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Cytophotometrical Measurement of Nuclear DNA Content in Some Coniferous and Deciduous Trees*

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Summary. The DNA content of nuclei from meristematic root tip cells of five coniferous and one deciduous tree species and, for comparison, of *Vicia faba* was cytophotometrically determined. The DNA values of diploid nuclei from *Ginkgo biloba* are approximately a quarter lower than those from *Vicia faba*. The nuclear DNA values of the other tree species are merely a third to a ninth part of those of *Vicia faba*. In three tree species, as well as diploid, we have found nuclei of different polyploid level.

The reliability of different cytochemical methods, which are used for determination of the nuclear DNA content, is critically analyzed. The DNA values of the investigated tree species are discussed in connection with the evolution of the DNA content in higher plants.

Key words: Conifers – Deciduous tree – Polyploidy – Endoploidy – Evolution – Cytophotometry – DNA content

Introduction

Cytophotometrical and densitometrical nuclear DNA determinations of plant cells are of great importance in clarifying the following three problems: a) evolution of higher plants (Price, Sparrow and Nauman 1973; Price 1976; Rees 1972; Sparrow and Nauman 1973); b) relation between nuclear DNA content and minimum generation time (Bennett 1972; Bennett and Smith 1976); c) procedures of endopolyploidisation, nuclear DNA amplification and under-replication and nuclear DNA content, which is tissue specific and dependent on the degree of the differentiation (e.g. Nagl 1976; Frisch and Nagl 1979).

The following investigations were carried out to determine cytophotometrically the nuclear DNA content of meristematic root tip cells from selected tree species. Our own nuclear DNA values were compared with values of other authors to test the reliability of the different staining and measuring methods. The nuclear DNA values were also used to compare nuclear DNA content and 'minimum generation time' and to identify the very early appearance of endoployploid cells. In the following investigations we used five coniferous and one deciduous tree species.

Material and Methods

We used root tip cells of the following five tree species: 1.) *Ginkgo biloba*; 2.) *Larix leptolepis*; 3.) *Metasequoia glyptrostroboides*; 4.) *Pinus nigra austriaca*; 5.) *Taxodium distichum* and 6.) *Ligustrum ovalifolium*. The young trees were obtained from the firm Flora 2000 GmbH., 7024 Filderstadt FRG. We used the plant species *Vicia faba*, variety 'Kleine Thüringer', for comparison. We received the seeds of *Vicia faba* from 'Landessaatzuchtanstalt der Universität Hohenheim, 7000 Stuttgart 70'. The root tips originated from single trees, which were cultivated in the greenhouse. The root tips of *Vicia faba* came from primary roots of germinated seeds. The cultivation of the *Vicia faba* seeds, the fixation and Feulgen-staining of the root tips of the trees and *Vicia faba* seeds were carried out using in the main points the same methods as have been described by Hesemann (in preparation). In the following points we deviated from this method. All test groups are presented in Table 1. Cells of a root tip of one tree species and cells of a *Vicia faba* root tip, using the same fixation and staining methods for both root tips, were on the same microscope slide. The cell material of both root tips of only the test group XIa and b were treated with 5% pectinase for 60 minutes at room temperature. The time of hydrolysis was in all test groups the same as in the publication of Hesemann (in press). The test group VIIIa and b is an exception: in this case we prolonged the time of hydrolysis to 70 minutes. The root tips were squashed in 45% acetic acid, normally for no longer than 50 minutes. Only for the test groups IIIa and b, Va and b and Xa and b were times extended up to 70 minutes. In all other points the production of the preparations was the same as described by Hesemann (in preparation).

The quantitative determinations were performed by a 'Universal-Mikro-Spektralphotometer (UMSP I)' combined with a

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Table 1. Survey of the test objects I – XI in regard of object, pre-treatment with colchicine, date of the staining series and number of the measured nuclei

Number of the test objects	Object	Pre-treatment with 0.02% colchicine (in minutes)	Staining series from	Number of the measured nuclei n
I a	*Ginkgo biloba* L.	–	16.3.78	27
I b	*Vicia faba* L.	–	16.3.78	6
II a	*Larix leptolepis* Gord.	90	22.3.78	18
II b	*Vicia faba* L.	120	22.3.78	10
III a	*Larix leptolepis* Gord.	90	22.3.78	49
IV a	*Metasequoia glyptrostroboides* Hu et Cheng	–	14.3.78	35
IV b	*Vicia faba* L.	–	14.3.78	10
V a	*Metasequoia glyptrostroboides* Hu et Cheng	90	14.3.78	50
V b	*Vicia faba* L.	120	14.3.78	10
VI a	*Pinus nigra* Arn. *austriaca* (Hoess) A. et Gr.	90	15.3.78	25
VI b	*Vicia faba* L.	120	15.3.78	10
VII a	*Pinus nigra* Arn. *austriaca* (Hoess) A. et Gr.	90	15.3.78	25
VII b	*Vicia faba* L.	120	15.3.78	10
VIII a	*Pinus nigra* Arn. *austriaca* (Hoess) A. et Gr.	–	24.4.79	50
VIII b	*Vicia faba* L.	–	24.4.79	20
IX a	*Taxodium distichum* (L.) Rich.	–	25.10.78	19
IX b	*Vicia faba* L.	–	25.10.78	7
X a	*Taxodium distichum* (L.) Rich.	90	22.3.78	72
X b	*Vicia faba* L.	120	22.3.78	5
XI a	*Ligustrum ovalifolium* Hassk.	75	2.3.78	50
II b	*Vicia faba* L.	–	2.3.78	10

'Schnell-meßzusatz XD 50', Carl Zeiss, Oberkochen. The dates of the instrument, the threshold value and the number of the repeats of every measuring point are specified in the publication of Hesemann (in press). The measured nuclear DNA values are listed in arbitrary units.

We used telophases for the determination of the nuclear DNA content. In the test groups IVa and XIa the amount of telophases was small, so in these test groups we also used metaphases and anaphases. The total number of all measurements was nearly 900 without taking into account the number of repeats of every measuring point. We measured the two equal parts of every telophase and listed only the total DNA value of every telophase (4C niveau).

Results

The nuclear DNA content of meristematic root tip cells from five coniferous and one deciduous tree species was determined by scanning microspectralphotometry. For comparison we also measured the nuclear DNA content of *Vicia faba* root tip cells. The DNA values are graphically demonstrated in the Figure 1a-k. In comparison with *Vicia faba* the coniferous tree species *Ginkgo biloba* had the highest nuclear DNA content. On the grounds of the distribution of the values in *Ginkgo* we came to the conclusion that all the measured cells of this subject were diploid. In comparison with *Ginkgo* the tree species *Larix leptolepis* and *Ligustrum ovalifolium* had much lower DNA values. The DNA values of these two species also demonstrate that all the measured cells were diploid. The 4C-DNA values of the rest of the investigated tree species came between the values of *Ginkgo* and *Larix*. The distribution of the DNA values in these three coniferous tree species demonstrates, that their root tips contain cells with diploid and also with polyploid niveau. The variations of DNA values within these three tree species do not admit precise information on the additional appearance of DNA underreplication and amplification.

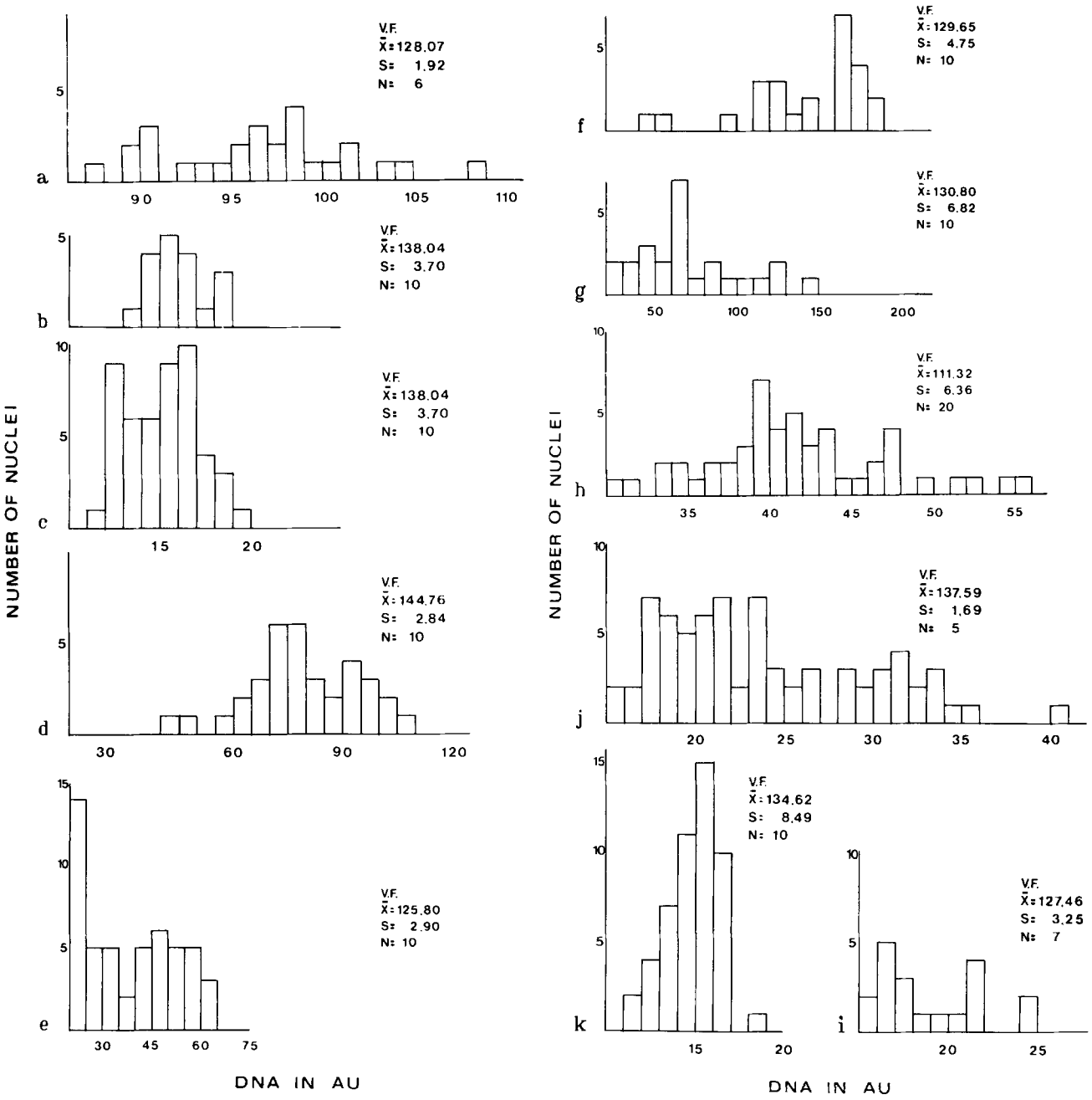

Fig. 1a-k. The nuclear DNA values of the test objects I-XI from five investigated tree species are graphically represented; in comparison with the tree species of every test object the DNA mean value of *Vicia faba* is represented. The figure symbols mean: a test object Ia + b; b test object II a + b; c test object III a; d test object IV a + b; e test object V a + b; f test object VI a + b; g test object VII a + b; h test object VIII a + b; i test object IX a + b; j test object X a + b; k test object XI a + b; abscissa: DNA content in AE (arb. units), ordinate: number of measured nuclei

Discussion

The DNA content of the root tip cells from the four coniferous tree species has already been cytochemically determined (Price, Sparrow and Nauman 1973). The authors gave the DNA contents as absolute values, but they did not give values of a well known plant species for comparative investigations. Therefore it is impossible to compare directly the absolute values of the other authors and our own values, which were given in arbitrary units. There are big differences between our results and those of the other authors, especially the higher *Ginkgo* and the

lower *Larix* values of our own measurements. Explanations for these discrepancies could include the following points. Firstly, the American authors listed the DNA mean values without deviations, so that it is not clear whether the values came only from nuclei of diploid niveau. Price and co-workers secondly used a very inaccurate method of DNA determination. The DNA content was determined by measurements of the nuclear volume. This method, a proportionality between nuclear volume and DNA content, was first described by Baetke et al. (1967) and Miksche (1967, 1968). The results of both publications by Miksche show very big discrepancies between the nuclear volume values of the same subject, *Picea glauca*. It is very probable that the discrepancy cannot be explained only by the different methods of volume determination. In the first publication the author does not give information on the condition of the cell material in measuring the nuclear volume. We assume that the nuclear volumes were determined on squashed cell material. In the second publication the author gives precise information that the nuclear volumes were determined from section preparations. The author did not consider the possibility that nuclei were measured which were not medianly cut, in this section material, so the mean values of nuclear volumes in the second publication should have been lower than those in the first publication. Curiously, the author listed higher values in the second publication than in the first, and does not give any explanation of these discrepancies. In considering such differing results, Nagl (1976) very carefully commented in his book 'Zellkern und Zellzyklen', in the chapter on nuclear structure, that the nuclear volume can only give an indication of the nuclear DNA content. Price and co-workers (1973), thirdly, supposed that the measured interphase-nuclei of the meristematic shoot and root tip regions belonged to a mixture of cells in G_1, S and G_2. Therefore the authors assigned the nuclear values to the 3C niveau. In this way the inaccuracy of the nuclear data increased.

Another result is also surprising. We found, contrary to Price et al. (1973), cells of different polyploid levels within the meristematic root tip region in three coniferous trees. At the moment we cannot give a plausible explanation of these findings. We cannot exclude the occurrence of specific DNA amplification. The variability between the nuclear DNA values is great. In this case the Feulgen-cytophotometry does not allow an exact explanation of such DNA extra-replication procedures.

Bennett and Smith (1976) have very accurately assorted the DNA contents of 753 angiosperms. In the same publication the authors discussed many possible sources of error in the Feulgen-photometry. As yet, a similar grouping of DNA values from gymnosperms is not to hand, because the DNA values of 236 gymnosperms, which Price et al. (1973) published, have a lower degree of reliability than the angiosperm values from Bennett and Smith (1976).

Therefore we have started a program of renewed measurements of nuclear DNA content from gymnosperm species, using cytophotometrical technique. The first results on only a few coniferous and deciduous tree species are presented in this publication. We proceed on the assumption that the conditions of hydrolysis and staining, which we used in the same way for the tree species and *Vicia faba*, allow an exact comparison of the measured values. The DNA values of the few tree species demonstrate that the DNA contents of diploid niveau are relatively low compared with those of *Vicia faba*. The low DNA content of the tree species is inconsistent with the scheme of Bennett (1972), who found a correlation between nuclear DNA content and minimum generation time in herbaceous plants. In continuation of the ideas of Price (1976), who has represented in great detail the evolution of the DNA content from higher plants, we have arrived at the following explanation for the occurrence of low DNA content in the analysed tree species. The DNA contents of higher plants on the diploid niveau do not pass upper and lower limiting values. The few numbers of 753 analysed angiosperms indicate the limit points of the DNA values. The DNA values of the five analysed tree species are in these limit regions. Price (1976) propounds the hypothesis that the variability of the DNA content from plant species is partly explained by selection processes of duplication or deletion of regulative reiterated DNA sequences. We consider that in this part of Price's hypothesis two aspects are very important. We must firstly take into account the percentages of the different types of reiterated DNA sequences, which form, together with the non-reiterated DNA sequences, the DNA content of a nucleus. We must secondly take into account the frequency and the length of the different types of reiterated DNA sequences between the non-reiterated sequences within the total genome. The five tree species which we have investigated have a relatively low nuclear DNA content, but this content is obviously sufficient to fulfill all necessary functions of the genetical material, the structure genes, the genetical regions of regulatory role, and the genetic regions of special functions in chromatin condensation or the course of mitosis and meiosis. We hold the hypothesis that these tree species are very well adapted plant types. They possess a lower percentage of especially constitutive heterochromatic regions than other plant species, which have a greater nuclear DNA amount. At the moment we cannot decide whether these tree species, which possess a low nuclear DNA content, have lost heterochromatic areas or have not formed large heterochromatic regions in the process of evolution.

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