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FUNCTIONAL AND MORPHOLOGICAL FEATURES OF DEVELOPING POLLEN GRAINS IN *NICOTIANA TABACUM* L.*

by V. P. BANNIKOVA, O. A. KHVEDYNICH *and* O. V. OVSYANNIKOVA

The N. G. Kholodny Institute of Botany of the Academy of Sciences of the Ukrainian SSR, Kiev 252 601

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Functionally and morphologically the nuclei of vegetative and generative cells of tobacco pollen grains differ markedly. During development the vegetative nucleus shows signs of degeneration. On the contrary, the generative nucleus becomes more pronounced and compact. By cytophotometric method the DNA synthesis is not revealed in the vegetative nucleus of the developing pollen grain. The generative nucleus of the mature pollen grain contains 2c DNA. DNA replication in the generative nucleus is timed to the beginning of interphase, and lasts more-than-a-half of it. The formation of male gametes is accompanied by a considerable change of mitotic cycle parameters in comparison with those of somatic cells.

INTRODUCTION

The pollen grains of many angiosperms have been described (Poddubnaya-Arnoldi, 1964), but no adequate studies were carried out on the functional aspects of developing pollen grains and only the cytoplasm has been examined using modern methods of investigation (Mascarenhas, 1971).

The available information about DNA synthesis in the nuclei of developing pollen grain is controversial. According to Hesemann (1971) and Bolchovskikh (1973), the DNA-content in the vegetative nucleus increases during the course of pollen development. On the other hand, Woodar (1958) and Reznikova and Bugara (1975) have shown the absence of DNA replication.

Prior to mitosis the DNA-content in the nucleus of the generative cell doubles (Swift, 1950; Bolchovskikh, 1973). However, end-time of this synthesis and its timing to the definite stage of development, maturation (Woodard, 1958; Pipkin & Larson, 1973), or germination (Morozov *et al.*, 1975) of pollen grain, or growing pollen tubes (Hesemann, 1971) are open to question. There are no data of mitotic cycle parameters in the generative cell of the plant pollen grain.

MATERIALS AND METHODS

The material was fixed in Carnoy fixative, followed by cold hydrolysis in 5 N HCl, and stained in Schiff reagent for an hour.

The DNA content in the nuclei of generative and vegetative cells was determined by 2 wave length method of cytophotometry. The taking off of absorption spectrum

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of DNA-Schiff complex was preceded by the choice of working wavelengths. It gave the opportunity to confirm quantitatively the reaction, and to exactly determine the wave lengths that satisfy the relation of absorption coefficients 2 : 1. In addition, the right determination of wavelengths was verified by the test of nought indication.

The working length was 502 and 575 nm, and nearly 200 vegetative and 500 generative nuclei were analysed.

DNA-content (m) was determined in accordance with Sherudilo's method :

$$m = \frac{1}{k} \bar{D}S$$

where S is area of probe, used in photometry of nucleus; K — coefficient of absorption, adopted for 1; and D — middle optical density.

$$\bar{D} = 2D_1 - \frac{D_2}{2}$$

where D_1 and D_2 are less and more effective optical densities.

The condition that $\beta \leq 1$, is the criterion of correctness of the analysis.

Results

The vegetative and generative nuclei that have just been formed hardly differ from each other in their chromatin structure (Fig. 1). Later, when the vacuole disappears, the generative cell moves to the centre and the nuclei become more prominent. In the course of development to the pollen grain the vegetative nucleus loses its clear contour, shows signs of degeneration; chromatin threads are fragmented, and cytoplasm becomes strainable. DNA synthesis does not take place.

On the contrary, the generative nucleus becomes more compact and responds more intensely to Feulgen reaction. In mature pollen grain it becomes much elongated, shows signs of prophase, and contains 2 c DNA. The data on DNA-content in the vegetative nuclei are statistically analysed. The results of photometry of 200 vegetative nuclei are presented in the form of histogram (Fig. 2a). The histogram shows that it is subjected to the law of normal distribution. With the method of the third moment the coefficient of asymmetry is determined as 5.0%. On the basis of the above data, it is concluded that there is no DNA synthesis in the nucleus of vegetative cell.

The duration of the interphase periods in the nucleus of the generative cell has been determined, based on the data obtained from 500 generative nuclei.

The mass of nuclei that showed DNA-content at different stages of their development varied from 3.21 to 12.78.

The data are summarized into 10 classes with the class interval of 1.06.

The summation into classes was made in such a way that the average nuclei mass of the second class corresponded to the haploid DNA value (c) and the average nuclei mass of the last but one class corresponded to the diploid DNA value (2c). The average nuclei mass of intermediate classes showed the dynamics of DNA synthesis,

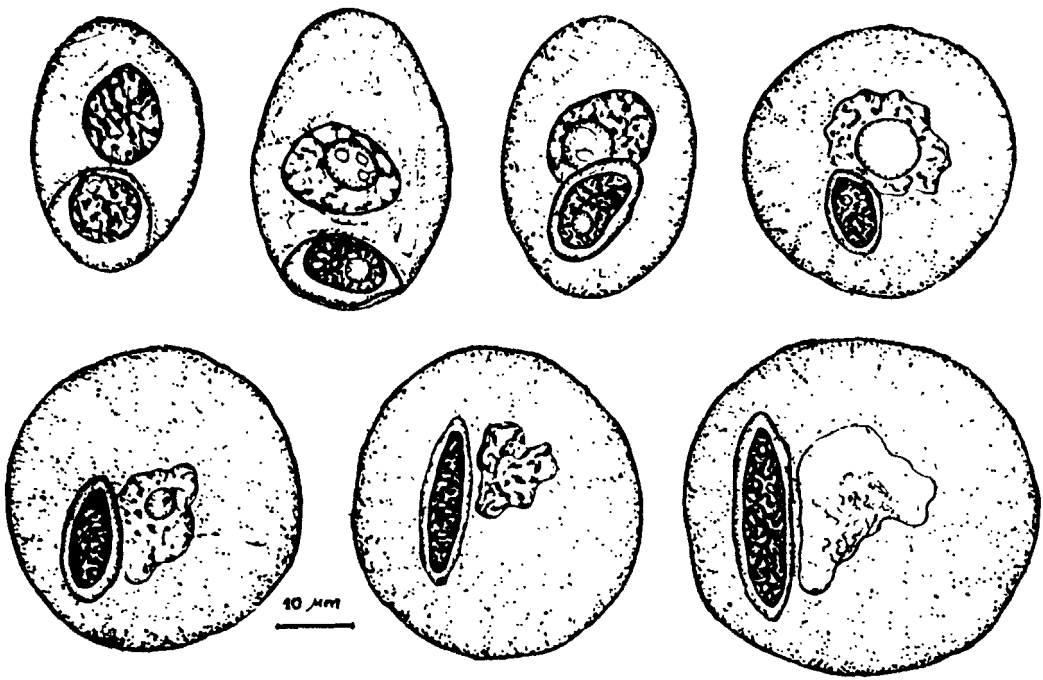

FIG. 1. *Nicotiana tabacum*. Successive stages of development of pollen grain.

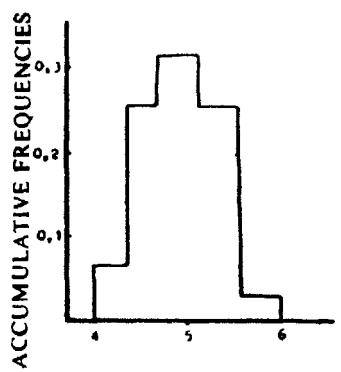

FIG. 2a. *Nicotiana tabacum*. Histogram of DNA distribution in the vegetative nuclei.

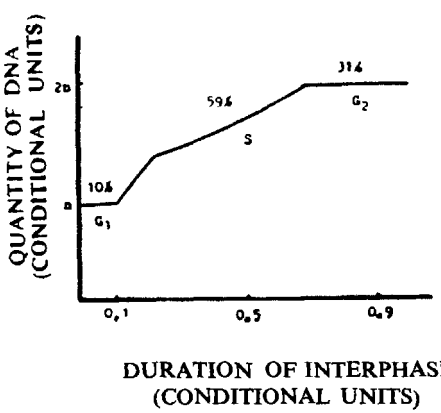

FIG. 2b. *Nicotiana tabacum*. Motion of DNA distribution in the vegetative nuclei.

On the basis of data distribution into classes, the interphase periods were calculated in relative interphase portions and, accordingly, the curve of DNA synthesis dynamics (Fig. 2) was prepared.

From this calculation and curve it follows that the pre-synthetic period (G_1) of the formation of generative nucleus takes 10%, the period of DNA synthesis (S)— 59%, the post-synthetic period(G_2) — 31%. DNA is synthesized at two stages. At the first stage 24% of DNA is replicated, at the second one—the rest.

Thus, DNA replication in the nucleus of the generative cell is timed to the beginning of the interphase and lasts more-than a half of it.

In comparison to the morphological and cytophotometrical data based on our studies, one can determine the timing of synthesis period to the definite morphological phases.

The DNA synthesis in the nucleus of the generative cell begins after its separation from the cell-wall of the pollen grain. The first period of synthesis is attained, obviously, in the process of generative cell moving from the membrane of the pollen grain towards the centre. At that time the nucleus is oval; chromatin, previously in the state of diffusion, begins displaying itself in the form of threads. The second stage of DNA synthesis is timed to transition of the nucleus from oval form to oblong one. DNA synthesis in the generative nucleus ends up when the latter acquires the oblong form.

Discussion

The contradictory information of DNA synthesis in the vegetative nucleus is conditioned by differences in methods applied and, partly, their imperfections (Swift, 1950; Bryan, 1951; Woodard, 1958; Bolchovskikh, 1973). However, the data obtained during morphologic and cytophotometric investigations show quite clearly that DNA replication in the vegetative nucleus does not take place.

DNA synthesis at the beginning of interphase, increase of post-synthetic period, duration and decrease of pre-synthetic period, are characteristics of the developing generative nucleus. The timing of DNA synthesis at the beginning of interphase in the generative nucleus has also been observed in other species of angiosperms (Woodard, 1958; Reznikova & Bugara, 1975). Correlation of mitotic cycle parameters, obtained in *Nicotiana tabacum*, coincides with that of *Lilium regale* (Bannikova *et al.*, 1975).

On the basis of the above data, one can suggest that correlation of periods of the mitotic cycle is regular in the formation of male gametes. In comparing our data with the results of the analogous investigations of somatic plant cells, one can conclude that in the process of male gamete formation of tobacco some changes of mitotic cycle parameters are observed (Grif & Ivanov, 1975). These changes are manifested by increase of S and G_2 periods, and the visible shortening of G_1 period. The same regularity is found during spermatogenesis in animals (Dondua, 1967). Consequently, the gametogenesis in plants is characterized by peculiarity of mitotic cycle, and accompanied by essential transformation of its kinetics.

In our study we take for conditional units such units that determine quantity of stain connected with DNA molecules. This quantity of stain is measured in photometer in units of absorption intensity of light that passes through the object.

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