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KARYOTYPE ANALYSIS OF *PINUS PEUCE* GRISEB

INTRODUCTION

The species of the genus *Pinus* are almost exclusively spread throughout the northern hemisphere, mostly in the boreal zone, but some species occur in equatorial regions.

The species, *Pinus peuce* Griseb belong to the subgenus *Strobus*, section *Strobus*, subsection *Srobi*. This species is a relic form, and endemic in mountainous areas of the Balkan peninsula. It occurs in the mountain regions of Yugoslavia, Bulgaria, north-eastern of Albania and northern Greece.

In Yugoslavia, *Pinus peuce* as a natural population, occurs in the mountains of Montenegro (Sekirica, Komovi), Serbia (Proklerie, Shar) and Macedonia (Pelister, Nidgje, Kožuv and Rudoka).

Because it is of economic value and of taxonomic importance this species has been intensively investigated. Research is especially towards improving quantitative and qualitative characteristics by employing different breeding methods such as intra and inter-specific hybridisation. Latterly, more and more attention has been given to new methods of obtaining improved varieties of commercial pine species or fast growing hybrids. For such methods to succeed knowledge of the cytogenetics of the tree is necessarily.

The cytogenetics of the subgenus *Strobus*, has been investigated by many authors. Saylor (1961) for example has investigated many *Pinus* species, including *Pinus strobus*. Series of investigations have been done by Muratova (1978, 1979) on the related species: *Pinus sibirica*, *P. korainsis* and *P. pu-*

mila. Our investigations on *Pinus peuce* are a contribution to the cytogenetics of this genus and to the cytogenetic of the genus *Pinus* in general.

MATERIAL AND METHODS

The cytogenetic investigations were carried out on seedling from seed collected from the trees natural habitats on the high mountainous region of Pelister, Macedonia.

Seeds were germinated in optimum germination conditions to allow normal development of the seedlings. When the roots were 1—2 cm long, their tips (2—5 mm) were cut and pretreated in oxyquinoline solution (0,002 mol/l), for 4—5 hours. The roots were then fixed in acetis alcohol (1:3) overnight and stained in Feulgen.

For morphometric analysis, the mitotic chromosomes were analysed. Measurement were taken on the mikrophotographyes. The length (relative and absolute) and arm index were determined. The measurements were made on the ten metaphase plates, only with good arrangement of the chromosomes. Chromosome are arranged to decreasing order.

RESULTS AND DISCUSSION

The cytogenetics analysis on the genus *Pinus* were carried out by many authors. Sax and Sax (according to Saylor) for the first time investigated cytogenetically this genus and concluded that the basic number is $n = 12$. Serries of karyotype analisis were done by Saylor (1961, 1964, 1970) who investigated different pine species. He presented evidence that among some species are present karyotype variation. So, some species of Laricones group contain two heterobrachial schromosome instead of one. He found that the second small chromosome had submedian location of the centromere. Karyological date of 46 species of the subgenus *Pinus* indicated that the investigated karyotype are similar, but not identical. Karyotype investigations in subgenus *Strobus* was done by Muratova (1978, 1979). She analysed the karyotype of the *P. sibirica*, *P. koraiensis* and *P. pumila* and concluded that karyotypes in this group contains only one chromosome which is distinguished from other chromosomes, which are homobrachial and almost homomorphic.

Our investigations demonstrated that the basic number of the chromosomes is $n = 12$ (Tab. 1 and plate 1), number which was established for other species of this subgenus and genus *Pinus* too; (Saylor, Muratova).

According to the morphology we concluded that in this species, the karyotype is rather symmetrical. Eleven chromosomes are isobrachial type and only the smallest chromosome in the complement are heterobrachial. Thus it can be distinguished morphologically from other chromosome. However, according to the classification of Levan (1964) all of the chromosomes are metacentric, because the arm index (long: short arm) are less than 1,7.

For investigation of the chromosomes a suitable feature is the number and position of the secondary constrictions. From our analysis, we can see that the number of chromosome having secondary constrictions ranging from four to eight. More frequently, secondary constrictions were found on the 3, 5, 7, 8 and 10 chromosome, but rarely in chromosome 9. In some cases secondary constrictions were found on the 12th chromosome. Plate 2 and 3. This conclusion we have possibility to decide with analysis of interphase nucleus which permit a more detailed analysis the number of nucleoli. Plate 4.

Previous investigations showed that number and location of secondary constrictions in *Pinus* vary, not only from species to species, but also from population to population, and even from plant to plant in the same species (Saylor 1961, 1964) Muratova (1978, 1979) who has investigated three species in the subgenus *Strobus* as *P. peuce*, found that in all of the analysed species they consist a more than four pairs with secondary constrictions. About *P. sibirica* the above mentioned author found that different populations consist polymorphism among chromosome with regular secondary constrictions. In this species, eight pairs of the chromosomes has regular secondary constrictions, but from population to population there are differences. In *P. pumila* 7 to 8 chromosomes contain secondary constrictions, but in some plant this number is must be 14.

This investigation suggests that *Pinus peuce* has a similar karyotype as other species of the subgenus *Strobus*, *P. sibirica*, *P. koraiensis* and *P. pumila* (Muratova, 1978, 1979) and *P. strobus* (Saylor, 1961). In these species the previous authors have found that only one pair of chromosomes has distinguished morphology from other chromosome on the complement. This pair of chromosomes is heterobrachial, and the rest are homobrachial. These features are present in other species of the genus *Pinus*, except in the *Laricaceae* group (Saylor 1964) where two pairs of chromosomes are heterobrachial.

This investigation very clearly demonstrates that despite geographical barriers and sexual isolation, the genus *Pinus* it seems has very stable karyotype, and that chromosome rearrangement does not play a big role in the speciation on this genus

MORPHOMETRIC CHARACTERISTICS OF *PINUS PEUCE*

Chr. number n	Long absolute L um a	Long relative L r	Arm index A i
1	10,63	9,11	1,00
2	10,36	8,89	1,05
3	10,28	8,77	1,05
4	10,10	8,68	1,06
5	10,04	8,62	1,03
6	9,97	8,46	1,05
7	9,83	8,43	1,05
8	9,77	8,39	1,03
9	9,54	8,20	1,03
10	9,39	8,00	1,05
11	9,32	7,78	1,07
12	7,48	6,42	1,49

KARYOGRAMEE OF PINUS PEUCE GRISEB

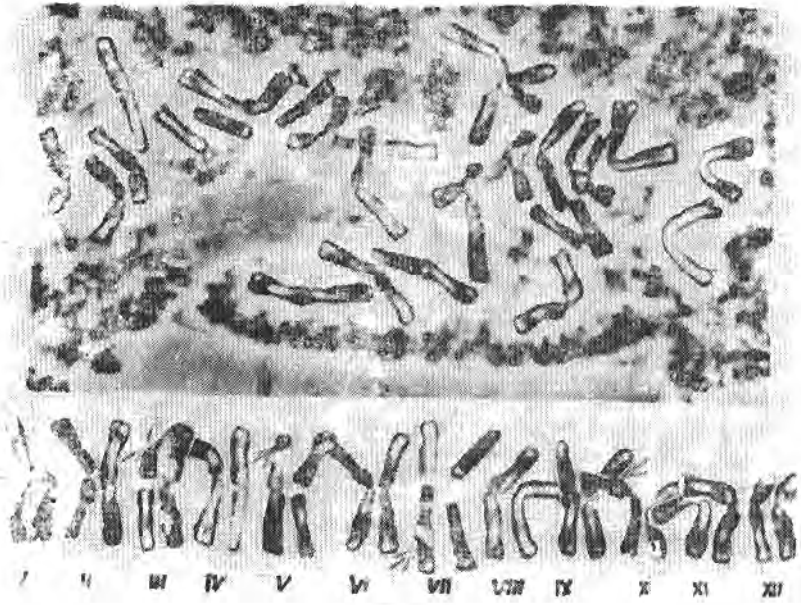


Foto 1



Foto 2

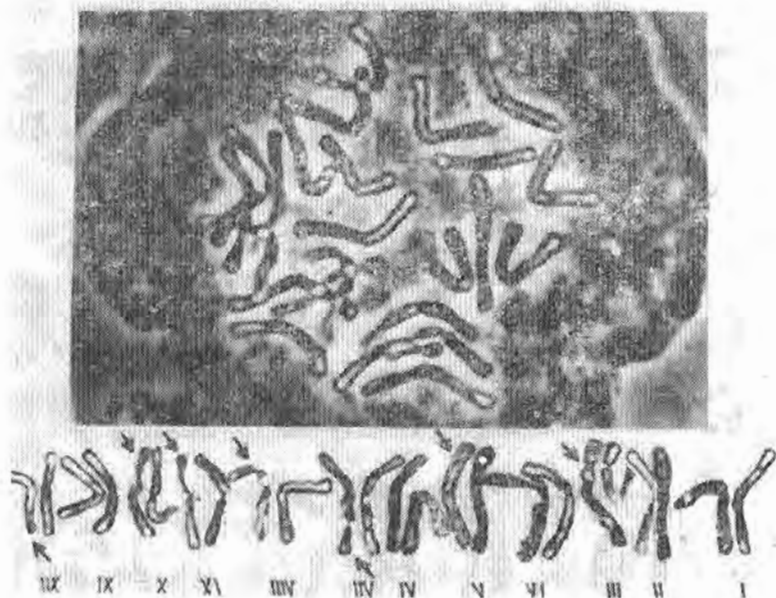
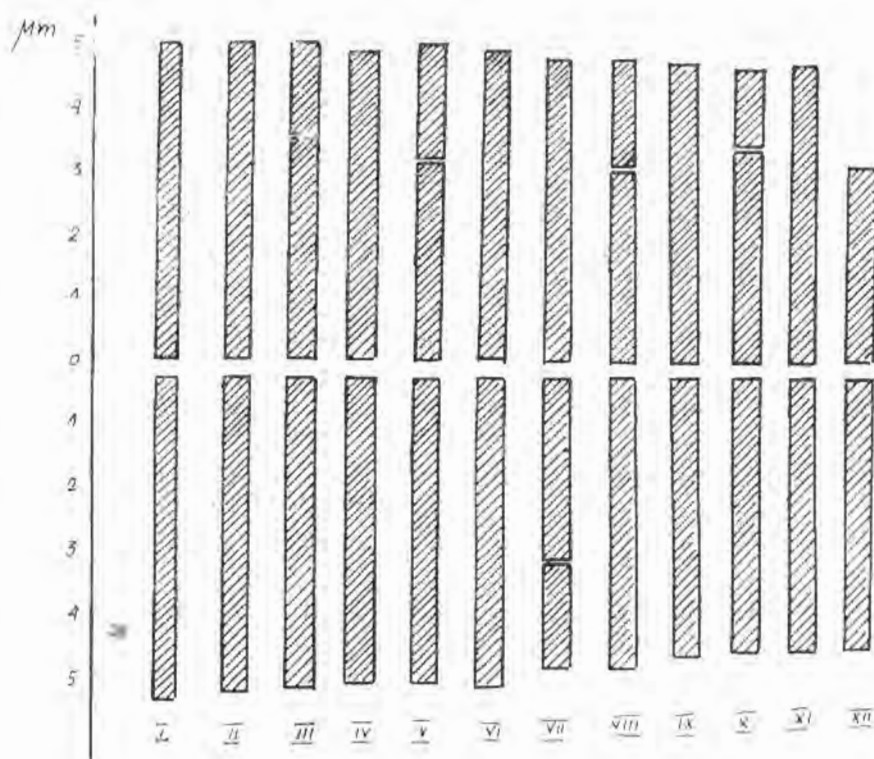


Foto 3



Plate 4

Karyotype of *Pinus peuce* Griseb. 1. Cell with four visible satellites. 2. Cell with five visible satellites. 3. Cell with seven visible satellites. 4. Inerphase nuclei with six visible nucleoli.



KARYOTYPE ANALYSIS OF *PINUS PEUCE* GRISEB

by

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We have analysed the karyotype of *Pinus peuce* Griseb. From the investigations we can conclude that the species has $2n=24$.

The length of the chromosomes is ranged from $10,63 \mu m$ to $7,48 \mu m$ and arm index from 1,00 to 1,47. That means that all chromosomes are metacentric, and that the karyotype is rather symmetrical. Only one pair which is smallest in the karyotype is heterobrachial. The rest eleven chromosomes are rather homobrachial.

The karyotype of *P. peuce* is similar to the other species of the subgenus *Strobus* despite geographical barriers and sexual isolation.

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KARIOTIPSKA ISTRAŽIVANJA VRSTE *PINUS PEUCE* GRISEB

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REZIME

Analizirali smo kariotipove vrste *Pinus peuce* Griseb. (molika) Utvrdili smo da diploidan broj iznosi $2n = 24$, kao i ostale vrste subgenusa *Strobus*.

Dužina hromozoma kreće se u granicama od 7,48Mm do 10,63 Mm. Indeks krakova je od 1,00 do 1,49, što ukazuje da su svi hromozomi metacentrični. Najmanji hromozom u garnituri, iako je metacentričan (indeks krakova 1,49) heterobrahijalan je, dok su svi ostali hromozomi nomobrahijalni.

Broj hromozoma sa sekundarnom konstrikcijom kreće se od 4 do 8. Najčešće sekundarna konstrikcija je utvrđena kod hromozoma 3, 5, 7, 8 i 10.

Upoređujući kariotip molike sa kariotipovima ostalih vrsta subgenusa *Strobus*, možemo da konstatujemo da postoji velika sličnost u morfologiji hromozoma. Ovo ukazuje da u specijaciji molike (*Pinus peuce*) hromozomski rearanžmani nisu imali primarno mesto.