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DNA synthesis in the nuclei of *Pinus silvestris* embryos during germination

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Abstract

DNA synthesis starts earliest in the apical meristem of the shoot, and latest in the cotyledons. Mitoses appear simultaneously in the apical meristem and in the hypocotyl cortex. Synthesis continues in the mother cells of vascular elements and cotyledon parenchyma when mitosis ceases. In the cotyledons DNA synthesis is rather synchronous and leads to the elimination of 2 C nuclei.

INTRODUCTION

Interest in the DNA content in nuclei of resting embryo arose in connection with the problem of radiosensitivity of plants (Brunori and D'Amato, 1967; Bogdanov et al., 1967). The knowledge of the population of embryo nuclei is essential, because the eventual occurence in it of nuclei in the radiosensitive phase S may be connected with the radiosensitivity of seeds, and then the changes in the proportion of nuclei in phase S might, at least in some degree, explain the variability in the radiosensitivity of seeds. Differentiation of cells and tissues and the development of plants are problems which all arouse interest in the DNA content in the resting and germinating embryo nuclei.

MATERIAL AND METHODS

Seeds of *Pinus silvestris* were germinated in petri dishes on filter paper moistened with tap water at 19—20°C in darkness.

On the 3rd, 4th and 6th day after soaking the embryos were separated of seed coat and endosperm, fixed in a mixture of glacial acetic acid and ethyl alcohol (1:3) and embedded in paraffin. Microtome longitudinal sections 20 μm thick were hydrolysed for 10 min in 1 N HCl at 60°C, stained with Schiff's reagent for 3 h and mounted in Canada balsam.

DNA content in the cell nuclei was measured by the double-wave method described by Sherudilo (1966) on a cytophotometer devised by this author (1964) in the Institute of Cytology and Genetics in Novosybirsk (U. S. S. R.). The measurements were taken at light wavelength 510 and 550 nm and extinctions were calculated by the formula of Sherudilo (1965, 1968) which gives an error not exceeding 2 per cent.

The use of sections for nuclear DNA measurement might suggest that the results may be burdened with errors due to sectioning of nuclei. With this in mind a microscopic selection was performed and for the measurements only intact nuclei were taken. Moreover, cytophotometric measurements of the nuclear remnants were taken, which might have not been remarked at the time of selection. The data obtained showed that the mean error caused by the overlooked defects could not exceed 6,25 per cent. This would, of course, be a systematic error without major importance, which would always reduce the obtained value as compared with the true one.

For measurements the median section of the embryo and one or two neighbouring ones on both sides were taken. Histograms were prepared separately for the nuclei from the cotylodon base, hypocotyl cortex, apical meristem of the shoot and elongated cells of the central cylinder (Fig. 1). In each case 50—200 nuclei were taken in dependence on the number of nuclei suitable for measurement. In order to have a comparable distribution, the frequencies are presented on the histograms as the per cent of all the measured nuclei. The DNA amount = 2C was first generally calculated from 50 measurements of DNA of the late anaphases and telophases on one preparation, and then the mean value of 2C was verified on each preparation by at least 10 measurements. Besides, the situation of the 4C values on the histograms was checked by appropriate cytophotometric measurements of the pro- and metaphases.

RESULTS

Fig. 1 shows histograms of the relative DNA contents in the cell nuclei of 3-, 4-, and 6-day seedlings taken separately from the cotyledons, hypocotyl cortex, shoot apex and elongated cells of the central cylinder. The relative DNA contents are expressed on the histograms in C units.

Cotyledons. The frequency distributions of the relative DNA content in the nuclei of the cotyledons of 3- and 4-day-old seedlings are almost identical. Nearly all DNA values are grouped on these histograms close to the value 2C. On the histogram of a 6-day seedling there are no value 2C; most of them are grouped in the interval 3C—5C, and a small number reaches values 6C.

Comparison of these histograms leads to the conclusion that in the nuclei of cotyledons DNA synthesis starts as late as 4 days after germination. The absence of 2C and the narrow frequency distribution on the histogram of the 6-day seedling suggest that DNA synthesis begins almost simultaneously in all the nuclei of cotyledons. When mitoses at the 4C level occur in the synchronous population of nuclei, the frequency of 2 C nuclei should considerably increase on the histograms, since each

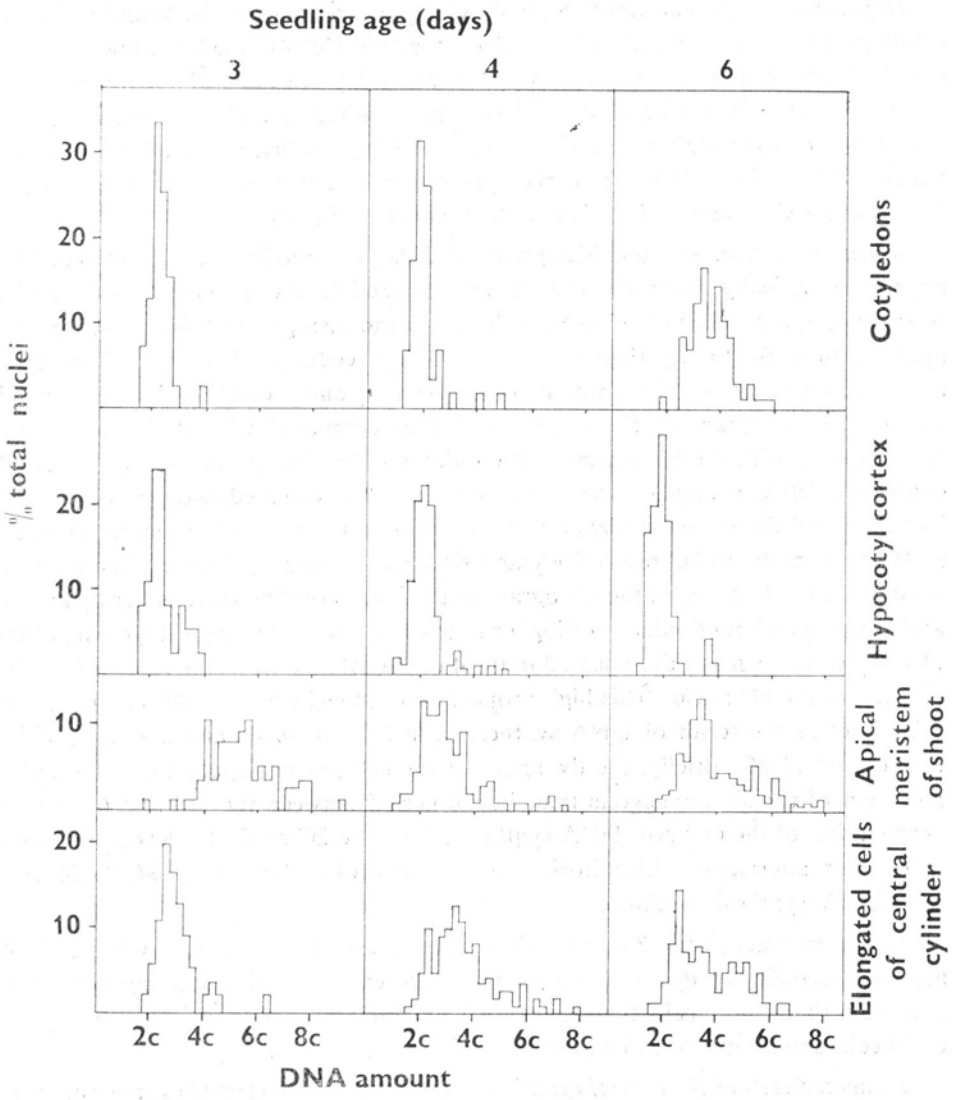

Fig. 1. Histograms showing the distribution of nuclear DNA in the tissue of 3-, 4- and 6-day-old seedlings of *Pinus silvestris*

particular division at the 4C level reduces the number of 4C cells by one and causes the appearance of two 2C cells. The occurrence on the histogram of 6-day seedling of cells containing DNA up to 6C but no 2C nuclei suggests that in the nuclei of cotyledons DNA synthesis occurs from 2C to 8C with the omission of mitosis at the level of 4C. This conclusion finds support in the results of cytological observations, namely, in the period from 3rd to the 6th day of germination the nuclei of the cotyledon cells do not divide although they synchronously increase their volume. The mitoses observed at this time are sporadic and localized exclusively at cotyledon base.

Hypocotyl cortex. The DNA contents of hypocotyl nuclei of 3-, 4- and 6-day-old seedlings are grouped mostly at 2C. The proportion of 4C nuclei is about 20 per cent in 3-day seedlings, about 8 per cent in the 4-day ones and about 6 per cent in 6-day ones. The diminution of 4C nuclei during germination and the absence of nuclei with DNA content higher than 4C in older seedlings indicate that all nuclei which reached the 4C level divide, that the synthesis is in some extent synchronous and that it occurs simultaneously only in a small per cent of nuclei.

Apical meristem. On the histogram of 3-day-old seedling 2C DNA contents appear sporadically, most of contents are grouped in the interval 4C—6C and a relatively large number close to 8C values. On the histogram of 4-day seedling 2C appears more frequently than on that of 3-day seedlings. The 2C—4C contents are more frequent on histogram of 4-day seedling and the 4C—8C contents much less on the histogram of the 4-day seedling as compared with that of 3-day one. As compared with the histogram of the 4-day seedling the proportion of 2C DNA contents is left unchanged in the 6-day one, 2C—4C decreased and that of 4C—8C increased. All the contents higher than 2C or 4C are the transitory states from 2C to 4C or from 4C to 8C respectively and thus can be simply discussed like the final states 4C and 8C. Since in the histogram of the 3-day seedling there is hardly any 2C and the proportion of values not higher than 4C is low, therefore a large part of the 2C—4C contents may have apeared in the 4-day seedling only as the result of double division of the 8C nuclei. This high proportion of nuclei with 2C—4C content maintains later as the result of DNA synthesis at a 2C—4C level, and nuclear division at the level of 8C. Briefly, the dynamics of the cell nuclei population in the apical meristem of the shoot suggests that, in the period between the 3rd and 6th day of germination of the embryo DNA synthesis, from the 2C to the 8C level, may occur twice, not alternated with mitosis as well as twofold mitosis from 8C to 2C level with DNA synthesis omitted.

In the meristem of a 3-day seedling mitoses are almost absent, whereas in 4- and 6-day seedlings they are frequent. The meristem of a 6-day seedling consists of about 3,5 times more cells than that of a 3-day seedling, therefore almost every one of its cells should divide twice in this time interval.

Elongated cells of the central cylinder. There occur in the central cylinder approximately isodiametric cells with nuclei more or less spherical, and elongated cells with elongated nuclei. The spherical nuclei preserve the ability to divide, whereas those the length of which is twice the breadth usually do not divide any further. These longer cells are the precursors of the vascular elements.

On the histogram of the 3-day seedling the proportion of 2C and 4C—8C is law, and most of the contents are grouped in the interval 2C—4C. In the 4- and 6-day seedlings the proportion of 2C does not change, but the 2C—4C contents diminish and the 4C—8C progressively increase. It results from the data obtained that the whole population of elongated nuclei of the central cylinder is very active as regards DNA synthesis which does not stop at the 4C level, but, without mitosis, starts a new cycle, increasing the amount of nuclear DNA to the 8C level.

DISCUSSION

In studies on DNA content in cell nuclei squashes were preferably used. Investigations of nuclear DNA content in connection with cell differentiation (List, 1936) led to the introduction of cytophotometry of sectioned (tissues in spite of the difficulties associated with this method. On sections there is always a certain number of split nuclei, therefore, there is a possibility that the DNA content will be estimated too low. Such an error may be prevented by microscopic evaluation of the completeness of the nuclei. The second difficulty consists in that the nuclei situated close to one another cannot be measured, particularly by the double-wave method. This difficulty can be avoided by reducing the thickness of the sections and summating the amounts of DNA calculated for the successive segments of the same nucleus on the neighbouring sections. A doubtless adventage of DNA measurements on sections is the easy and precise localization of each measured nucleus in the tissue and organ.

According to Nagl (1967), there occur in the embryo of *Pinus silvestris* nuclei containing 2C, 4C and intermediate DNA contents. Brunori and D'Amato (1967) found in resting embryos of *Pinus pinea* 2C and 4C DNA contents without any intermediates. In this study in 3-day-old seedlings the first mitoses could be found only sporadically, this suggesting that values higher than C2 appeared at the germination time.

In the germinating embryo of *Pinus silvestris* DNA synthesis starts earliest in the apical meristem of shoot, and latest in the cotyledons. Mitoses appear simultaneuosly in the apical meristem and hypocotyl cortex, whereas in the cotyledons mitoses are scarce during this whole time, and in the elongated cells of the central cylinder they are not seen at all. The nonsimultaneous beginning of DNA synthesis as well as the variable synthetic and mitotic activity in the particular parts of the embryo may be explained by the fact that in these parts development occurs at different rates. The cotyledons are organs which function temporarily and for a short time, therefore blocking of mitosis may occur in them very early. Also in the hypocotyl cortex low synthetic and mitotic activities may be connected with limited longitudinal growth and early beginning of tissue differentiation in this part of the plant. DNA accumulation in the nuclei of the elongated cells of the central cylinder and the absence of mitoses are well known symptoms of differentiation of vascular elements (List, 1963).

Most interesting is the activity of the apical meristem of the shoot. In the 6-day-old seedling no distinct leaf primordia are as yet visible, their initiation has not started yet. The high activity of DNA synthesis, however, appearing twice, at least in part of the meristematic cells, without mitosis in between and the maintanance of the ability of division of 8C nuclei may be a preparation to this morphogenetic process. Since in 3-day embryos mitoses are only starting, it would seem that the delayed unblocking of mitosis, as compared with the unblocking of DNA synthesis, is the cause of the renewed DNA synthesis in the nuclei which already attained the 4C level.

These results are in agreement with the general conclusion of Patau and Das (1967) and Patau and Swift (1953) that DNA synthesis and mitosis may be independently blocked in the course of normal development.

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Synteza DNA w jądrach zarodka *Pinus silvestris* w czasie kiełkowania

Streszczenie

W zarodku *Pinus silvestris* w trzecim, czwartym i szóstym dniu kiełkowania zachodzi intensywna synteza DNA w jądrach komórek merystemu wierzchołkowego pędu i wydłużonych komórek walca osiowego. W korze pierwotnej synteza DNA daje się stwierdzić w jądrach sporadycznie. W szóstym dniu kiełkowania jądra liścieni mają DNA w ilości większej od 2C, a więc syntetyzują DNA raczej synchronicznie.

Pierwsze mitozy pojawiają się w trzecim dniu kiełkowania równocześnie w merystemie wierzchołkowym pędu i w korze pierwotnej hypokotylu. W liścieniach mitozy spotyka się rzadko, a w wydłużonych komórkach walca osiowego wyjątkowo.

Gdy zaczynają się pojawiać pierwsze mitozy, znaczna ilość jąder merystemu wierzchołkowego pędu ma DNA w ilości 8C. Te jądra dzielą się, po odblokowaniu mitoz, dwukrotnie z pominięciem syntezy DNA w przejściu od poziomu 8C do poziomu 2C.