Cytogenetic Studies on Norway Spruce [*Picea abies* (L.) Karst.]

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Summary


Since 1989 we used a plant test system by the classification of chromosomal aberrations in the root tip meristems of young spruce trees [*Picea abies* (L.) Karst.] for an early detection of environmental influences on forest tree species. The results obtained from different natural sites and from fumigation experiments recommended this test system to be an easy and sensitive screening method for damages caused by environmental mixtures on spruce trees, for non-accumulating compounds as ozone, too.

In the connection with these studies of the structural chromosome alterations further techniques were performed to get information about the heterochromatin behaviour of the chromosomes. Therefore C-banded preparations were done and as a further dividing tissue female gametophyte tissue was used. First results of the use of the interphase nuclei for cytogenetic studies and the first steps to explore the pattern of the heterochromatic parts of the interphase nuclei are also presented here.

Introduction

The classification of chromosomal aberrations comprise a sensitive method for the assessment of genotoxic effects caused by chemical treatments. The cytological studies are relatively simple to perform and give valuable information to effects of cell division and chromosomes (LEVAN 1938, FISKESJÖ 1985). In our search for an easy and reliable test assay for the detection of genotoxic substances we have carried out a test system by the classification of chromosomal aberrations

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with the spruce tree as bioindicator plant. Some other test systems with forest tree species still exist (Fiskesjö 1989, Matschke & al. 1994, Schubert & Rieger 1994). According to the basic work with spruce trees of a Slovenian working group (Drusković 1988), in 1989 we performed our plant test system by the classification of chromosomal aberrations with the spruce tree as bioindicator plant. We used this method at natural sites (Grill & al. 1993, Müller & al. 1991, 1992, 1994, Müller & Stabentheiner 1996) and under defined conditions in climate chambers (Müller & Grill 1994, Müller & al. 1996), greenhouses and open-top chambers (Müller & al. 1995) for the assessment and evaluation of genetic risks of trees from environmental influences. These studies showed that the classification of chromosomal aberrations in spruce trees is a sensitive measure to investigate various environmental effects on trees.

In the connection with these studies of the structural chromosome alterations further techniques were performed and a further dividing tissue was used to get information about the heterochromatin behaviour of the chromosomes. Karyotyping of gymnosperm macrogametophytes (= female gametophyte tissue, endosperm tissue in development) has been rarely used. This haploid endosperm tissue in development was investigated by Sax & Sax 1933 (different conifers), Santamour 1960 (Pinus and Picea), Pedrick 1967, 1970 (several Pinus species), Borzan 1977a, 1977b, 1981, 1988 (several Pinus species), Borzan & Papěš 1978 (Pinus), MacPherson & Filion 1981 (Pinus) and by some other cytogeneticists. Norway spruce (Picea abies (L.) Karst.) was investigated by Sax & Sax 1933 and Santamour 1960. Köhler & al. 1995 used digital image analysis to form a karyotype from this material. The revealed quantitative karyotype can be used as a reference for comparison with Norway spruce karyotype analyses from studies to detect intraspecific variation and interspecific differences.

Different schemes of classification of plant interphase nuclei have been proposed by several authors (see e.g. Heitz 1929, Tschermak-Woess 1963). According to Nagl 1979 the organization of plant nuclear chromatin is nearly stable and species specific. Tschermak-Woess 1963, Nagl & Fussenig 1979, distinguished three nuclear types based on the structure: diffuse nuclei with small chromocentres; chromomeric nuclei with condensed chromatin; and chromonematic nuclei with eu- and heterochromatin packed in chromocentres. Cremonini & Funari 1993 investigated the plant chromatin organization by using cytophotometric analysis.

In this paper the results are presented of the classification of chromosomal defects as a test system for environmental studies from 1989 to 1996 and discussed on the one hand. On the other hand the cytogenetic studies with the female gametophyte as C-banded preparations and the use of the interphase nuclei and 3-D reconstructions are shown.
The classification of chromosomal defects of spruce trees as an alternative in environmental studies

**Material and Methods**

For our investigations young spruce trees \([Picea abies (L.) Karst.]\) either on-site trees or clonal trees were used. The plants were potted and irrigated regularly. Sampling, preparation and classification of tissue was described by MÜLLER & al. 1991.

**Results and Discussion**

From 1989 to 1996 we have applied our plant test system at natural spruce stands in Austria. The data show that the classification of chromosomal aberrations is a valuable tool in environmental monitoring under natural conditions. Our method works with young spruce trees and the results of the young trees can be correlated to the results of the older trees. Therefore it is possible to determine the vitality of spruce stands (MÜLLER & al. 1991, 1992). The data suggest that an intensive site effect is more significant than an effect of the soil or of the provenance of the individual (MÜLLER & al. 1992, 1994). The results of our plant test system bring valuable information about the condition of the trees, especially also of spruce stands at higher elevation sites (MÜLLER & STABENTHEINER 1996). Plants at higher sites have to cope with lower temperatures, higher irradiation and higher concentrations of naturally occurring photooxidants with the leader substance ozone as compared to plants growing at lower sites (TRANQUILLINI 1979, BOLHAR-NORDENKAMPF & LECHNER 1989). At natural sites it is difficult to decide which factors are the most decisive in affecting the plants. In experiments each different environmental factor may be varied while other factors are kept constant, thus enabling the researcher to test the plant's reaction to the particular factor (FANGMEIER & al. 1992).

In all fumigation experiments no visible evidence of injuries or of stunted growth due to the effect of increased ozone was observed in any of the plants at the end of the fumigation experiments and also up to two years later (MÜLLER & GRILL 1994, MÜLLER & al. 1996). The data showed that the spruce clones used in our experiments were not sensitive to ozone treatments.

The results of all investigations under defined conditions of enhanced levels of ozone (80 to 100 nl l\(^{-1}\)) showed an intensive influence of ozone on the genetic material in the root tips of spruce. The amount of chromosomal defects increased with increasing ozone dose (e.g. MÜLLER & al. 1995, 1996). Up to two years later after the fumigation had stopped a so called long-term after-fumigation effect of ozone on the genetic material of spruce plants could be observed in an increased number of chromosomal aberrations (MÜLLER & al. 1994, 1996). If no long-term after-fumigation effects were manifested in the genetic material of the former fumigated variants all series of our experiments would show the same frequency of chromosomal defects under field conditions. In our experiments the
former ozone-fumigated variant responded under normal air and weather conditions with an increased amount of chromosomal defects compared to the former control variants. Post-fumigation long-term effects of ozone are well known and among others they were observed for plant growth and vitality in loblolly pine (Spence & al. 1990), for secondary metabolites and antioxidants as catechin (Langebartels & al. 1990) and for pigments in spruce needles (Lütz 1992). Thus indicating the genetic material of the root tips of spruce trees is a sensitive measure for oxidative stress, although they did not come in contact directly with ozone. From other studies with different test systems by the classification of genetic defects ozone has been identified as an agent that is genotoxic to plants (e.g. Ma & al. 1982). The investigations concerning genotoxicity of ozone to higher plants were carried out with high ozone concentrations at first. In an early study (Fetner 1958) Vicia faba was exposed to 4000 μl l⁻¹ ozone in the air for 15, 30, and 60 minutes and this treatment induced chromosome-type aberrations in the root meristem cells. The effects of ozone on Vicia were also studied by Janakiraman & Harney 1976 by investigations of the meiotic chromosomes of the buds. These exposures to concentrations of 2 μl l⁻¹ for 4 or 8 hours caused chromosome-type aberrations, too. Further test systems for studying the genotoxic effects of volatile air pollutants in the laboratory and for field monitoring were carried out with Tradescantia. Either somatic mutations in stamen hairs (Schaier & al. 1979) or micronucleus formation in the meiotic pollen mother cells as test systems were used (Ma & al. 1982). Ozone exposures for 6 hours at 5 μl l⁻¹ was positive in the stamen hair system, whereas the test for micronucleus formation (5.5 hours at 5 μl l⁻¹) was negative. In many of these investigations high ozone concentrations were added to the respective test system and the direct effects produced in response to the ozone treatment were then determined after a certain time. In our experiments we worked with concentrations found also in our environment under so called natural conditions. In this connection must be taken also into consideration the distance from the side of ozone influence (the top of the tree) to the root meristems.

Summing up we can conclude: The plant test system by the classification of chromosomal aberrations is a sensitive measure for environmental influences on spruce trees, for non-accumulating compounds as ozone, too. Despite the widespread occurrence of ozone injuries, the mechanisms of the damaging process and the plant defence systems against ozone attacks are still poorly understood. Considering the importance of our forest trees it is essential to characterize their responses to a variety of environmental and pollution impacts.
Cytogenetic studies using digital image analysis and computer 3-D reconstructions

Materials and methods

The macrogametophytes of Norway spruce were collected at the botanical garden of the University of Graz and prepared as described by KÖHLER & al 1995. Sampling and preparation of the root tips of young spruce trees was described by MÜLLER & al. 1991.

The preparations were evaluated by an image analysis system (see KÖHLER & al. 1995). 3-D reconstruction was done using the data language IDL 3.6.1b for Windows (Research Systems Inc.).

Results and Discussion

The use of the image analysis equipment allows for a very fast and exact analysis of a large number of chromosomes. Using image arithmetic, one can de-calibrate the images for the effects of reflected light and/or uneven illumination for making accurate measurements (GUTTENBERGER & MÜLLER 1996).

Therefore we were able to make statistic analyses of the karyogramms. These quantitative karyogramms of roots and from the metaphases of the endosperm are similar, but they show some little differences: the most evident difference is that in root meristems a second constriction was found at the short arm of chromosome IV, in macrogametophyte metaphases we found a second constriction on the long arm of chromosome V. The other two second constrictions are on the same chromosomes (II and IX), but in root meristems is the second constriction on the short arm, in macrogametophyte tissue on the long arm (GUTTENBERGER & MÜLLER 1996, KÖHLER & al. 1995).

Using the dividing material of macrogametophyte tissue has many advantages: It is haploid, the tissue is separated. Staining techniques can therefore tested best by using this material (KÖHLER & al. 1995). Of this material one disadvantage is the short time of availability (only few weeks in spring).

We found that metaphases were very rare in dividing tissues (roots, macrogametophyte). According to NAGL 1979 the organization of plant nuclear chromatin is species-specific but not tissue-specific. Therefore we were reconstructing the morphology of the interphase nuclei. We measured the area of the nuclei, and the number and area of the heterochromatic regions of each nucleus. Following the classification of NAGL & FUSENIG 1979 the interphase nuclei of Norway spruce belong to the 'diffuse nuclei with small chromocentres' - type (Fig. 1a, b, c). We were also able to reconstruct 3 D images of these nuclei (Fig. 1d). We hope that we can use in future interphase nuclei for some general questions of the cytogenetic bioindication.
Fig. 1. Interphase nucleus of *Picea abies* from the root meristeme after Feulgen staining; a) normal view; b) enhanced and in six distinct grey intensities separated image; c) 3-D view of grey intensities of the heterochromatin; d) 3-D reconstruction of the whole nucleus. Bar = 5 μm, * indicates the nucleolus.

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