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Inhibition of cytokinesis in the microspores of *Tradescantia bracteata* Small. by caffeine*

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Abstract

In isolated stamens of *Tradescantia bracteata* planted in nutrient medium cytokinesis in microspores was inhibited by caffeine and binucleate pollen grains were formed. Both the nuclei were of similar shape, size and structure and did not undergo further changes. It seems that separation of the polarly differentiated microspore protoplast by a cell wall is a necessary condition for differentiation of the gametophyte nuclei to generative and vegetative.

INTRODUCTION

Asymmetric division of the microspore in angiosperms, involving mitosis and cytokinesis gives two cells, a generative and a vegetative one, separated temporarily by a callose wall and surrounded by sporoderm. The most striking differences between the generative and the vegetative cell are manifested in the shape and structure of the nuclei of these cells, becoming more pronounced as the pollen grains develop.

The purpose of the present study was to establish whether in the binuclear pollen grain of *T. bracteata*, after inhibition of cytokinesis in the microspore, the nuclei will differentiate to a vegetative and generative one. For cytokinesis inhibition caffeine was used, long known as a sufficiently specific cytokinesis inhibitor (Shigenaga, 1937).

Cytokinesis in higher plants is characterized by cell plate formation. The latter arises, as demonstrated in root meristem cells, by coalescence of vesicles produced by the Golgi apparatus (Whaley and Mollenhauer, 1963; Frey Wyssling et al., 1964; Benbadis et al., 1974).

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In the continuous process of cytokinesis López Sáez et al. (1966) distinguished 4 phases: production, accumulation, arrangement and coalescence of the vesicles. The first three phases may be selectively blocked by various cytokinesis inhibitors (Risueno et al., 1968). Caffeine inhibits cytokinesis by preventing arrangement of the Golgi vesicles in the equatorial plane, but it does not disturb chromosome transformation and the activity of the anaphase spindle. The above mentioned authors believe that caffeine inhibits the transverse currents in the cytoplasm decisive for vesicle arrangement in the plane of the future cell plate. They do not discuss the role of phragmoplast in cell plate formation, and the fixative used does not reveal the microtubules of the cytokinetic spindle on EM pictures.

MATERIAL AND METHODS

The experiments were performed with a clone of *Tradescantia bracteata* Small. Stamens were isolated from flower buds of plants growing in a greenhouse at 23°C. On account of the lack of synchronization in the development of microspores and pollen grains in the two stamen whorls of *T. bracteata*, stamens were taken for the experiments from one whorl only. An acetoorcein smear was made from one anther for determination of the stage of development, while the two remaining ones were planted in nutrient medium. Within the *T. bracteata* anther the microspores, and later the pollen grains do not develop simultaneously and therefore in each sample the microspore population within the anthers was not completely homogeneous.

As a nutrient medium the liquid medium for culture of pollen tubes (Brewbaker and Kwack, 1963) was used. A loose cotton wool layer immersed in the liquid medium was placed on the bottom of glass vessels. The isolated stamens with filaments were placed perpendicularly to the surface of this layer so that only the filaments were immersed in the medium. The vessels were placed in a culture chamber at 23°C with a day-cycle of 16-h light of 2000 lux intensity. Caffeine concentration in the medium was 0.1 per cent. The experiment was performed during 48 h in three series: continuous incubation in caffeine, 28 h incubation and 20 h postincubation without caffeine, and the control in pure liquid medium.

The smears were prepared in acetoorcein and modified Ehrlich's haematoxylin. Callose was identified by the method of anilin blue fluorescence (Eschrich and Currier, 1964).

RESULTS

In the *Tradescantia* stamens planted into pure medium during the stage of vacuolated microspores differentiating division occurs in the microspores and normal two-celled pollen grains develop. The mitotic spindle in normally developing microspores is situated perpendicularly to the microspore long axis, and after cytokinesis the nuclei of the two cells lie on a common axis.

We have demonstrated earlier that in the isolated stamens normal pollen grains develop from microspores, if these microspores have reached previously at least the late G_1 stage. Stage G_1 and S were distinguished autoradiographically with ^{3}H-thymidine (results in preparation for publication).

In the period of DNA synthesis the microspore nucleus lies in the cytoplasm layer of the vacuolated microspore at the end of the cell long axis. After the DNA synthesis the nucleus is displaced to the proximal pole of the microspore and after several hours mitosis begins.

Stamens containing in the anthers vacuolized microspores with laterally situated nucleus (ca. 40% of microspores) and microspores with nucleus at the proximal pole (ca. 60%) were used for the experiment. After 14-h culture on medium with caffeine about 50 per cent of the microspores were in advanced mitosis. The configuration of about 70 per cent of the mitotic spindles observed did not differ from those in the controls, that is the spindles were in perpendicular position to the cell long axis (Plate I, Photo 1). In telophase phragmoplasts were not visible and the position of the telophase nuclei in respect to one another was in most microspores the same as in the controls (Plate I, Photo 2). The remaining 30 per cent of the observed spindles were in slanting or perpendicular position to the short microspore axis (Plate II, Photos 1 and 2). In some mitoses occurring in a changed plane a retardation in chromosome segregation to the poles was noted (Plate II, Photo 3). In microspores with one vacuole the nuclei entered sometimes mitosis without previous translocation to the proximal pole (Plate II, Photo 4).

After 20 h of the experiment the sample contained binucleate pollen grains (ca. 60% of cells in anther) and microspores in the course of mitosis. In the microspores which entered mitosis late, the dislocations of the spindle are more numerous than after 14 h. They are of the same character as the previous ones.

After 28 h nearly all the pollen grains are binucleate. Both nuclei have similar dimensions and structure, a spherical shape and they stain equally. In most pollen grains the nuclei lie on the shorter cell axis (Plate I, Photo 3). In the remaining ones they are located on the inclined or long axis (Plate II, Photos 5 and 6). The abnormally situated nuclei are sporadically connected with chromosome bridges (Plate II, Photo 7).

Sometimes pollen grains may be found with one nucleus of irregular shape (Plate II, Photo 8).

During the further 20 h of binucleate pollen grains development on medium with caffeine, no changes were observed as regards the position of the nuclei in respect to one another, their shape and structure (Plate I, Photo 4).

The disturbances caused by 28 h of exposure to caffeine persisted after transferring the stamens for 20 h to medium without caffeine. In the anthers all pollen grains remained binucleate with nuclei and vacuoles in the same position as after 28 h of exposure to caffeine (Plate 1, Photo 5). On the other hand, in the control anthers kept for 48 h on medium without caffeine, binuclear pollen grains developed with a vegetative cell containing a spherical nucleus and a generative cell with a slightly elongated one (Plate I, Photo 6).

Another experiment was carried out with older anthers containing at the moment of starting the experiment about 60 per cent of microspores with mitoses, about 30 per cent of two-celled pollen grains and about 10 per cent microspores with nucleus at the proximal pole. After 48 h on medium with caffeine about 90 per cent of two-celled normal pollen grains were found and only about 10 per cent of binucleate ones.

In the experiment with anthers containing only microspores in mitosis and two-celled pollen grains, the effects of caffeine were not noticeable, all the microspores developed to two-celled normal pollen.

In all the experimental combinations in which inhibition of cytokinesis was achieved, phragmoplast was not observed in telophase, and in pollen grains with equal nuclei a callose wall could not be revealed by the fluorescence method.

DISCUSSION

The asymmetric division of microspores in angiosperms is preceded by a polar translocation of the nucleus, and characterized in all examined cases by a constant orientation of the mitotic spindle.

A long time ago, the view was advanced (Geitler, 1935; Sax and Husted, 1936) that the orientation of the spindle in the microspore is determined by the position of the microspores in the tetrad, its position depends therefore on the arrangement of division spindles in the meiocyte.

The cause of disturbances in the development of pollen grains may be genetic or external factors, among which the influence of low and high temperatures of short duration was demonstrated in the experiments of Sax (1935, 1937). Disturbances in pollen development may also be produced by the action of low colchicine concentrations on the meiocyte or microspore (Bischop and McGowan, 1953; Heslop-

-Harrison, 1971; Dover, 1972). In both cases it seems that the disturbances are the consequence of destruction of the microtubules of the mitotic spindle or of microtubules of a different type which have been lately revealed by Dickinson (1971) in young microspores released from tetrads. The latter are supposed to play a role in the polar orientation of the prophase microspore nucleus.

It has been well documented in the light and electron microscope investigations that cytokinesis in the microspore is associated with the formation of phragmoplast and that formation of the cell plate after mitosis in the microspore occurs usually in the same way as in normal somatic cells (Heslop-Harrison, 1968; Angold, 1968; Burgess, 1970). The only difference is that the cell plate separating the generative cell from the vegetative one contains callose (Górska-Brylass, 1967; Heslop-Harrison, 1968).

If cytokinesis in the microspore is of the same character as in somatic cells, it should be expected that the action of caffeine will prevent cytokinesis like in the latter cells and a binucleate gametophyte will form. In the present study the process of cytokinesis was inhibited experimentally for the first time at the stage of asymmetric division of the microspore, without disturbing the course of mitosis. As compared with the data concerning the root apical meristem, where the action of 0.1 per cent caffeine for as little as 1 h is sufficient for producing a population of binucleate cells (Garcia-Herdugo et al., 1974), the time of incubation in caffeine of *T. bracteata* stamens necessary for obtaining the same effect is much longer. It seems that caffeine penetrates through the stamen filament into the pollen sac and hence into the microspores much slower than directly into the meristem cells. This may explain the lack of a noticeable effect of caffeine in the experiment with stamens containing microspores with advanced mitosis. Cytokinesis in this case was ended before caffeine penetrated into the microspores.

Heslop-Harrison (1968) attributed to the phragmoplast microtubules the morphogenetic function of ordering the dictiosome vesicles merging during cytokinesis in the microspore into a hemispherical cell plate. Our results also point to a significant role of phragmoplast in the process of cytokinesis in microspores. In *Tradescantia bracteata* microspores after inhibition of cytokinesis by caffeine phragmoplast did not form, although the anaphase and interzonal spindles were present earlier.

The changed position of the mitotic spindles observed in a large number of microspores and the sporadic disturbances in chromosome segregation during mitosis in the microspores show an influence of the caffeine concentration used (0.1%) on the mitotic spindle of the microspore.

The ultrastructural manifestation of the polarity of the microspore during mitosis is the unequal segregation of organelles initiated already

in prophase (Sanger and Jackson, 1971). Most mitochondria and usually plastides translocate to the vegetative cell pole, and after formation of the cell plate each of the nuclei lies in a different cytoplasm (Jensen et al., 1974; Mascarenhas, 1975). The mechanism of polar segregation of organelles remains obscure.

The present results are apparently contradictory to those of Terasaka and Tanaka (1974) who described in the light microscope the differences in the degree of chromosome condensation of the future vegetative and generative nuclei in the microspores of some species, visible as early as anaphase. On this basis they claim that polarization of the microspore is so strong that it makes possible unlike of two nuclei differentiation within the common cytoplasm.

It results from the present experiments that after inhibition of cytokinesis by caffeine in *T. bracteata* microspores, pollen grains arise with two identical nuclei even in those cases in which microspore nucleus before mitosis was polarly oriented and the mitotic spindle was situated as in typical differentiating division.

This fact suggests that for differentiation of two nuclei to a generative and a vegetative one, there are necessary not only preliminary polar differentiation of the microspore and selective segregation of the cell organelles to the vegetative and generative poles, but also separation by a cell wall of the two cells.

So far we have not performed EM investigations and it is not clear when, under the influence of caffeine, depolarization occurs in the distribution of the cell organelles in the microspore. The possibility cannot, therefore, be ruled out that as early as telophase both nuclei lie in polarly undifferentiated cytoplasm. This, however, does not seem probable.

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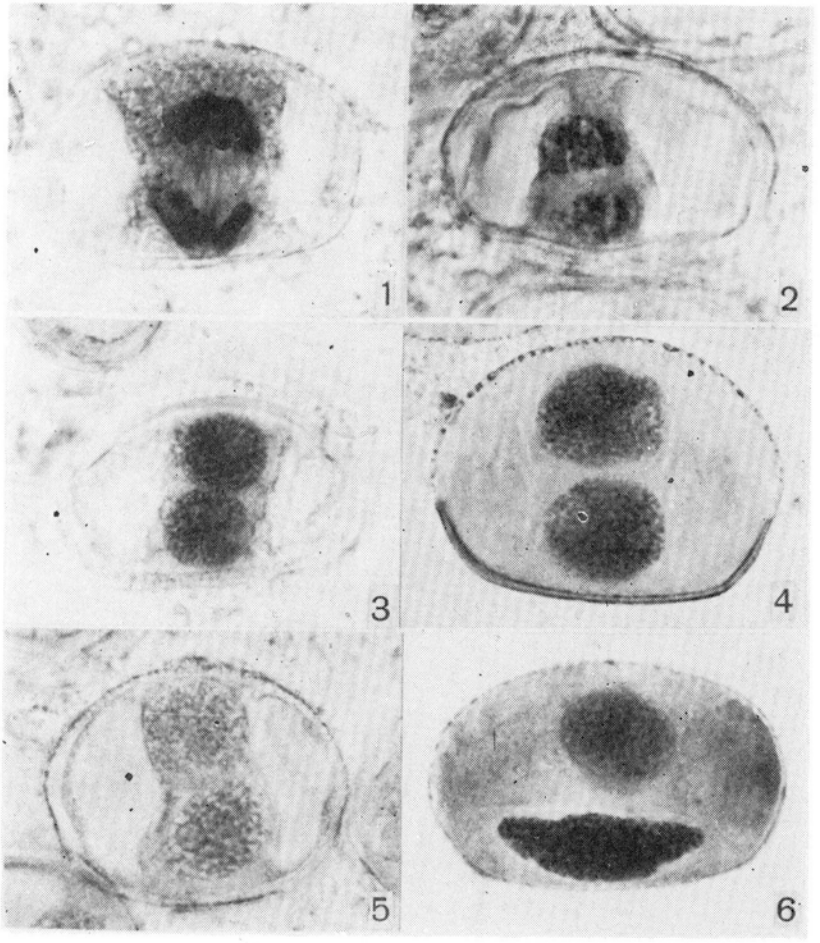
Inhibicja cytokinezy przez kofeinę w mikrosporze Tradescantia bracteata Smal.

Streszczenie

W izolowanych na pożywkę pręcikach *Tradescantia bracteata* zahamowano kofeiną cytokinezę w podziale różnicującym mikrospor i otrzymano dwujądrowe ziarna pyłku. Obydwa jądra mają podobny kształt, wielkość i strukturę i nie ulegają dalszym zmianom.

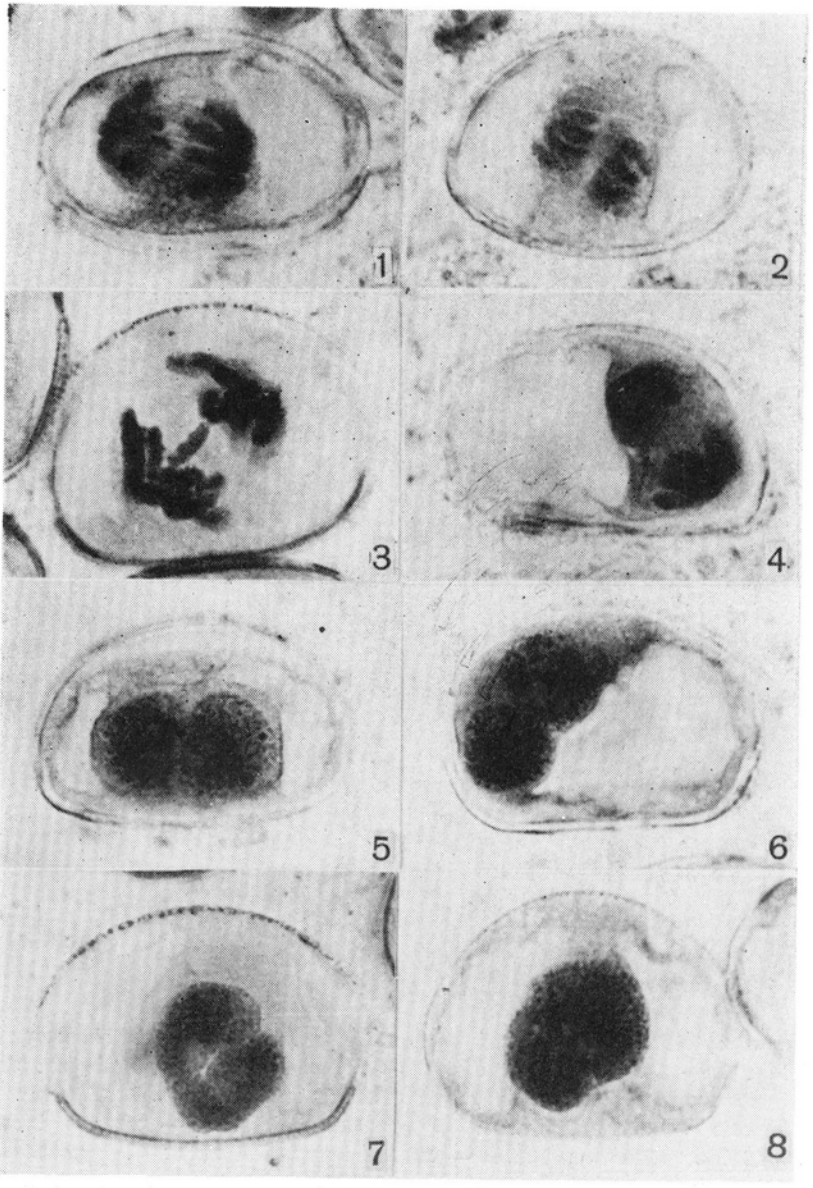
Należy sądzić, że rozdzielenie biegunowo zróżnicowanego protoplastu mikrospory ścianą komórkową jest niezbędnym warunkiem zróżnicowania jąder gametofitu na generatywne i wegetatywne.

Plate I



Inhibition of cytokinesis induced by 0.1% caffeine in *Tradescantia bracteata* microspores. Fig. 1 — late anaphase, Fig. 2 — telophase (the phragmoplast and cell plate are not formed). Binucleate pollen grains: the stamens with the microspores before mitosis were treated with 0.1% caffeine: Fig. 3 — 28 hrs incubation, Fig. 4 — 48 hrs incubation, Fig. 5 — 28 hrs incubation and 20 hrs postincubation in nutrient medium without caffeine, Fig. 6 — two celled pollen grain after 48 hrs development of stamen (isolated with microspores before mitosis) in nutrient medium (ca ×1200)

Plate II



The changes in the course of microspore mitosis *T. bracteata* sporadically induced by 0.1% caffeine (Fig. 1—4) following the aberated pollen grains (Fig. 5—8) ca ×1200