CHROMOSOME BANDING FOR IDENTIFICATION OF THE CHROMOSOMES OF NORWAY SPRUCE

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ABSTRACT

Images of *Picea abies* (L.) KARST. chromosomes ($2n = 2x = 24$) were taken through a light microscope equipped with a video camera and digitized by image analysis equipment. The original image was enhanced with electronic filters and arithmetic operations, *e.g.*, background correction. A scaling option offered the possibility of enlarging the chromosome region of interest by 8×. Precise measuring and statistical evaluation of chromosomes were then possible. Another method we used to identify chromosome pairs was pattern recognition. Using this approach, five chromosomes could be identified: chromosomes I, II, IV, IX, and XII. The other chromosomes could be separated only into two groups. In subsequent experiments, chromosome banding was used to increase the efficiency of chromosome recognition using this system. Different banding methods were attempted using megagametophytic tissue as the experimental material. The use of this material is advantageous because of the smaller haploid number of chromosomes, and because the cells are easily separated. Banding patterns were compared by examining the distances and intensities of the bands. Using the combination of image analysis and chromosome banding, specific recognition of all chromosomes of *Picea abies* was possible. Because nuclei in division (especially metaphase plates) are very rare in megagametophyte tissue and also in root meristems, we discuss the use of interphase nuclei and their 3-dimensional reconstruction for future evaluation.

Key words: karyotypes, *Picea abies* (L.) KARST., chromosome banding, digital image analysis, pattern recognition system

INTRODUCTION

Norway spruce (*Picea abies* [L.] KARST.) is the most important forest species in Austria. Decline of the spruce trees is a complex problem and many scientists are working toward solutions to this problem. Detection and assessment of the damage are the first steps. DRUŠKOVIČ (1988) showed that disturbances of the chromosome set in dividing root meristems can be used as bioindicators of decline in Norway spruce. This method was developed and standardized by MÜLLER *et al.* (1991, 1992, 1994a, 1994b). They showed not only that changes concerning the whole chromosome set can appear, as by their stickiness or clumping, but also that single chromosomes can be affected, for example by gaps, breaks, or deletions. Therefore it was very important to recognize all of the chromosomes of Norway spruce. GUTTENBERGER & MÜLLER 1995, KÖHLER *et al.* 1995 were only able to identify five of the twelve chromosomes using the Feulgen squash method. A computer-based system using pattern recognition did not give better results. In subsequent experiments, chromosome banding was used to increase the efficiency of chromosome recognition. Different banding methods were attempted using the female gametophyte as the experimental material. This material has the advantage of including a haploid number of chromosomes, and the cells are easily separated. Banding patterns were compared by examining the distances and intensities of the bands. Using the combination of image analysis and chromosome banding, specific recognition of all chromosomes of *Picea abies* was possible.

Among the papers on karyotypes of Norway spruce are those by SAX & SAX (1933), BIAŁOBOK & BARTKOWIAK (1967), PRAVDIN *et al.* (1976), TERASMAA (1971, 1972, 1975), DRUŠKOVIČ (1988), GUTTENBERGER (1993), GUTTENBERGER & MÜLLER (1995), and KÖHLER *et al.* (1995). All karyotypes in these papers are slightly different.

In this paper we also use of new kinds of data presentation for banded chromosomes and for the 3-dimensional reconstruction of interphase-nuclei.

MATERIALS AND METHODS

Plant material and preparation

For root tip preparation, the actively-growing root tips of potted four-year-old Norway spruces, *Picea abies* L. (KARST.), (one clone of 1039/116 – Forstliche

Versuchsanstalt Hannover–Münden) were collected for examination. Samples were treated as in MÜLLER *et al.* (1991). The preparation of the female gametophyte followed the procedure of KÖHLER *et al.* (1995). During May 1995, cones from a single tree of Norway spruce growing in the Botanical Garden of the University of Graz were collected. Ovules were extracted, fixed in 3:1 ethanol : acetic acid for 24 hours at room temperature and stored in 70 % ethanol at 4 °C.

Three different staining procedures were used: (1) Feulgen squash technique (after KÖHLER *et al.* (1995); (2) Giemsa C-banding, a combination of a method used by four authors, including SCHWEIZER (1973, 1974), MARKS & SCHWEIZER (1974), MARKS (1975) and BORZAN & PAPEŠ (1978) – see KÖHLER *et al.* (1996); (3) fluorescent staining with chromomycine A3 (CMA) and 4',6-diamidino-2-phenylindole (DAPI), modified from SCHWEIZER (1976).

Technical equipment and software

An Axioplan microscope (Zeiss Inc.) was used for the observations, equipped with a C-mount adapter for the Sony DXC 930 P video camera (Sony Inc.). Images were captured by the frame grabber ITI MFG–3M–V (Imaging Technology Inc.), including an AM–VS–VP variable scan module and an AM-CLR–VP color recording module. Optimas 4.02 image analysis software (BioScan Inc.) was used. For more details see KÖHLER *et al.* (1995). Image manipulations (autotracing, rotations, arrangements, inscriptions, etc.) were carried out with graphic package Corel Draw 5.0, Corel Photo-Paint 5.0 (Corel Inc.), and Picture Publisher 3.1 (Mikrografix Inc.). Prints were made with a Hewlett Packard Laserjet 4/4M. The data were transferred by DDE (dynamic data exchange) directly to the Excel 5.0 spreadsheet program (Microsoft Inc.).Three-dimensional (3D) presentation of data and 3D reconstruction of nuclei was done with the data language IDL for Windows 3.11 (Research Systems Inc.).

The computer-based recognition system was Autoklas 5.05 (Schindler+Partner Inc.) version S, with additional modules Z1 (compression of foreign data and their classification), Z2 (evaluation test of parameters), Z3 (sorting and printing of array-structures), Z4 (concentration of knowledge bases) and Z5 (analysis of the components). The program is able to manage 90 knowledge bases and to connect them. Each knowledge base can consist of 50 classes, 125 parameters, and 5000 data records.

RESULTS AND DISCUSSION

The methods applied in this work greatly facilitated the identification of the haploid chromosome set in Norway spruce, by a more precise analysis of the karyotype. Many papers have been published on karyotypes of Norway spruce, including SAX & SAX (1933), BIAŁOBOK & BARTKOWIAK (1967), PRAVDIN *et al.* (1976), TERASMAA (1971, 1972, 1975), DRUŠKOVIČ (1988), GUTTENBERGER (1993), GUTTENBERGER & MÜLLER (1995), and KÖHLER *et al.* (1995). However, all karyotypes in these papers are slightly different. GUTTENBERGER (1993) and GUTTENBERGER & MÜLLER (1995) introduced image analysis and a computer expert system in Norway spruce karyotyping. The advantages of these new tools, used in the present work, are that a large number of metaphases can be measured and rapidly set up for statistical analysis in a short time. Using image arithmetic, one can correct the images for the effects of reflected light and/or uneven illumination to enable accurate measurement. The 8× zoom optical equipment is very helpful for precision in measurement.

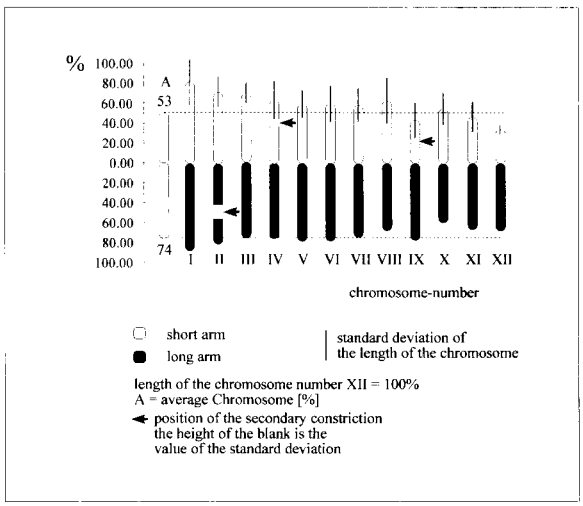

Figure 1 Quantitative idiogram of the root tip meristem of Norway spruce

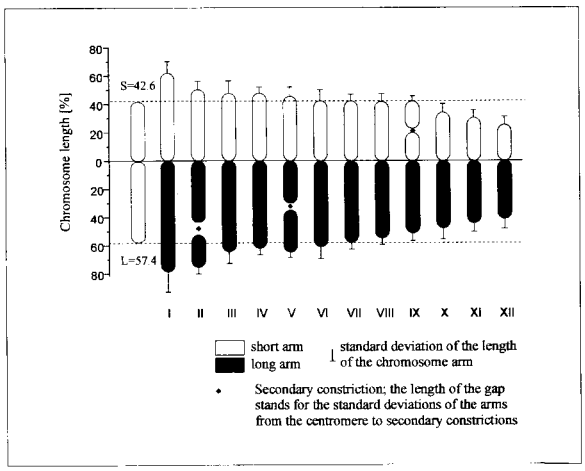

Figure 2 Quantitative idiogram of female gametophyte tissue of Norway spruce.

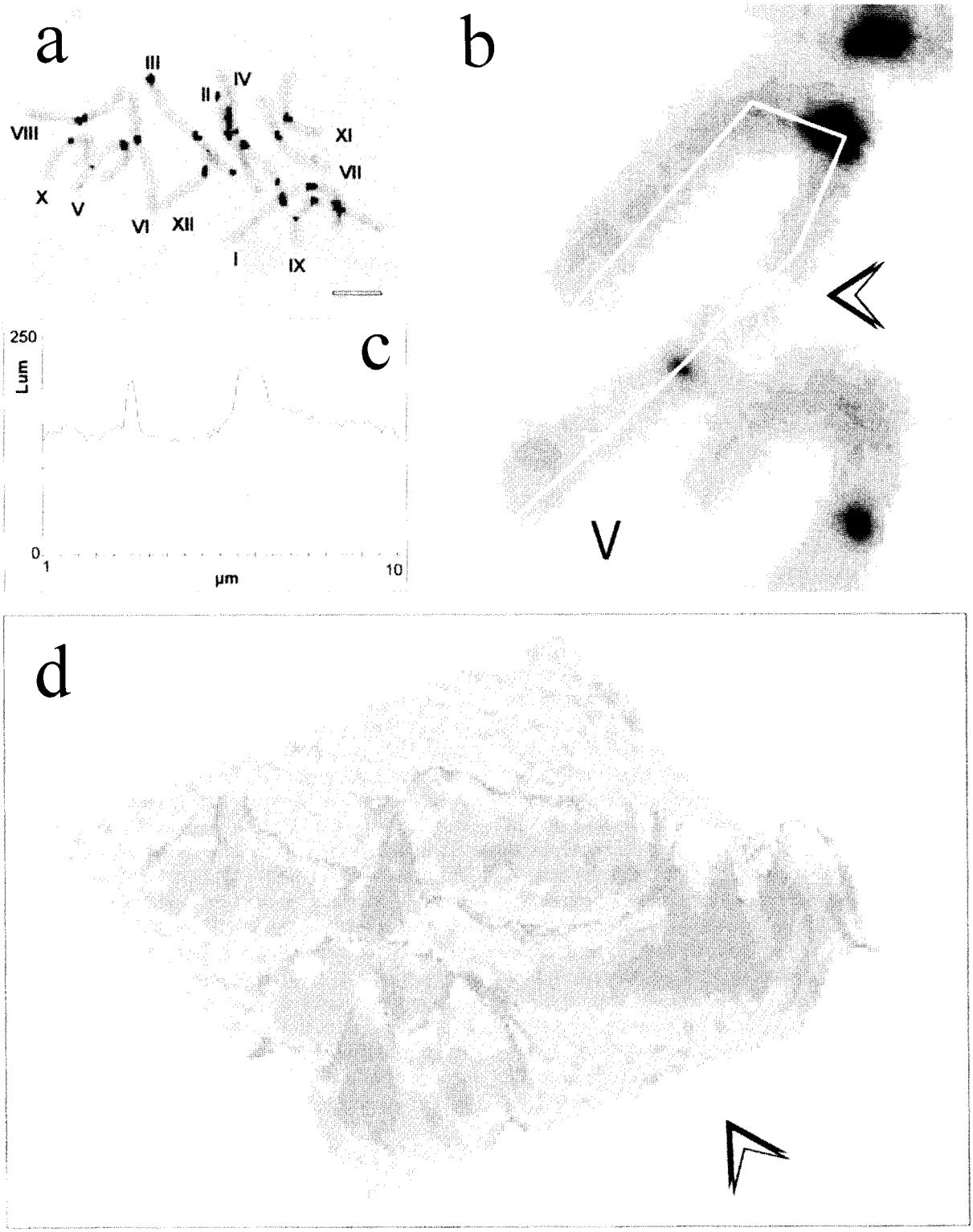

Figure 3 a – Giemsa-C-banded metaphase plate of Norway spruce, bar = 5 1m, chromosome numbers are written in Roman letters (after KÖHLER *et al.* 1996), **b** – enlarged chromosome V of 3a, **c** – density values (Lum) along the white line in 3b, **d** – 3D reconstruction of the density values of the whole chromosome. Arrowheads mark the direction of view (**3b** and **3c**)

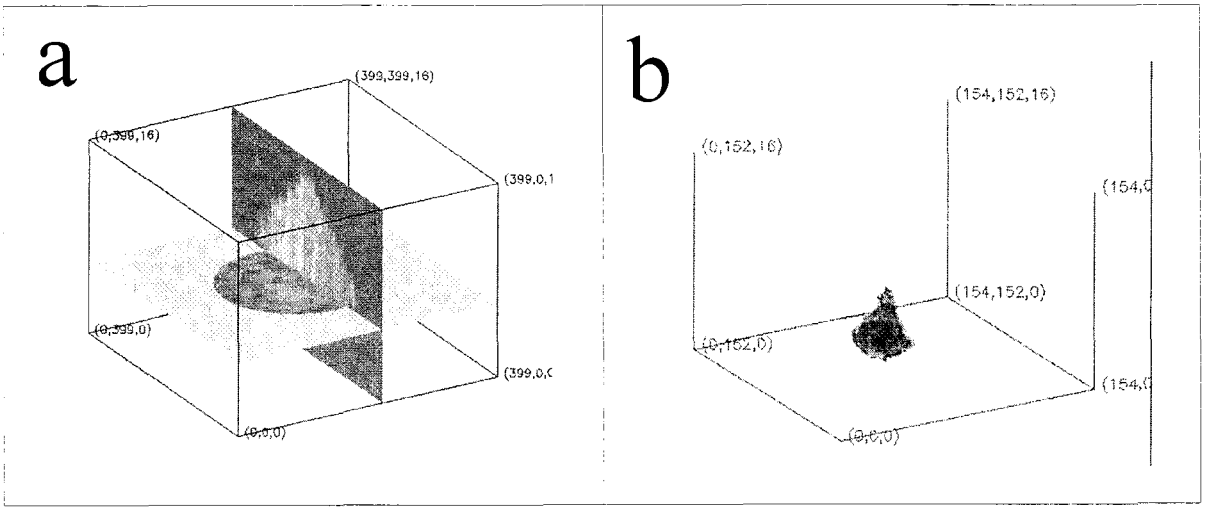

Figure 4 3D reconstruction of an interphase nucleus of the female gametophyte tissue of Norway spruce; **a** – Slices through the nucleus, **b** – Isosurface of the nucleolus

The result of an enlarged image of a chromosome is presented in Figure 3. The advantage of the pattern recognition system is that it is possible to connect the data and to recognize single chromosomes automatically, even if the data of a parameter are missing. Using these tools, we constructed a quantitative idiogram (Figure 1). The shortest chromosome length was set at 100 % and all other chromosome lengths were calculated as percentages of its length. For discussion on this topic see BORZAN *et al.* (1995). However, even with these tools it was impossible to recognize all chromosomes. Therefore, the next approach was to change the tissue for our evaluation and to use the female gametophyte. Advantages of this tissue are the haploid state of the cells and a lesser dependency on chemical procedures for preparation (BORZAN *et al.* 1995). In Figure 2, we propose a new type of representation of the quantitative idiogram. Slight differences are apparent between the idiograms shown in Figures 1 and 2. The question is whether the different tissue or the different treatment causes these differences.

In the next step, we used different banding techniques. Figure 3a shows a metaphase plate of the megagametophyte, stained with Giemsa C. In Figure 3b, chromosome V is enlarged by the optical zoom and the background is removed. Precise measurement is now possible. Figure 3c shows the values of densities along the white line represented in Figure 3b. The density values were between a 0–255 range (8-bit depth). Figure 3d shows a 3D reconstruction of the density values of the whole chromosome. One can get much more and better information than from one single line; it can be seen that bands often do not cover the whole width of the chromosome. With the Giemsa C method it was possible to identify a further chromosome: chromosome number III (KÖHLER *et al.* 1996).

Following these experiments, we tried a lot of different banding techniques to identify all chromosomes. Finally, we found that with a sequential staining technique (CMA, DAPI, and Feulgen) of the same preparation it was possible to distinguish between all twelve chromosomes of Norway spruce (KÖHLER & GUTTENBERGER, in preparation). Mitotic phases are seldom found in root tips in even as high a frequency as 1%. They are still less frequent in megagametophyte tissue, where they are difficult to find even as a fraction of a percent. Well-spread metaphases are therefore, very seldom observed. For this reason, we intend to examine interphase nuclei in the future. With a newly-created technique (GUTTENBERGER, in preparation) it is possible to make "theoretical sections" through an image. These slice series can be combined by the use of the software tool IDL, and 3D reconstruction and volume calculation of isosurfaces are feasible. Figure 4 shows 3D reconstruction of an interphase nucleus of the endosperm of Norway spruce. Figure 4 b shows the reconstruction of the nucleolus. Using this technology, we hope that in the future we can use interphase nuclei for our investigations.

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