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Investigations on Chromosomes of Siberian Spruce (Picea obovata Ledeb.)

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Summary

38 individuals of the species *Picea obovata* Ledeb. were studied with special regard to their chromosome number and chromosome morphology. The chromosome number within the diploid (somatic) set is 2n = 24. Socalled accessory or B-chromosomes were not found.

Within two specimens of the investigated plants, a pair of homologous chromosomes (no. 11), was found which differed in length. Thus there exists polymorphism in chromosome length of pair no. 11.

Key words: *Picea obovata*, karyotype, polymorphism, B-chromosomes.

Zusammenfassung

38 verschiedene Bäume der Art *Picea obovata* Ledeb. wurden in bezug auf Chromosomenzahl und -morphologie untersucht. Die Chromosomenzahl beträgt im diploiden (somatischen) Satz 2n = 24. Sog. akzessorische oder B-Chromosomen konnten nicht nachgewiesen werden.

Es wurden bei zwei Exemplaren der untersuchten Pflänzchen unterschiedlich lange Chromosomen beim Paar Nr. 11 gefunden, d. h. es liegt hier ein Polymorphismus der Chromosomenlänge vor.

1. Introduction

Since its designation as a species by Ledeb. (1833) dissension exists as to the systematical position of Siberian Spruce in the genus *Picea* (Schmidt-Vogt, 1974). Teplouchov (1898) concluded that of the characteristics described by Ledeb., i.e. the position of the cones and the form of needle-tips and cone-scales, only the form of the cone-scales allows a clear differentiation.

As to further macroscopic morphological characteristics, there are recent indications in the literature that *Picea obovata* differs from *Picea abies* in the occurrence of so-called „B- chromosomes" in addition to the regular 24 chromosomes in the somatic set (Kruklis, 1971; Pravdin *et al.*, 1976; Pravdin and Rostovtsev, 1979).

In the present paper, the karyotype of Siberian Spruce (origin Altai-mountains, 52° northern latitude, 86° eastern longitude, 1500 m above sea level) was investigated with special regard to the number and morphology of the chromosomes.

2. Material and Methods

The material for the studies were 4-year-old plants, grown from a seed lot from the above-mentioned origin[1]).

A total of 482 samples (root tips) from 47 different trees were investigated. Thereof, 109 samples from 38 different trees contained useful metaphase-stages for karyotype-analysis.

The root tips were taken between late July and late September from potted plants, rinsed with Aqua dest. and subsequently treated as follows:

In order to increase the number of metaphase-stages, the root tips were incubated in colchicine-solution (0.01 to 0.05%) at 24° C for 4 to 24 hours. After the colchicine-treatments, the samples were rinsed throughly in Aqua bidest. and subsequently fixed in ethanol-acetic acid (3:1) at 4° C for 8 to 24 hours. The samples were macerated in different ways, the most favourable method proving to be the acetic-treatment (45%) for one hour at 60° C. Other methods, such as the treatment with 45% acetic acid for only 10 minutes at 60° C (Kondo and Hizume, 1982) or incubation with 0.2 to 1 N hydrochloric acid at different temperatures (Mergen and Novotny, 1957; Greilhuber, 1973 and 1974; Wochok *et al.*, 1980; D'Amato *et al.*, 1981; Mac Pherson and Filion, 1981; Schlarbaum and Tsuchiya, 1981) showed less satisfactory results. Also, the use of cellulase

[1]) The author is greatly indebted to Doc. Dr. Leon Mejnartowicz, Institute of Dendrology, Kórnik, Poland, for providing the seed.

(4% solution) or cellulase plus macerozyme (4 + 2% solution) was unsatisfactory, since in addition to the dissolution of the cell wall, single chromosomes were usually lost. Squashed preparations were made in acetic acid (45 %) or in ethanol-acetic acid using refigerated slides. Staining of the chromosome preparations was done according to the method described by Wochok *et al.* (1980) for differential heterochromatin staining. The metaphase chromosomes were observed under the microscope at 1000-fold magnification under oil immersion and photographed. The enlargements of these photos were used for assembling the karyotypes in *Figs. 1* and *2*.

3. Results

The usable samples showed a somatic chromosome set of 2n = 24 *(Fig. 1)*. B- or accessory chromosomes were not found.

The longest chromosome pair (no. 1) is meta- or submetacentric (in the sense of the definition by Nagl, 1980) and because of its length unequivocally identifiable.

The next five pairs (nos. 2—6) are also meta- resp. submetacentric. However due to slight differences in length, they cannot be identified with certainty, all the more so as the arm-length-ratios differ only slightly or not at all.

Moreover, the extant bands (clear C-bands on the telomeres and in the centromeric region as well as indistinct G-banding along the chromatids) are not sufficiently specific for an exact determination of homologous chromosomes.

The pairs 7—10 are shorter than the pairs 1—6 and differ in armlength-ratio.

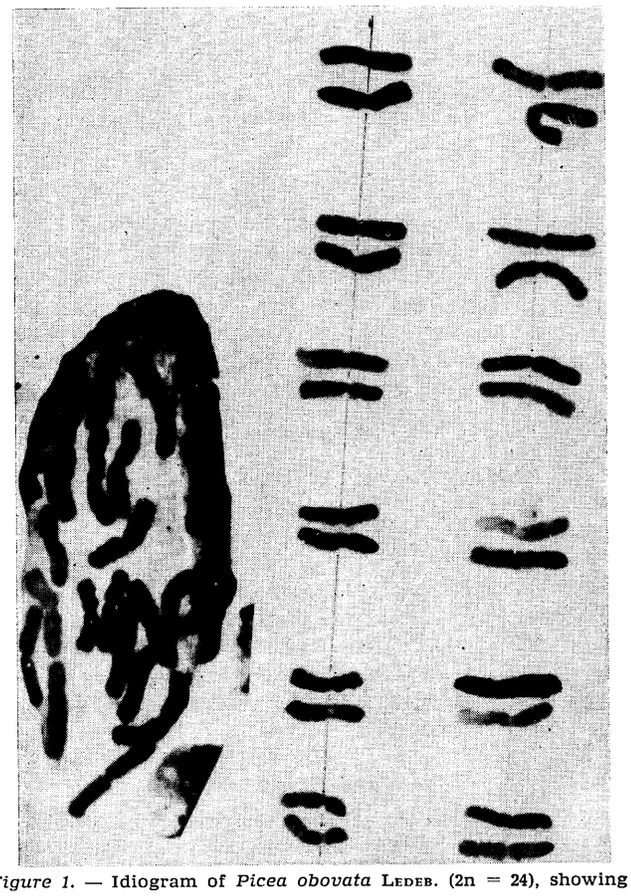

Figure 1. — Idiogram of *Picea obovata* Ledeb. (2n = 24), showing a "normal" pair no. 11.

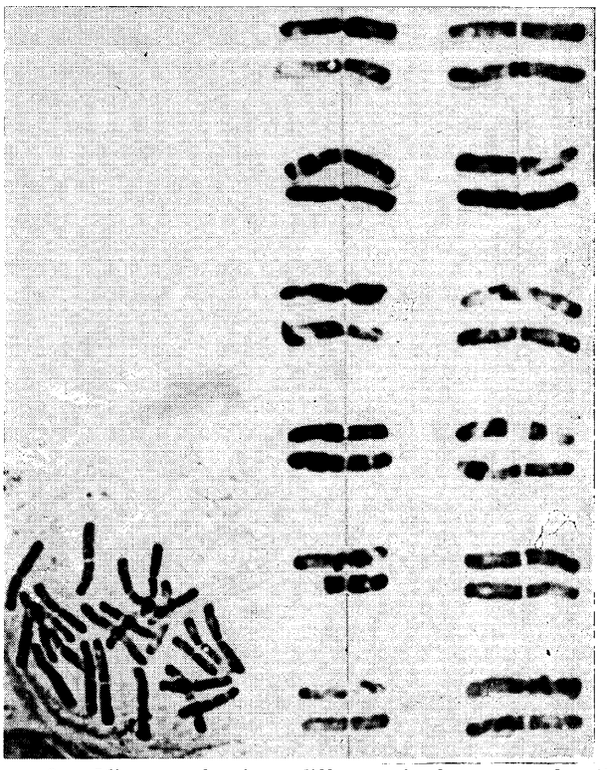

Figure 2. — Idiogram, showing a difference in chromosome length of pair no. 11.

In two trees, pair no. 11 showed a peculiarity: it was composed of one submetacentric and one shorter, probably subtelocentric, chromosome *(Fig. 2)*.

Chromosome pair no. 7 is submeta- or subtelocentric, the pairs 8 and 10 are meta- or submetacentric, while pair 9 is subtelocentric.

The chromosome pair no. 11 is normally submetacentric. The shortest pair in the somatic set (no. 12) consists of two submeta- or sub-telocentric chromosomes.

The above-mentioned results are qualitative assertions which must be quantified by measurements on a large number of samples; for this purpose, the single chromosomes should be easily identifiable by a characteristic banding pattern.

4. Discussion

B-chromosomes as described by Kruklis (1971) and Pravdin *et al.* (1976) as well as by Pravdin and Rosovtsev (1969) were not found within the test material. One possible explanation for this result is the comparatively small number of individuals available for the present studies. While Pravdin *et al.* (1976) used more than twice as many trees for their investigation, comparable numerical data are missing in Kruklis (1971).

Kruklis investigations apply to two populations near Kransnojarsk, whereas Pravdin *et al.* also studied a population in the Altai mountains (49° northern latitude, 87° eastern longitude). In this population, they found that 17 per cent of the plants contain B's. Due to these differing results, the question as to the cytogenetical evolution of such accessory chromosomes arises. To date accessory chromosomes have been described within the forest tree species *Picea obovata* Ledeb. and *Picea sitchensis* (Bong.) Carr. (Moir and Fox, 1972). For the last-named species, more

detailed studies exist, especially concerning the mechanism of meiotic segregation as well as the effects of B-chromosomes on flowering (KEAN *et al.* 1982) and growth (MOIR and Fox, 1976; KEAN, 1981) As a result, it was found that the accumulation of the B-chromosomes is probably due to their preferential migration at the first meiotic division in the female to the pole giving rise to the single functional megaspore (KEAN *et al.*, 1982).

These findings are of interest for the present study insofar as the absence of B-chromosomes in the test material may be explainable by their absence in the mother trees.

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Short Note: An albina-type natural Chlorophyllmutant in Azadirachta indica A. Juss

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Abstract

Occurrence of albina-type chlorophyll mutant in Neem is reported. This is perhaps the first report of achlorophyllus seedlings detected in the half-sib progenies of ten selected Neem trees.

Key words: Neem, Albino-Seedlings.

Zusammenfassung

Es wird über das Auftreten von Albino-Sämlingen (Chlorophyll-Mutanten) bei *Azadirachta indica* A. Juss berichtet. Dies ist möglicherweise der erste Bericht über Albino-Sämlinge, die bei dieser Baumart in Halbgeschwister-Nachkommenschaften von 10 selektierten Einzelbäumen entdeckt wurden.

Introduction

Azadirachta indica A. Juss., the Indian Neem tree is wide-spread in this country, both in wild state as well as under plantations. It is a common avenue tree and highly valued as medicinal plant and as a timber. The Neem seed oil is also used as an agent for pest control. Neem has been employed as a rule for dry zone afforestation in various states. For large scale plantations under social forestry programme, various experiments are being conducted at nursery stage and from one such experiment, Neem seedlings exhibiting chlorophyll mutation is reported in this paper.

Material

Open pollinated seeds from ten mature selected trees having dense crown and profuse fruiting were collected during the months of June and July. The seeds were germinated immediately after collection as Neem seeds do not retain viability for a longer period. From each individual tree, 2000 seeds were kept for germination and nearly 12510 seedlings were raised from ten individual trees *(Table 1)*. After completion of the germination period, seedlings were transplanted into polybags containing soil, sand and farm yard manure and were kept under observation.

Observations

The Neem seeds germinate epigeously and the period of germination was 20 to 27 days. The germination percentage obtained in ten trees varied from 60 to 68 per cent. Bed No. 7 (Tree No. 7) showed three seedlings having milky

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