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Callose in the cell walls of the developing male gametophyte in *Gymnospermae*

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In earlier investigations the periodical occurrence of a callose wall in newely formed generative cell of pollen grains has been observed in several species of monocotyledon plants (Górska-Brylass 1957).

Further investigations of the author proved that this phenomenon is common both in mono- and dicotyledon plants (in preparation).

After establishing this fact it seemed interesting to check whether callose also appears in male gametophytogenesis in gymnosperm plants. According to the so far prevailing opinion the generative cell of gymnosperms is surrounded by a plasma membrane (see Vazart 1958).

The present paper reports studies on *Larix decidua, Ginkgo biloba, Taxus baccata* and *Thuya occidentalis.* These species represent various types of development of the male gametophyte in *Gymnospermae.* In *Larix* and *Gingko* the pollen is characterized by the formation of two prothallial cells, whereas in *Taxus* and *Thuya* they are lacking. Anthesis in the species studied occurs at various development stages of the gametophyte. In *Larix* pollen shedding occurs after division of the generative cell, i.e. after formation of a stalk and body cells, and in *Ginkgo* it takes place before division of the generative cell, whereas in *Taxus* and *Thuya* the microspores are shed. The generative cells of the latter are formed as late as during the germination of microspores.

The investigations comprised the succesive stages of pollen development in *Larix* and *Ginkgo* from the microspore to the period of anthesis. Formation of generative cells in *Taxus* and *Thuya* was studied after germination of the microspores on a medium containing 3% sucrose in 0,8% agar with an addition of H_2BO_3 (0,001%) and $CaCl_2$ (0,003%).

The observations were made on living or fixed in 5% glutaraldehyde material. Callose was detected by two methods: staining with resorcine blue and by the fluorescence method with the use of an aqueous aniline blue solution (Eschrich and Currier 1964).

RESULTS AND DISCUSSION

The results obtained by both methods were concordant and proved that callose occurs in the process of male gametophytogenesis in the gymnosperms examined.

Callose appears in the telophase of each successive division of the gametophyte. At first it forms a small plate within the cytokinetic spindle. This plate extends and separates by a thin layer the succesively forming cells, thus prothallial cells, the generative cell and also the stalk and body cells.

Larix decidua.

The first unequal division of the microspore in *Larix* yields the first parietal prothallial cell (Fig. 5). It is separated from the larger cell by a callose wall in the shape of a bowl with its edge supported by the wall of future pollen grain (Fig. 1).

The second prothallial cell formed adhering closely to the first one becomes separated by a similar callose wall from the antheridium initial cell (Fig. 2). After formation of the prothallial cells at one of the poles of the initial cell, the latter continues to divide. This division runs along the polar axis (Fig. 3). The generative cell resulting from this division closely adheres to the second prothallial cell (Fig. 6). The border between it and the vegetative cytoplasm of the pollen grain is also delimited by

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Plate I

(Explanations)

Figs 1—4. Fluorescence of callose in the successive development stages of the male gametophyte in *Larix decidua*. Fluorochrome — aniline blue. Ca. ×350.

1 — Callose bowl enveloping first prothallial cell

2 — Callose at stage of two prothallial cells

3 — Callose plate over forming generative cell

4 — Callose bowl enveloping newly formed generative cell adjacent to the other prothallial cell.

Figs 5—7. Developmental stages of *Larix decidua* pollen as seen in light microscope. Ca. ×350.

5 — Stage of one prothallial cell

6 — Young pollen grain with second prothallial cell and generative cell

7 — Ripe pollen grain. Above degenerated prothallial cells a stalk and a body cell are visible

Figs 8, 10, 12. Callose fluorescence in the successive developmental stages of the pollen grain in *Ginkgo biloba*. Ca. ×800.

8 — Callose wall at the border of the first prothallial cell

10 — Two callose walls separating the prothallial cells

12 — Three callose walls separating the prothallial cells and the generative cells

Figs 9, 11, 13. Developmental stage of *Ginkgo biloba* pollen as seen in light microscope. Ca. ×800.

9 — Stage of one prothallial cell

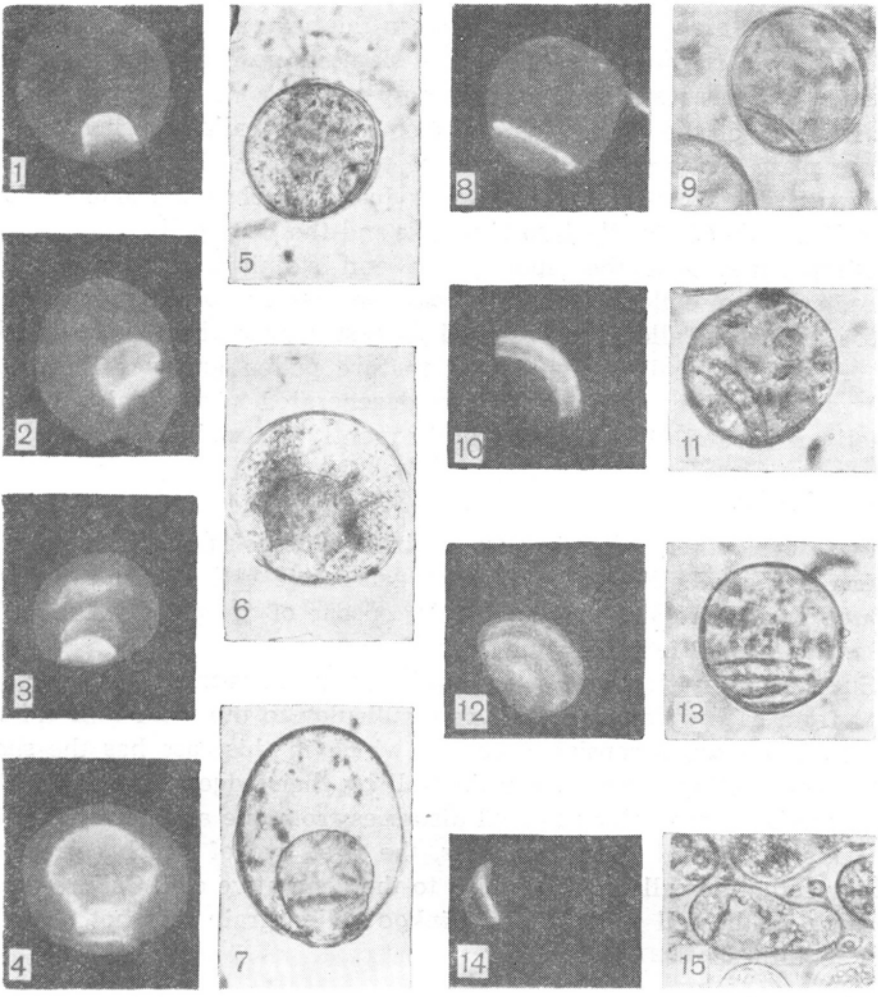
11 — Stage of two prothallial cells

13 — Young pollen grain with two prothallial cells and generative cell

Fig. 14. Germinating pollen grain of *Taxus baccata*. Fluorescence of callose plate separating the generative cell from the vegetative cytoplasm of the pollen. Ca. ×550.

Fig. 15. Germinating pollen grain of *Taxus baccata* as seen in light microscope. Ca. ×550.

Plate I



a callose wall which, similarly as in the prothallial cells has the shape of a bowl supported at its edge on the second prothallial cell (Fig. 4).

The division of the generative cell in the *Larix* pollen grain is generally unequal giving a smaller stalk cell and a larger body cell. This division in *Larix* was observed to occur as a rule perpendicularly to the polar axis of the grain (Fig. 7). A slanting position of the cytokinetic spindle giving a slanting septum between the body and the stalk cell was observed less frequently, although a slanting position of this wall is assumed as characteristic of all *Gymnospermae.*

Division of the generative cell also involves callose formation. The callose plate appears in the period of telophase in the middle part of the generative cell, and extending gradually reaches the side walls of the cell dividing it finally into the stalk and the body cell.

Disappearance of the fluorescence and staining in resorcine blue characteristic of callose is observed only in mature pollen grains shortly before anthesis. Callose is preserved longest around the first prothallial cell. A certain small percentage of mature pollen grains still exhibits distinct callose fluorescence in the degenerated prothallial cells and sometimes around other cells, most frequently the stalk ones.

Ginkgo biloba.

The development of the pollen grain in *Ginkgo* is similar as that in *Larix.* Two prothallial cells and the generative cell adhere closely to each other and are arranged layer-wise on one of the poles of the pollen grain, the only difference being their shapes.

Successive division yields prothallial cells and generative one separated from each other by a wall perpendicular to the polar axis (Fig. 9, 11, 13). These walls consist of callose which in this case has the shape of discs contacting the pollen grain wall by their edges (Figs. 8, 10, 12). These walls lie generally at equal distances from one another and divide the pollen grain into four segments, the first two of which correspond to the prothallial cells and the third to the generative cell. Callose disappearance in the cell walls of the *Ginkgo* pollen grain was not observed prior to anthesis.

Taxus baccata, Thuya occidentalis.

The microspores of *Taxus* germinated 6—7 and those of *Thuga* 12—14 days after their sowing on the medium. In both these species the generative cell is formed in pollen grains which have already developed a small pollen tube (Fig. 15). Quite frequently, however, the generative cell may occur before formation of the pollen tube, in microspores which have reached a size 2—3 times larger than that of the microspores sown on the medium.

The generative cell forms in *Taxus* and *Thuya* next to the wall at the

opposite pole of the pollen grain to that bearing the pollen tube. Here too the generative cell is isolated from the vegetative cytoplasm of the pollen by a callose layer (Fig. 14). Similarly as in the other cases described, the callose does not encompas the generative cell on the side neighbouring with the pollen wall.

The occurrence of callose in the generative cell walls of the species of gymnosperm plants investigated points to an analogy with the findings in angiosperm plants. Not only the fact of callose occurrence in the walls of generative cells is a common feature but also its periodical appearance. The periodical appearance of callose regularly localized in the wall of the newly formed cell during the process of gametophyte development bears evidence to the physiological role of this substance. It seems that callose is here the factor associated with cell differentiation. Callose is present during the unequal division giving rise to two different cells. It may serve as a barrier isolating these two newly formed cells and thus facilitating their further diverging lines of development.

SUMMARY

The periodical occurrence of callose walls between the newly formed cells of the developing male gametophyte was demonstrated by staining with resorcine blue and by the flurescence method with the use of aniline blue in four species of gymnoseprm plants.

Callose in a thin layer separates the successively forming prothallial cells, the generative cell and also the stalk any body cells arising from its division.

It is suggested that callose is associated with cell differentiation in the forming gametophyte.

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Kaloza w ścianach komórek rozwijającgo się gemetofitu męskiego u Gymnospermae

Streszczenie

Stosując barwienie błękitem rezorcynowym oraz metodę fluorescencji przy użyciu błękitu anilinowego wykazano okresowe występowanie ścian kolozowych na granicy nowopowstałych komórek rozwijającego się gametofitu męskiego u czterech badanych gatunków roślin nagozalążkowych.

Kaloza oddziela cienką warstwą kolejno powstające komórki przedroślowe, komórkę generatywną, a także powstające z podziału tej ostatniej komórki — sterylną i plemnikotwórczą.

Wysunięto przypuszczenie, że kaloza w formującym się gametoficie jest czynnikiem związanym z różnicowaniem się komórek.