Bobrov 1972; Milyutin et al. 1988, 2004; Barchenkov et al. 2012; Ariunbaatar and Jamyansuren 2015).

This paper includes the results of Siberian larch (*L. sibirica*) karyological study. *L. sibirica* belongs to series, *Eurasiaticae* of section *Pauciseriales*. This species has an extensive area in Eastern Kazakhstan and Siberia, growing to the lower reaches of the river Yenisei in the North, throughout Altai in the South, to Southern Transbaikalje (Bobrov 1972). In the North of Mongolia, Siberian larch is the most common forest species and occupies about 80% of the country's forested territory. In this region, larch forests grow on the territory of 7553.3 thousand ha with a timber reserve of 1074.9 million m^3 (Dulamsuren et al. 2010). This research is of interest because isolated (marginal) populations of Siberian larch are near the southern border of rangeand in extreme conditions (Milyuitin et al.1988, 2004).

Materials and methods

The seeds of mixture of Siberian larch population were collected from North-East of Mongolia (Mungun Mort, Khentii Mountains, 48°20'N, 108°39'E) and used in the study. The seed material was prepared and analyzed according to generally accepted techniques for coniferous plants with some modifications (Pravdin et al. 1972; Muratova 1991,1994; Muratova et al. 2007; Muratova and Karpyuk 2021). Seeds

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were germinated under laboratory conditions on moist filter paper in Petri dishes. The germinating seeds were pretreated in 1% colchicine solution for 4-5 hrs at room temperature, fixed in 3:1 ethanol : acetic mixture and stained with acetohematoxylin. The slides were prepared from root tip meristem cells using the improved squash technique.

The slides were analyzed using microscopes MBI-6 and Mikmed-6 with Digital microscope camera DCM510. Suitable cells were selected for analysis and the chromosomes were measured. The parameters determined were: absolute length of the chromosomes (L^a) in micrometers (μm); the total diploid complement chromosome length ($\sum L^a$) in micrometers; relative chromosomes length (L^r) in percentages *i.e.*, the ratio of absolute length of the chromosome to the total chromosome length; centromeric index (I^c) in percentages *i.e.*, the ratio of the short arm length(s) to absolute chromosome length, in percentages; and localization of secondary constriction (sc) *i.e.*, the ratio of the distance from constriction to centromere to the length of arm, in percentages. The chromosomes were classified according to nomenclature developed by Grif and Agapova (1986).

Two-colour Fluorescent *in-situ* hybridization (FISH) were performed according to the standard method with some modifications (Goryachkina et al. 2013). Two wheat DNA probes, pTa794 (5S rDNA) and pTa71 (45S rRNA) provided by E.D. Badaeva (Vavilov Institute of General Genetics RAS), were used for *in situ* hybridization. The slides for staining of nucleoli were treated with a 50% solution of $AgNO_3$ for 1-2 hours at 60°C (Muratova 1995). The results were analyzed using standard statistical methods.

Results and discussion

Siberian larch (*L. sibirica*) from Mongolia has 24 chromosomes ($2n = 24$, Fig. 1), like other representatives of the genus *Larix* (Il'chenko 1973; Hizume 1988; Hizume et al. 1988, 1993, 1994; Muratova 1991, 1994; Milyutin et al. 2004; Muratova et al. 2007; Goryachkina et al. 2013; Ariunbaatar and Jamyansuren 2015; Muratova and Karpyuk 2021). It is diploid species with $x = 12$.

Fig. 1. Metaphase plates of *Larix sibirica*root-tip meristems from Mongolia ($2n$=24). Material stained with acetohematoxylin. Magnification: X 900 (10 x 90). Scale bar 5μm.

Table 1. Morphometric parameters of *L. sibirica* chromosomes from Mongolia

Chromo-some numbers	Absolute length(L^a), mm		Relative length(L^r), %		Centromeric index(I^c), %		Secondary constrictions (sc) localization: ratio of the distance between centromere and secondary constriction to the total length of the arm (sc), %	
	Mean ± SD	CV, %	Mean ± SD	CV, %	Mean ± SD	CV, %	Mean ± SD	CV, %
							s –sc_1 32.9±2.22 frequency 20,0%	19,1
							s –sc_2 51.9±3.13 frequency 22,5%	13,5
I	12.8±0.13	13.8	5.0±0.04	12.0	47.2±0.15	4.4	s – $sc_3$69.5±1.48 frequency 25,0%	6,7
							l –$sc_1$35.6±1.16 frequency22,5%	9,8
							l –$sc_3$68.5±1.82 frequency22,5%	7,5
II	12.8 ± 0.13	13.8	5.0 ± 0.04	12.0	47.2 ± 0.15	4.4	l –66.7± 1.31 frequency40%	7,8
III-IV	12.8 ± 0.13	13.8	5.0 ± 0.04	12.0	47.2 ± 0.15	4.4	–	–
V	12.8 ± 0.13	13.8	5.0 ± 0.04	12.0	47.2 ± 0.15	4.4	l – 34.3±2.30 frequency15%	16,4
VI	12.8 ± 0.13	13.8	5.0 ± 0.04	12.0	47.2 ± 0.15	4.4	l –56.2 ± 0.11 frequency35%	7,4
VII	8.5 ± 0.18	19.9	3.3 ± 0.06	18.9	34.0 ± 0.36	10.1	l –35.0 ± 3.09 frequency 17,5%	7,0
VIII	8.5 ± 0.18	19.9	3.3 ± 0.06	18.9	34.0 ± 0.36	10.1	–	–
IX	8.5 ± 0.18	19.9	3.3 ± 0.06	18.9	34.0 ± 0.36	10.1	l – 55.1 ± 1.66 frequency 17,5%	7,9
X	8.5 ± 0.09	10.2	3.3 ± 0.03	9.0	27.9 ± 0.26	8.7	–	–
XI	8.5 ± 0.09	10.2	3.3 ± 0.03	9.0	27.9 ± 0.26	8.7	l – 66.0 ± 2.45 frequency 15,0%	9,1
XII	8.5 ± 0.09	10.2	3.3 ± 0.03	9.0	27.9 ± 0.26	8.7	l – 52.8 ± 1.66 frequency 20,0%	8,2

Total length of a diploid chromosome set ($\sum L^a$) is 255,7 ± 4,12 mm; s – short arm, l – long arm.

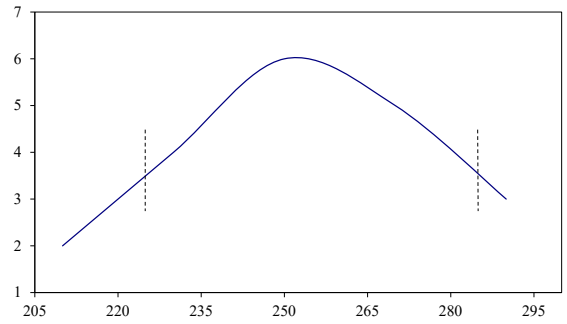

Fig. 2. Variation of the total lengths of the diploid chromosome complements of *L. sibirica* from Mongolia. The abscissa axis is the total lengths of the chromosome complements (ΣL^a); the ordinate axis is the metaphase plates (n). Marked interval shows total lengths selected for statistical analysis

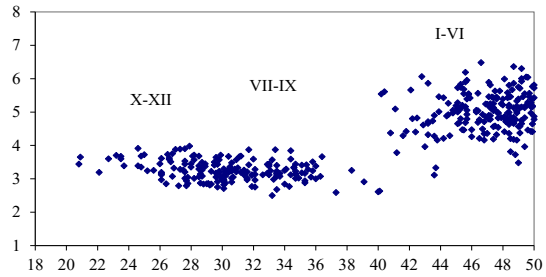

Fig. 3. The polykaryogram of *L. sibirica* from Mongolia. The abscissa axis is the centromeric index (I^c, %), the ordinate axis is the relative length of the chromosomes (L^r, %). I – XI, VII – IX, X – XII are the numbers of the chromosomes

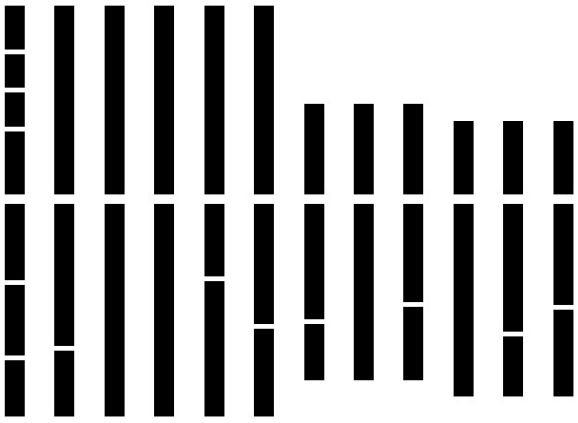

Fig. 4. Idiogram of *L. sibirica* from Mongolia. Gaps in the chromosomes show the secondary constrictions

In 20 metaphase plates, the total length of a diploid chromosome set varied from 209 to 290 μm (Fig. 2). Sixteen plates (interval ΣL^a = 233–282 μm) were used for construction of the polykaryogram (Fig. 3). On the polykaryogram of *L. sibirica,* two chromosome groups could be distinguished. The long metacentric chromosomes of I-VI pairs form one group with similar parameters: L^a=12.8 ± 0.13μm, L^r=5.0 ± 0.04%, I^c=47.2 ± 0.15%. The short sub-

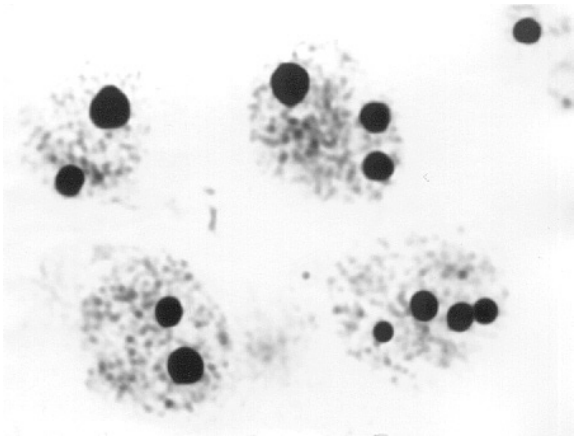

Fig. 5. Different numbers of nucleoli in the interphase nuclei of *L. sibirica*from Mongolia. Material stained with silver nitrate. Magnification: X 900 (10 x 90)

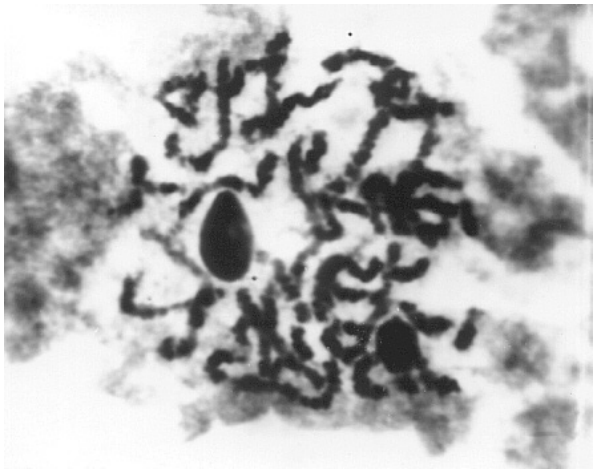

Fig. 6. Connection between the regions of the secondary constrictions and the nucleoli in prophase chromosomes of *L. sibirica* from Mongolia. Material stained with acetohematoxylin. Magnification: X 900 (10 x 90)

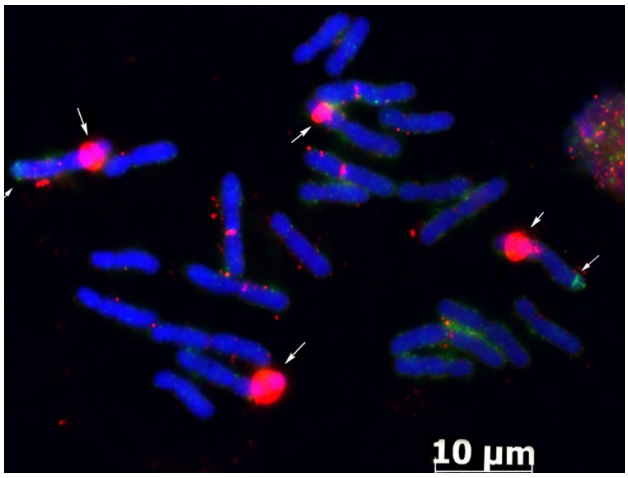

Fig. 7. Physical mapping of 45S and 5S rDNA genes using bicolor FISH on metaphase chromosomes of *L. sibirica* from Mongolia. Arrows point to 45S rDNA (red signals) and 5S rDNA (green signals). Scale bar 10 μm.

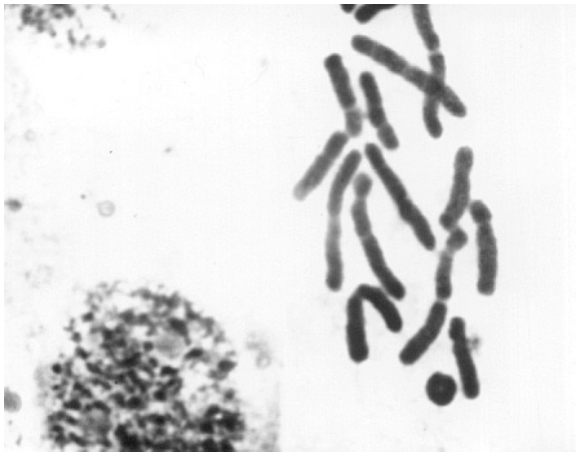

Fig 8: Part of metaphase plate of *L. sibirica*from Mongolia with ring chromosome (arrow points). Material stained with acetohematoxylin. Magnification: X 900 (10 x 90

metacentric chromosomes of VII-IX pairs form second group with similar parameters: $L^a = 8.5 \pm 0.18\mu m$; $L^r = 3.3 \pm 0.06$ %; $I^c = 34.0 \pm 0.36$ %. And X-XII pairs form third group with similar parameters: $L^a = 8.5 \pm 0.09\mu m$; $L^r = 3.3 \pm 0.03$ %; $I^c = 27.9 \pm 0.26$ %. Morphometric parameters of *L. sibirica* chromosomes from Mongolia are given in Table 1. Average total length of a diploid chromosome set ($\sum L^a$) was 255.7 ± 4.12 μm.

As many as 8 pairs of *L. sibirica* chromosomes had the secondary constrictions. In the mitotic metaphase stage, secondary constrictions are revealed as sites stained lighter than other chromosome parts. Longest chromosomes (Ipair) had the secondary constrictions in proximal, medial and distal parts in the short (sc_1=32.9%; sc_2=51.9% sc_3 = 69.5%) and long (sc_1=35.6%; sc_3=68.5%) arms with different frequencies. Constriction in the distal part of the long arms (sc=66.7%) observed in II chromosome pair. Secondary constrictions with different frequencies were observed in the medial parts of the long arms in chromosome pairs VI (sc = 56.2%), IX (sc = 55.1%), and XII (sc = 52.8%). Chromosome pairs V and VIII had constrictions in the proximal parts of the arms (sc = 34,3%; 35,0%). According to Grif and Agapova (1986) nomenclature, chromosome pairs I-VI were metacentric and chromosome pairs VII-XII were sub-metacentric. In Fig.4, the idiogram of *L. sibirica* from Mongolia is illustrated. Ther karyotype formula is 2Msc+2M+2Msc+1Smsc+1Sm+4Smsc.

The occurrence of the secondary constrictions often can be related to nucleolus organization in the cell. Nucleolus formation is due to the function of nucleolar organizing regions (NORs). The NORs are localized at the secondary constrictions (Prokof'eva-Belgovskaya 1986). The interphase nuclei of *L. sibirica* from Mongolia contained 1 – 8 nucleoli; examples nuclei with nucleoli are given in Fig.5; the average number of nucleoli was 2.4 ± 0.04. The connection between the nucleoli and the secondary constrictions is shown in Fig. 6.

These regions are of great importance in the cell protein metabolism. The genes of ribosomal RNA and formation of ribosomes are localized here. Using FISH was shown for conifer chromosomes that loci of 18S and 25-26S rDNA were localized in the regions of secondary constrictions (Hizume et al. 1988, 1993, 1994; Goryachkina et al. 2013). They represent sites of nucleolus organizing activity and form nucleoli in telophase of mitosis and the interphase nuclei. Fluorescence *in situ* hybridization (FISH) revealed major 45S rDNA loci in regions of secondary constrictions of II and VI pairs of metacentric chromosomes, which occur with the highest frequency. Hybridization *in situ* showed that the 5S rDNA loci are positioned in a sub-telomeric region of the short arm of chromosome II, which also carries a major 45S rDNA site in the long arms (Fig. 7). Minor 45S rDNA sites were detected in the pericentromeric regions of some chromosomes.

Some seedlings exhibited mixoploidy; they had triploid ($2n = 3x = 36$) and tetraploid ($2n = 4x = 48$) cells, but the majority of the cells possessed diploid chromosome number ($2n = 2x = 24$). The karyological studies revealed the ring chromosomes in some cells (Fig. 8). They were also observed in some *L. sibirica* populations from different regions *i.e.*, Tyva, Buryatia, Khakasia and Kazakhstan's Altai (Milyutin 1980; Muratova 1991; Milyutin et al. 2004; Muratova et al.2007). Furthermore, the populations of *L. sibirica* in Mongolia and Southern Siberia were found to have some similar karyological features in the number of nucleolar regions in chromosomes and nucleoli in interphase nuclei. It can be explained by close proximity of the two regions. Therefore, results obtained in the present investigation confirm the supposition of a close relationship between *L. sibirica* populations in Mongolia and Southern Siberia. The cytological analysis showed that similarity in the biodiversity exists aming the conifers from Mongolia and Southern Siberia. A possible explanation is the great degree of floristic propinquity between these territories. They form a common Mongolian – Southern Siberian group (Malyshev 1965). This material gives the new information on variability of *L. sibirica* near southern border of area. Furthermore, this data can serve as the foundation for selection and plant breeding in Mongolia.

Authors' contribution

Conceptualization of research (ENM); Designing of the experiments (ENM, OVG, TVK); Contribution of experimental materials (ENM, OVG, TVK); Execution of field/ lab experiments and data collection (OVG, TVK); Analysis of data and interpretation (ENM, OVG, TVK); Preparation of the manuscript (ENM)

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