

Many authors observing differences in nutrient content between provenances, progeny groups or seed lots have discussed the causes of the differences. Differences in root efficiency for absorbing nutrients from the soil or growth medium, the size of the root absorption surface, efficiency of translocation and metabolism, or differential growth responses to other environmental influences, have all been postulated (MERGEN and WORRALL, 1964; WALKER and HATCHER, 1965; STEINBECK, 1966; GROVES, 1967; GIERTYCH and FOBER, 1967).

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#### Summary

Trees of six clones, whose parents were selected for overall phenotypic superiority, have been examined for crown dry weight and nutrient content characteristics. Variations between clones in branch number and size were similar to those reported previously. Regressions relating branch leaf or wood (inclusive of bark) weight to branch size differ little between clones; but because of varying branch numbers and average size, linear regressions of total crown weight on bole diameter differ significantly between clones, mainly in the value of the intercept.

The concentrations of the six mineral nutrients varied between clones, both in overall average for the tree crowns of each clone and for 1-year old leaves. Evidence is provided that genetic influence in the distribution of nutrients through the crown should be recognised when foliage samples are used to assess nutrient status of trees.

The crowns of trees of similar bole size varied markedly between clones in the amount of each nutrient accumulat-

ed. Differences between clones in crown dry weight contributed largely to differences in amounts of nutrients accumulated, but differences in concentration influenced the relationships between clones for each nutrient. In selecting trees for planting, particularly in areas of infertile soil, it could be important to select clones capable of rapid growth with relatively small nutrient uptake.

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## Karyotype Analysis of Norway Spruce *Picea abies* (L.) Karst.

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#### Introduction

Cytogenetical researches into the chromosome morphology in tree species provide us with the basis allowing to make a series of conclusions in connection of their cytogenetical evolution. It is essential to know what genetical differences result from changes in chromosome morphological constitution and to what extent variation is due to gene mutations.

In addition to the classical work of SAX and SAX (1933) concerning the chromosome morphology of conifers, a series of publications relating to the karyotype analysis of conifer species have appeared in the last years (MEHRA and KHOSHOO, 1956 a, b; AASS, 1957; SANTAMOUR, 1960; NATARIAIAN *et al.*, 1961; SAYLOR, 1961; SIMAK, 1962; MORGENSEN, 1962; CHRISTIANSEN, 1963; YIM, 1963; MERGEN and BURLEY, 1964; PRAVDIN, 1964; SAYLOR, 1964; SIMAK, 1964; BURLEY, 1965; TARNAVSCHI and CIOBANY, 1965; KUMAR *et al.*, 1966; SIMAK, 1966; KRUKLIS, 1967; PEDERICK, 1967; TOYAMA and KUROKI, 1967; SHISHNIASHVILI, 1968; THOMAS and CHING, 1968). Much work still needs to be done in this field.

The previously published papers on cytological investigations of conifers have amply demonstrated the importance of pretreatment methods of the material in making squash preparations (MERGEN and NOVOTNY, 1957; NATARIAIAN *et al.*, 1961; SAYLOR, 1961; MERGEN and BURLEY, 1964; WINTON, 1964; BURLEY, 1965; KEDHARNATH and UPADHAYA, 1965; SIMAK and HAPPEL, 1966).

The purpose of the research described in this paper was to determine a more suitable method of making squash preparations from root tips for chromosome morphology studies in Norway spruce and to establish the karyotype of the species.

#### Materials and Methods

The present investigation was made on seed material collected in Estonia from the Järvselja Forest District. All the trees from which the seed were gathered, were described from a taxonomical point of view, based on differences in the cones and cone scales, as a var. *acuminata* BECK.

Several combinations of pretreatments with 0.002 M aqueous solution of 8-oxyquinoline, with saturated aqueous

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solution of coumarin and paradichlorobenzene, with 0.1%, 0.2% and 1.0% aqueous solution of colchicine were tried out. It was found that after the pretreatment with 0.2% aqueous solution of colchicine for 5 hours at  $+20^{\circ}$  to  $+26^{\circ}$  C, the contraction of the chromosomes was the most suitable to distinguish satisfactorily the details of chromosome morphology of Norway spruce.

The following techniques were applied:

1. *Germination*: Seeds were germinated on a moist filter paper in a petri dish at the temperature of  $+26^{\circ}$  C.
2. *Colchicine pretreatment*: When the roots of germinating seeds had reached a length of 0.5 to 0.7 cm, the seeds were transferred to another petri dish containing the filter paper

moistened with 0.2% colchicine solution and germination continued there for another 5 hours.

3. *Fixing*: After the pretreatment the root tips were fixed in alcohol-acetic acid (3 : 1) for 0.5 to 2 hours.

4. *Hydrolysis and staining*: Root tips were stained at first in a mixture of 2% aceto-orcein and NHCl (9 : 1) for 30 minutes at  $+60^{\circ}$  C and in addition to this for 30 to 45 minutes in the same mixture at room temperature.

Then squash preparations were made.

The length of the chromosomes was measured from camera lucida drawings ( $\times 2100$ ) with dividers and engineer's ruler to the nearest 0.2 mm. All the chromosomes in a plate ( $2n = 24$ ) were measured. The regions of centromeres and secondary constrictions were omitted from



Figures 1—4: — Fig. 1 (above). Metaphase chromosomes ( $2n = 24$ ) of *Picea abies* ( $\times 1440$ ). One chromosome has broken from centromere region on squashing. — Fig. 2 (midst). Karyotype on the ground of the same metaphase plate as fig. 1 ( $\times 1800$ ). — Fig. 3 (below left). Idiogram of *Picea abies*. — Fig. 4 (below right). An interphase nucleus with 8 nucleoli ( $\times 2000$ ).

measurements. Then the absolute values of chromosome length were put in relation to the average chromosome (= 100 units) of the plate as it was earlier done by SIMAK (1962; 1964; 1966). Only such plates were selected for the measurements on which the spiralization index of chromosomes was approximately of the same value. The spiralization index was calculated as the ratio (in percentages) of the sum of the length of the two smallest chromosomes to the sum of the length of the two biggest one. This method of determining the spiralization index of the chromosomes was according to the idea of SASAKI (1961) successfully used in karyological investigations with mitotic chromosomes in man (GINDILIS, 1966) as well as with plant chromosomes in *Allium* species (PAVULSONE *et al.*, 1970).

For each chromosome, too, was determined the arm index as the ratio of the longer arm to the shorter one.

### Results and Discussion

The haploid chromosome number of the genus *Picea* showed earlier, is  $n = 12$  (SAX and SAX, 1933; KIELLANDER, 1950; MEHRA and KHOSHOO, 1956 a; SANTAMOUR, 1960; MORGENTERN, 1962; BURLEY, 1965).

The present investigation also shows  $n = 12$  chromosomes of which 10 are with centromere in the median region (arm ratio 1.0 to 1.7) and 2 are with submedian centromere (arm ratio is 1.7 to 3.0) (Fig. 1 and 2). The centromeric position of the chromosomes was described on the ground of the nomenclature published by LEVAN *et al.* (1964).

On the basis of 35 measurements the haploid karyotype in the form of an idiogram was constructed. In the idiogram the chromosomes were placed in order of their total relative length, the longest chromosome first and the shortest one last, and were arranged with the short arm directed upwards (Fig. 3).

Table 1. — Relative length and arm ratio of the chromosomes.

Chromosome Nos.	Relative length	Arm ratio
I	$126.4 \pm 0.5$	$1.07 \pm 0.01$
II	$114.3 \pm 0.6$	$1.10 \pm 0.01$
III	$111.3 \pm 0.6$	$1.12 \pm 0.01$
IV	$110.2 \pm 0.6$	$1.07 \pm 0.01$
V	$103.5 \pm 0.6$	$1.32 \pm 0.01$
VI	$103.4 \pm 0.5$	$1.10 \pm 0.01$
VII	$102.0 \pm 0.4$	$1.10 \pm 0.01$
VIII		
IX	$91.2 \pm 0.7$	$1.73 \pm 0.02$
X	$86.7 \pm 0.6$	$1.47 \pm 0.02$
XI	$79.1 \pm 0.5$	$1.26 \pm 0.01$
XII	$68.7 \pm 0.3$	$2.12 \pm 0.03$

Table 1 shows the average relative length of each individual chromosome in relation to the mean chromosome length of the karyotype and the average value of the arm ratio.

5 of the chromosomes with the centromere in the median region show to have a secondary constriction: chromosomes II and V have a secondary constriction on the longer arm and chromosomes III, VI and X on the shorter one. But it should be mentioned that the secondary constrictions were not always apparent on all the 5 pairs of abovementioned chromosomes. In some cases only one of the homologous chromosomes of the pair indicated a secondary constriction.

Secondarily constricted chromosome pairs (II, III, V, VI and X) as well as chromosomes I, IX, XI and XII are quite easily identified on the plate. Chromosome IV, VII and VIII are more less easily identified whereas chromosomes VII and VIII are impossible to distinguish from each other.

At least 4 pairs of the secondarily constricted chromosomes appear to be capable of organizing the nucleoli, since it was possible to count 8 nucleoli in the interphase nuclei (Fig. 4). But in most of the nuclei there was observed a varying number of nucleoli (4 to 7). The low number of nucleoli may be due to the fusion of several nucleoli. Such an opinion was earlier calculated also by NATARAJAN *et al.* (1961) in the paper which deals with the chromosome morphology of *Pinus silvestris*.

Of the literature cited in this paper in connection of chromosome morphology studies of conifers, only SAX and SAX (1933) have published the idiogram of *Picea abies* observed in the early development of the endosperm. But the paper contains no direct data on the length of the chromosomes and data of this kind for the comparison with results of the present study can only be procured by measurement on the published idiogram and calculating likewise the absolute length of each chromosome in relation to the average one. The comparison is presented in the form of the table 2.

Only the longest chromosome (I) has a clearly different length. In the idiogram published by SAX and SAX, it is longer than on the ground of the present investigation. But in the idiogram of SAX and SAX there are 3 chromosomes with submedian centromere since in the present idiogram there are only 2 of this kind.

No considerations have been taken in the analysis of SAX and SAX as to the occurrence of secondary constrictions. Unlike the present described 5 chromosomes each having a secondary constriction in haploid karyotype, TOYAMA and KUROKI (1967) have mentioned that *Picea abies* has 4 pairs of secondary constrictions.

### Summary

Solution of 0.2% colchicine was satisfactorily used for the pretreatment of root tips to determine the karyotype of Norway spruce. The results of the investigation were presented in the form of the idiogram and were compared with data of other authors.

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Table 2. — Comparison of the karyotype of Norway spruce with results of the investigation of SAX and SAX.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SAX and SAX	146	114	108	107	105	101	99	93	86	85	85	73
Present study	126	114	111	110	104	103	102	102	91	87	79	69
Difference	+20	±0	—3	—3	+1	—2	—3	—9	—5	—2	+6	+4

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## Inheritance and Correlation of Growth Characters in *Populus deltoides*

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### Introduction

Since unrooted cuttings rather than seedlings of eastern cottonwood (*Populus deltoides* BARTR.) are usually planted for timber production in the Lower Mississippi Valley (McKNIGHT, 1970), replicated clonal tests are ideally suited for selecting superior genotypes for planting there. These tests cannot be adequately designed without phenotypic and genetic correlations between measurements made over time and estimates of total genetic variance and covariance. The few data published to date have been based on first- and second-year measurements (WILCOX and FARMER, 1967; FARMER and WILCOX, 1968). We report here figures gathered over six growing seasons in a replicated clonal test.

### Materials and Methods

Clones were taken randomly from a natural stand of 2-year-old seedlings near Rosedale, Mississippi (Bolivar County). For the test, unrooted cuttings from these clones were planted at 9- by 9-foot spacing on a recently cleared site near Stoneville, Mississippi. The soil was a Sharkey clay, which BROADFOOT (1960) described as marginally suitable for cottonwood in the Mississippi alluvial plain. Details related to establishment were given by WILCOX and FARMER (1967).

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The test design was a randomized complete block with six replications, 49 clones, and single-tree plots. Data collected after 1 and 2 years were analyzed on the basis of this design and reported by WILCOX and FARMER (1967). Mortality and damage to trees by the end of six growing seasons led to the restriction of current analyses to 38 clones in five replications with no missing plots. The following variables were examined:

- 1–4. Total height after one, two, three, and five growing seasons.
- 5–6. Height growth in the second and third growing seasons.
- 7–11. Diameter at 1 foot after one and two growing seasons and at 4½ feet after three, five, and six growing seasons.
- 12–13. Diameter increment in the second and sixth growing seasons.

All heights were measured to the nearest 0.1 foot with the aid of a pole, and diameters were measured to the nearest 0.1 inch.

Following analysis of variance, the clone and error variance components were estimated from the mean square for all variables. Ratios of genetic to phenotypic variance (broad-sense heritability) and their confidence limits were calculated from two formulas:

$$H^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_e^2} \quad (1)$$

$$P [1 - K_{\alpha/2} \leq H^2 \leq 1 - K_{1-\alpha/2}] = 0.95 \quad (\text{BECKER, 1967}) \quad (2)$$

$$\text{where } K_x = \frac{r MS_2 F_x}{MS_1 + MS_2 (r-1) F_x}$$