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Callose localization in the walls of megasporocytes and megaspores in the course of development of monospore embryo sacs

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Abstract

The localization of callose in the cell walls of megasporocytes and megaspores was studied by means of fluorescence microscopy. It was found that the part of the meiocyte cell wall in which callose begins to appear corresponds to the region of active megaspore formation.
During the whole megasporogensis more of callose was found in walls of such cells which differentiate afterwards. For different species the pattern of callose distribution in walls during megasporogenesis is a constant specific trait.

INTRODUCTION

The polysaccharide callose is found in many types of cells, e.g. in sieve tubes, pollen tube walls and fungal hyphae.

In 1889 Mangin described special callose walls appearing at the time of microsporogenesis. Careful investigations on the appearance and disappearance of this polysaccharide in pollen mother cells and tetrads were undertaken by Waterkeyn (1962), Heslop-Harrison (1964, 1966) and Geneves (1969). During formation of generative cells a callose septum occurs transiently between the generative and vegetative cell in the pollen grain (Górska-Brylass 1967, 1970).

In megasporogenesis of an angiospermous plant (*Orchis maculata*), callose was detected for the first time in 1967 (Rodkiewicz, Górska-Brylass 1967). Further studies demonstrated that, in the course of megasporogenesis, callose appears transiently in the cell walls of a number of plants with a monospore type of development of the embryo sac. The polysaccharide is, however, absent in species with a tetraspore development type (Rodkiewicz 1967, 1968 a, b, 1970).

The purpose of the present work was to establish on more extensive plant material in how far the transient occurrence of callose in the walls is common in megasporogenesis of angiosperms, and to study in detail callose localization in megasporocyte and megasopre walls during monospore type development of the embryo sac. In one species, moreover, chemical analysis of the walls was performed during megasporogenesis.

MATERIAL AND METHODS

Fifty eight species belonging to 11 families of *Angiospermae* were examined: *Epipactis latifolia* (*Orchidaceae*), *Digitalis ambigua, D. lanata, D. purpurea, D. sibirica, Gratiola officinalis, Pentstemon aureus, Verbascum olympicum, V. phlomoides* (*Scrophulariaceae*), *Atropa beladonna, A. caucasica, Datura forex, D. quercifolia, D. stramonium, Hyoscyamus alba, Nicotiana affinis, N. alata, N. tabacum, Physalis alkekengi, Solanum sisymbifolium, S. tuberosum* (*Solanaceae*), *Agrostemma gitago, Cerastium lanatum, Dianthus arenarius, D. caesius, D. tatrae, Lychnis flos-cuculi, Saponaria ocymoides, S. officinalis, Silene inflata, S. italica, S. nutans, S. saxifraga, S. scendneri* (*Caryophyllaceae*), *Gesneria canescens, Saintpaulia ionantha* (*Gesneriaceae*), *Funkia umbellata, Hemerocallis flava, H. fulva, Ornithogalum umbellatum* (*Liliaceae*), *Laurentia longiflora, Lobelia dresdensis, L. syphilitica* (*Lobeliaceae*), *Manyanthes trifoliata* (*Menyanthaceae*), *Clarkia concinna, Epilobium hirsutum, Fuchsia corymbiflora, Gaura biennis, Oenothera fruticosa, O. glauca, O. grandiflora, O. missouriensis, O. muricata* (*Onagraceae*), *Chelidonium majus, Glaucium corniculatum, Papaver atlanticum, P. bracteatum* (*Papaveraceae*), *Monotropa hypopitis* (*Pyrolaceae*).

The ovaries were cut off at various stages of development and fixed in absolute alcohol with glacial acetic acid (3 : 1). The material was hydrolysed in 1 N HCl for 10 min, and then washed thoroughly until complete disappearance of acidic reaction. The embryos were placed in 0.05 per cent aniline blue solution in M/15 K_2HPO_4 (Arens 1949). Crushed preparations were used. They were inspected in a MUF—3M fluorescence microscope. Walls with callose were also investigated on paraffin section after treatment with fluorochrome. When treated with aniline blue, callose shows in the fluorescence microscope yellow-green fluorescence. In the ordinary microscope, walls with callose were inspected after applying a resorcin blue solution (Eschrich 1954).

Paraffin sections 5 μ thick from the embryos of *Epipactis latifolia* were used for investigation of the chemism of the cell walls by the method of selective extraction of the particular components (Jensen

1962), and for visualisation of insoluble polysaccharides with the use of the PAS-Schiff reaction (Hotchkiss, acc. to Pearse 1962). Reaction inhibition was achieved by acetylation according to the Gersh method (Pearse 1962).

For observation of glucose-^{3}H incorporation, fragments of the *Epipactis latifolia* ovary were incubated for 3 and 6 hours in an aqueous solution of D-glucose-6-^{3}H at a concentration of 6.0 μC/ml with specific activity 300 mC/mM. After incubation the material was quickly washed with water and fixed in a mixture of pure absolute alcohol and acetic acid (95 : 5 parts) at −6 to −10°C. Paraffin preparations were coated with L-4-Ilford emulsion and exposed for 30 days at 4°C.

OBSERVATIONS

I. Chemism of the cell wall in *Epipactis latifolia*

The walls of premeiotic cells, megasporocytes and megaspores are always PAS-positive (Photos 1, 2, 3, Plate I).

After the successive extractions of the magasporocyte wall components, the intensity of staining of the walls with Schiff's reagent in the PAS method decreases. In the first step of extraction (with 0.5% ammonium oxalate) pectins are removed, in the second (with 4% sodium hydroxide) hemicellulose, and after the third extraction (with 17.5% sodium hydroxide) only cellulose remains. Comparison of the stained preparation after successive removal of the cell wall components demonstrated a distinct decrease in the intensity of staining, no unstained preparations were, however, noted. The embryos were observed after removal of all components with the exception of cellulose. They also gave a positive PAS reaction although they stained very weakly.

After the first and second extraction steps, when callose is still present in the walls, it is visible as a strand unstaining in the PAS reaction, localised at the border of the degenerating megaspores. In preparations in which callose had been extracted and the remaining wall component was cellulose, this colourless strand was not seen. This observation agrees with the fact that callose without any admixtures is PAS-negative.

Photos 4 and 5 (Plate I) show the intensity of glucose-^{3}H incorporation. For the meiocyte, in the walls of which there is as yet no callose, the number of grains in autoradiograms is low. The walls of a mature megasporocyte are much stronger labelled, thus it is in this stage that synthesis of the wall components occurs, and probably at the same time intensive synthesis of callose takes place.

Callose appears in the first meiotic prophase stage in the chalazal wall of the meiocyte, and later the entire cell is surrounded by a luminescent wall (Photos 6 and 7, Plate I). Already in the course of formation of the primary septum, fluorescence can be observed in the latter, and it is most intensive after diad formation in the transverse wall (Photo 8, Plate I). Also the transverse walls developed after the second meiotic division show a stronger fluorescence than the longitudinal ones (Photo 9a, table I). In the chalazal wall of the active megaspore fluorescence still persists at the time of differentiation of the embryo sac.

II. The family *Scrophulariaceae*

The examined species of the family *Scrophulariaceae* develop according to the monospore *Polygonum* type. Callose appears in the meiocyte walls in the period of prophase I, and disappears after a short period from the wall of the chalazal pole (Photos 1—3, Plate II). In species of

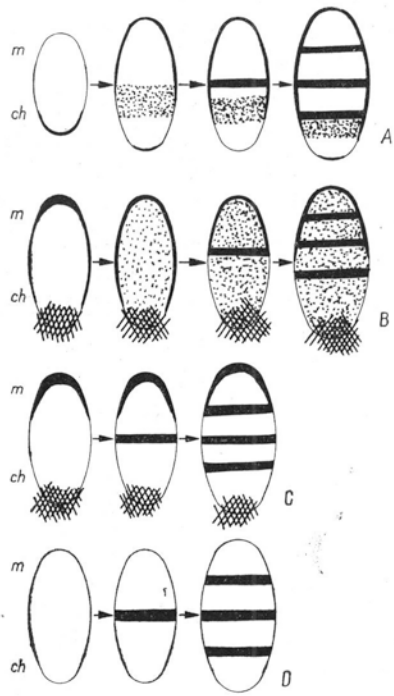

Fig. 1. Callose in cell walls during megasporogenesis in the examined species of *Scrophulariaceae* (Walls with callose denoted by thicker lines and dotting)

A — Gratiola officinalis, Digitalis ambigua; B — Digitalis lanata, D. sibirica, D. purpurea; C — Pentstemon aureus; D — Verbascum olympicum, V. phlomoides; m — micropylar pole, *ch* — chalazal pole. Hatched chalazal pole denotes presence of hypostasis

the genus *Digitalis and Pentstemon*, where the chalazal pole of the meiocyte is surrounded by the tissue of the fluorescent hypostasis (Photo 11, Plate II), the luminescence of the wall of the chalazal pole is not noticeable. Also at later development stages of these species, the chalazal pole remains masked by the fluorescent hypostasis (Photos 12, 13, Plate II; Fig. 1).

In the longitudinal walls, the intensity of fluorescence gradually increases. At first it appears in the shape of a luminescent ring situated in the subequatorial part of the cell (Photos 1—3, Plate II), and later cells are seen with longitudinal walls completely impregnated with callose (Photo 3, Plate II).

The first meiotic division occurs in the equatorial plane of the cell, it is followed by the appearance of a fluorescent transverse wall (Photos 4, 5, Plate II). The fluorescence intensity of the longitudinal walls in the diads varies depending on the stage of megasporogenesis (Photos 5—7, Plate II).

As the result of the asynchronic second meiotic division a triad arises after separation of the chalazal cell of the diad.

Certain differences are seen in the degree of impregnation of the longitudinal walls of the triads with luminescent material. In some triads fluorescence at the micropylar pole and in the longitudinal walls was very strong, particularly in the upper part of the wall of active megaspore. Photo 8 (Plate II) shows a strongly fluorescent triad of *Gratiola officinalis*.

After the second meiotic division tetrads form which usually are linear. The transverse walls and the micropylar pole are strongly fluorescent, but the luminescent segment of the longitudinal wall is not large (Photo 9, Plate II) or it may not be visible at all (Photo 10, Plate II).

In species of the genus *Verbascum* callose appears only in the transverse walls in the course of megasporogenesis in diads, triads and tetrads. The longitudinal walls show no fluorescence (Photos 14, 15, Plate II; Fig. 1D).

III. The family *Solanaceae*

In 12 species of the family *Solanaceae* the first callose layers were observed in the chalazal pole of the meiocyte. Photo 1 (Plate III) show the first fluorescence of the megasporocyte wall in *Atropa belladonna*. Generally after a short period fluorescence disappears in this zone, and at the same time it becomes visible in the wall of the micropylar pole and the longitudinal walls (Photos 3, 4, Plate III).

In the species examined, the segment of the longitudinal wall situated in the subequatorial part of the cell exhibits stronger fluorescence already in the meiocytes (Photos 2, 9, Plate III). This segment continues

to fluoresce in the subsequent division stages in the chalazal cells of diads, triads and tetrads (Photos 6, 7, 12—16, Plate III) except the chalazal pole in *Nicotiana tabacum* and *Atropa belladonna* strong uniform luminescence of the megasporocyte walls is observed, during the first meiotic division (Photos 10, 11, Plate III). In the newly formed diads of all *Nicotiana species* and in *A. belladonna* fluorescence of the longitudinal walls was equally intensive in both cells of the diad, only the chalazal pole was deprived of it (Photos 5, 11, Plate III).

The second meiotic division in the species of the family *Solanaceae* examined is as a rule asynchronic. Usually the chalazal cell divides first (Photos 7, 15, Plate III), but in *Nicotiana tabacum* this division takes place first in the micropylar cell of the diad (Photo 13, Plate III).

The tetrads are linear and the chalazal pole of the active megaspore contains as a rule no callose (Photo 8, Plate III). Only seldom tetrads can be found with fluorescence at the chalazal pole (Photo 16, Plate III). In the same species (*Datura forex*), however, tetrads prevail with a chalazal pole deprived of callose. In the active megaspore, the longitudinal walls fluoresce distinctly, but in some pictures the longitudinal walls of inactive megaspores also showed a high fluorescence (Photo 8, Plate III).

From the pictures observed it may be concluded that the luminescence in the longitudinal wall is intensified before the successive meiotic division, and particularly in the cell undergoing division at the given moment.

IV. The family *Onagraceae*

The species of the family *Onagraceae* which were studied developed according to the *Oenothera* type (monosporous, fournucleate). In this development type fluorescence appears first in the micropylar pole of the meiocyte (Fig. 2). This stage lasts but a short time and soon callose appears in the longitudinal walls, giving, a much stronger reaction (Photos 1, 2, Plate IV). The next "callose" stage through which the meiocytes pass is full impregnation of all the cell walls (Photo 9, Plate IV). This stage is short, the meiocytes have mostly strongly fluorescent longitudinal walls, and the micropylar pole is already deprived of fluorescence (Photos 3, 10, Plate IV). In several of the species examined (*Fuchsia corymbiflora, Epilobium hirsutum, Clarkia concinna, Oenothera glauca and O. grandiflora*), gradual disappearance of callose from the longitudinal walls of the megasporocyte may be observed (Photos 5, 11, 12, Plate IV). The megasporocytes of *Oenothera muricata* do not show any noticeable fluorescence.

Callose localization and the intensity of fluorescence in the diads agrees in broad line with the callose pattern of meiocytes. The species in which meiocytes had walls highly impregnated with callose exhibit

strong fluorescence of the longitudinal walls in the diads (Photos 4, 13, Plate IV). The diads of *Oenothera muricata* are visualised in the fluorescence microscope owing to the distinct reaction in the transverse wall formed after the first meiotic division (Photo 15, Plate IV).

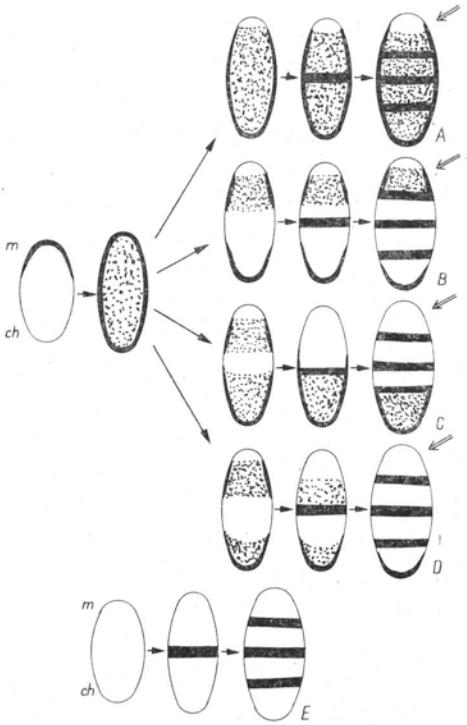

Fig. 2. Cell walls with callose in exmained representatives of the family *Onagraceae* (Walls with callose denoted by thicker lines and dotting)

A — Fuchsia corymbiflora, Gaura biennis, Oenothera fruticosa; B — Epilobium hirsutum, Oenothera glauca; C — Oenothera grandiflora; D — Oenothera missouriensis; E — Oenothera muricata; m — micropylar pole, *ch* — chalazal pole. Double arrow indicates the localization of the active megaspore

The second meiotic division in some species is asynchronic. The resulting triads may be found in *Oenothera glauca, Gaura biennis, Epilobium hirsutum, Fuchsia corymbiflora* and *Clarkia concinna* (Photos 6, 7, Plate IV). Callose distribution in the longitudinal walls of the triad follows the pattern found at earlier stages. The micropylar pole continues to show no fluorescence.

In all the species studied the tetrads are linear. Fluorescence of the longitudinal walls, like in the diads and triads is distributed according to the pattern found in meiocytes (Photos 8, 14, Plate IV).

The tetrads of *Oenothera muricata* have fluorescence distinctly localized in the transverse walls, sometimes also in the poles (Photos 16, Plate IV).

V. Other species

The species of the remaining families of *Angiospermae (Caryophyllaceae, Lobeliaceae, Liliaceae, Papaveraceae, Pyrolaceae, Gesneriaceae, Menyanthaceae)* studied develop according to the *Polygonum* type. In these species the first fluorescence appears in the chalazal pole of the megasporocytes, but callose does not disappear from this region in all species. The chalazal pole remains involved in at least weak fluorescence in numerous species of the families *Papaveraceae, Gesneriaceae, Liliaceae, Lobeliaceae, Caryophyllaceae* (Photos 1—6, 9, 11, Plate V). In most of these species segments of longitudinal walls with intensified fluorescence were not noted. On the other hand, in the species where a callose segment can be distinguished in the longitudinal walls, disappearance of early fluorescence from the chalazal pole region is generally observed (Photos 7, 8, 10, Plate V). Besides, some few pictures have been found in which callose in the chalazal pole does not disappear, and simultaneously a callose segment is visible (Photos 4, 5, Plate V). The callose segment in such cases is contiguous to the chalazal pole wall. It should be added that in older stages in this species (*Laurentia longiflora*) fluorescence of the longitudinal wall is uniform (Photo 6, Plate V).

The family *Caryophyllaceae* was an exception among the angiosperms examined, since the second meiotic division took place only in the chalazal cell of the diad. Thus, the triad frequently with a greatly elongated micropylar cell, was the last stage of megasporogenesis in this family (Photo 11, Plate V).

No fluorescence was found in the longitudinal walls in the only representative of the family *Menyanthaceae — Menyanthes trifoliata.* The reaction for callose was positive only in the transverse walls of the diads, triads and tetrads.

DISCUSSION

In the development of monospore embryo sacs two contrasting types of localization of the active megaspore in the tetrad can be distinguished: the *Polygonum* and the *Oenothera* one. Megasporogenesis of the *Polygonum*-type is most common among angiospermous plants. In this type of development, after meiotic division, a megaspore tetrad forms, the three micropylar cells of which degenerate, and from the chalazal megaspore, after mitotic division the embryo sac is formed. In the *Oeno-*

thera-type the three chalazal megaspores undergo degeneration, and the embryo sac is formed from the micropylar megaspore. From among the species investigated, the *Oenothera*-type occurs only in the *Onagraceae* family, the remaining species belonging to various families develop according to the *Polygonum*-type.

Studies of the chemism of cell walls in the course of megasporogenesis in a species with development of *Polygonum*-type (*Epipactis latifolia*) showed that, in the young archaesporial cell, the wall consists of hemicellulose, cellulose and a large amount of pectins. In the course of development up to tetrad formation, these constituents are found in the logitudinal and transverse walls. Even in young megasporocytes, after complete extraction of all noncellulose components, a positive though weak PAS reaction was obtained. In the microspore mother cells, on the contrary, before the appearance of callose in them, the primary wall of the young microsporocytes contains mainly pectins and no cellulose. Later thick callose form in the walls (Waterkeyn 1962).

In megasporogenesis callose appears in the first meiotic prophase, as it does in microsporogenesis. During megasporogenesis of the orchid (*Orchis maculata*), the first pictures with fluorescence were noted in the period of diakinesis (Rodkiewicz, Górska-Brylass 1967).

From the moment of callose formation in the cell walls during megasporogenesis, intensive processes of cell wall development start, resulting in thickening of the megasporocyte walls, and formation of transverse ones. The enhanced processes of synthesis of wall material seem to be indicated by the autoradiographic observations in which glucose-^{3}H is seen to incorporate intensively not earlier than at the stage of mature meiocyte. It is in this period that the callose content greatly increases in the walls of the latter. In earlier stages of megasporocyte development, incorporation of labeled glucose is low. The rate of tritium-labeled glucose incorporation is an index of synthesis of carbohydrate complexes (Peterson, Leblond 1964).

It results from these observations that, in most of the species investigated, the localization of the first callose layers in the meiocyte walls agrees with the localization of the active megaspore in the tetrad. Thus, the first site of callose appearance in *Polygonum*-type development is the chalazal pole of the meiocyte, and in the *Oenothera*-type the micropylar pole. Although Jalouzot (1971) found the earliest fluorescence in *Oenothera lamarkiana* in the chalazal part of the cell, it would rather seem that callose was not noticed at the micropylar pole in this species, on acconut of the short period of early fluorescence in this part of the meiocyte. Therefore, the primary localization of callose in the megaspore may be considered as connected with the polarity of the cell being the archaecell of the embryo sac.

Some species of the family *Scrophulariaceae* (*Polygonum*-type) seem

not to conform with the foregoing pattern. Fluorescence of the chalazal pole wall in the megasporocytes was not noticeable in them. These species, however, posses the tissue hypostase, encircling the chalazal pole; the hypostase cells after reaction with aniline bule exhibit fluorescence as strong as that of the meiocyte wall. Fluorescence of cell walls of the hypostasis has been described in another species of the same family (*Anthirrinum majus*) (Rodkiewicz 1967). Masking of the fluorescence of the meiocyte wall by the fluorescent hypostasis cells is all the more possible, since in *Digitalis ambigua* the hypostasis develops later at the time when the chalazal pole shows fluorescence.

The presence of callose in the wall of only one cell pole lasts a very short time. Callose soon forms in other meiocyte walls and often disappears completely from the initial site. This rapid appearance and disappearance of callose is possible owing to the high polymerisation and depolymerisation ability of this polysaccharide (Eschrich 1961).

The area of the longitudinal wall, in which callose can be detected, gradually extends. The moment of maximum saturation with callose of the longitudinal megasporocyte walls falls just before the first meiotic division and lasts for a short time after its end in the diad. The fluorescence intensity diminishes as the diad ages. Later strongly fluorescing triads may be seen. It may, therefore, be concluded that callose formed at the time of the first meiotic division is partly hydrolysed in the interphase, and then synthesis recommences in the second meiotic division period. Thus in the course of megasporogenensis increasing in the callose content takes place in the cellular walls ready to divide, as well as in the course of division I and II (Fig. 3).

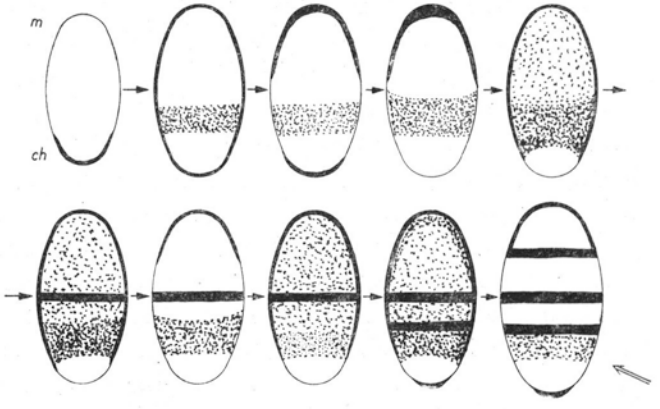

Fig. 3. Scheme of localization and fluorescence intensity changes characteristic for callose during megasporogenesis in *Gratiola officinalis* (Callose denoted by thicker lines and dotting)

Double arrow indicates active megaspore in the stage of tetrad. *m* — micropylar pole, *ch* — chalazal pole.

In many species an increased fluorescence was observed even before the first metiotic division, lasting over the entire period of megasporogenesis in the cell which after division will give the active megaspore. On the other hand, the longitudinal walls of the inactive megaspores show a gradual decline of fluorescence. In one species (*Nicotiana taba-*

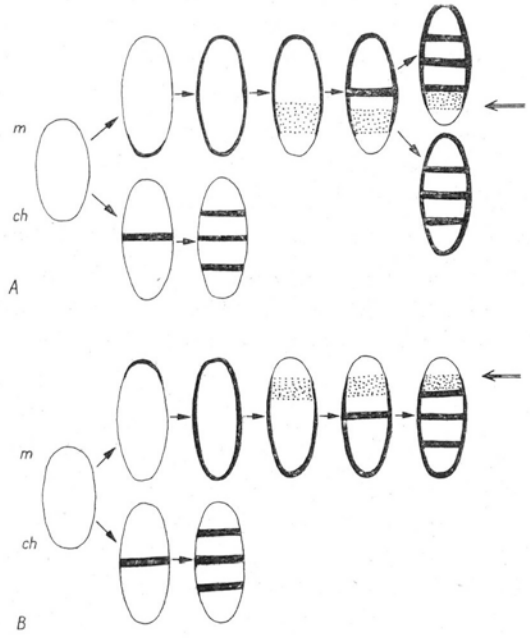

Fig. 4. Most frequent patterns of callose localization in megasporocyte walls and megaspores in monospore development of the embryo sac.

A — monospore type of *Polygonum* development; *B* — monospore type of *Oenothera* development; *m* — micropylar pole, *ch* — chalazal pole. Double arrows indicate active megaspores in both development types

cum), the second meiotic division occurs first in the micropylar cell of the diad, nevertheless, only the walls of the chalazal cell which is not yet dividing, that is the cell which by division will give the active megaspore, are strongly impregnated with callose.

In many of the species observed a segment of the longitudinal wall varying in shape and size has been noticed which shows an increased fluorescence. Such a segment may be seen already in the meiocyte, and its localization agrees with the later situation of the active megaspore. In the successive stages of megasporogenesis this segment persists in the chalazal region in the *Polygonum*-type and in the micropylar region in the *Oenothera*-type of development. The size of this segment, its shape and the intensity of fluorescence may vary from species to species (even within the same genus), but within one species this trait is constant.

This segment in the longitudinal wall of megasporocytes and active megaspores is more pronounced in the species in which callose disappears completely from the pole limiting the active megaspore.

The transverse walls in all the species examined show consistently the presence of callose. Fluorescence is already visible in the forming cell plate. Here the phenomenon is much more distinct than in the somatic cells where callose has also been described in the forming cell plate. During somatic cell division; however fluorescence is weak and disappears towards the end of telophase (Waterkeyn 1967).

Among all the plants with monospore development both of *Polygonum* and *Oenothera*-type, four species were found in which the meiocytes did not exhibit fluorescence characteristic for callose, these were: three species with *Polygonum*-type development (*Verbascum olympicum, V. phlomoides, Menyathes trifoliata*) and one species with *Oenothera*-type development (*Oenothera muricata*). During the megasporogenesis in these species only in the transversal walls the fluorescence was observed.

The fact that callose was present only in the transverse walls of diads, triads and tetrads in one of the species (*Oenothera muricata*) is probably connected with "Renner's effect" (Renner 1921), and it was interpreted in this way (Rodkiewicz, Kuran 1971). In the above named species, after meiotic division, one half of the megaspores receives lethal chromosome sets. The probability of passing of the lethal chromosome set to the chalazal or the micropylar megaspore is equal. Therefore in further development one half of the chalazal megaspores tetrads and one half of the micropylar megaspores tetrads are active megaspores. The megasporocyte cannot, therefore, be polarly determined since each of its poles may become the site of active megaspore formation.

In view of the commonly observed appearance of the first fluorescence in the pole of the meiocyte wall which by its situation corresponds to the later localization of the embryo sac, it may be concluded that callose deposition in the cell walls during megasporogenesis is genetically conditioned.

It results from observations in the investigated plant species that the pattern of callose distribution on the megasporocyte walls and megaspores is a character specific for the given species, but it is not as a rule uniform in the entire course of megasporogenesis. It may be stated, however, that, in general, there is more callose in the walls of cells which are going to pass into further stages of differentiation. The second common character of cells passing through megasporogenesis according to a definite development type (*Polygonum or Oenothera*) is the appearance of the first callose layers in the part of the meiocyte corresponding to the future region of embryo sac formation (Fig. 4).

According to this regularity, it is possible to establish, long before the formation of the female gametophyte, the type of megasporogenesis development of the given plant.

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Lokalizacja kalozy w ścianach megasporocytów i megaspor podczas rozwoju monosporowych woreczków zalążkowych

Streszczenie

Metodą mikroskopii fluorescencyjnej przeprowadzono badania dotyczące lokalizacji kalozy w ścianach megasporocytów i megaspor w monosporowym typie rozwoju woreczków zalążkowych.

Stwierdzono powszechność występowania badanego polisacharydu w tym typie rozwoju. Szczegółowe badania nad rozmieszczeniem kalozy w ścianach wykazały, że rejon ściany mejocytu, w którym kaloza pojawia się najpierw, jest zgodny z rejonem formowania się woreczka zalążkowego. Natomiast w ciągu dalszych stadiów megasporogenezy rozmieszczenie kalozy w podłużnych ścianach megasporocytów i megaspor jest nierównomierne. Można jednak stwierdzić, że prawie zawsze więcej badanego polisacharydu znajduje się w ścianach tych komórek, które będą przechodziły dalsze etapy różnicowania.

Dla poszczególnych gatunków wzór rozmieszczenia kalozy w czasie megasporogenezy jest cechą stałą.

U jednego z gatunków (*Epipactis latifolia*) zbadano ponadto chemizm ścian komórkowych metodą selektywnej ekstrakcji poszczególnych składników oraz metodą PAS. Stwierdzono w ścianach obecność pektyn, hemiceluloz i celulozy. Polisacharydy te występują w ścianach zarówno przed pojawieniem się składnika kalozowego, jak również w czasie jego występowania.

PLATE I

Epipactis latifolia

Figs 1—3. PAS-positive substance in cell walls during megasporogenesis (×700); fig. *1* — premeiotic stage, fig. *2* — diad stage, fig. *3* — tetrad stage of megaspore

Figs 4, 5. D-glucose-^{3}H incorporation into young and mature megasporocyte (×900)

Figgs 6—9. Callose fluorescence during megasporogenesis (× 700) P: fig. 6 — callose in wall of megasporocyte chalazal pole, fig. *7* — callose in megasporocyte walls, fig. *8* — callose in longitudinal walls and transverse wall of diad, fig. *9* — callose in cell walls at stage of megaspore tetrad

PLATE II

Fluorescence of callose in species of the family *Scrophulariaceae*

Figs 1—3. Megasporocyte of *Gratiola officinalis*

Figs 4, 6, 7. Diad stage of *Gratiola officinalis*

Fig. 5. Fluorescence of forming cell plate in *Digitalis lanata*

Fig. 8. Triad stage of *Gratiola officinalis*

Fig. 9. Tetrad stage of *Gratiola officinalis*

Fig. 10. Tetrad stage of *Digitalis lanata*

Fig. 11. Beside strong fluorescence of meiocyte walls in *Digitalis lanata* the fluorescence of the hypostatis walls is also visible

Figs 12, 13. Diad and tetrad stage of *Pentstemon aureus* megaspores, fluorescence is seen in the hypostasis cell walls

Figs 14, 15. Fluorescence of transverse walls in triad and tetrad stages of megaspores in the genus *Verbascum*
(× 700, photos 12, 13 × 300)

PLATE III

Callose fluorescence in species of the family *Solanaceae*

Figs 1—8. Callose in megasporogenesis in *Atropa belladonna* (×700): figs 1—3 — megasporocytes, figs 5, 6 — diad stage, fig. 7 — triad stage, fig. 8 — tetrad stage of megaspore

Figs 9—14. Callose in megasporogenesis in *Nicotiana tabacum* (×700): figs 9, 10 — megasporocytes, figs 11, 12 — diad stage, fig. 13 — triad stage, fig. 14 — tetrad stage of megaspore

Figs 15, 16. Walls with callose at triad and tetrad stage in *Datura ferox* (× 700)

PLATE IV

Callose fluorescence in species of the family *Onagraceae*

Figs 1—4. Megasporocytes and diads of *Fuchsia corymbiflora* (×500)
Figs 5, 6. Megasporocyte and triad of *Epilobium hirsutum* (×700)
Figs 7, 8. Triad and tetrad stages of *Oenothera glauca* megaspores (×700)
Figs 9, 10. *Oenothera fruticosa* megasporocytes (×700)
Fig. 11. Young megasporocytes of *Clarkia concinna* (×700)
Fig. 12. *Oenothera missouriensis* megasporocyte (×700)
Figs. 13, 14. Diad and tetrad stages of *Gaura biennis* megaspores (× 700)
Figs 15, 16. Fluorescence of transverse walls in *Oenothera muricata* diad and tetrad (×700)

PLATE V

Walls with callose during megasporogenesis in species of families *Liliaceae*, *Lobeliaceae* and *Caryophyllaceae*

Fig. 1. Megasporocyte of *Ornithogalum umbellatum* (*Liliaceae*) (×700)
Figs 2, 3. Diad and tetrad stages of *Hemerocallis flava* (Liliaceae) (× 700) (× 700)
Figs 4, 5, 6. Megasporocyte, diad and tetrad of *Laurentia longiflora* (*Lobeliaceae*) (×700)
Fig. 7. *Silene inflata* (*Caryophyllaceae*) megasporocyte (×700)
Figs 8, 10. Diad and triad stages in *Silene scendneri* (*Caryophyllaceae*) (× 700)
Fig. 9. Diad stage of *Lychnis flos-cuculi* (*Caryophyllaceae*) (×700)
Fig. 11. Triad stage of *Dianthus arenarius* (*Caryophyllaceae*) (×700)

Plate I

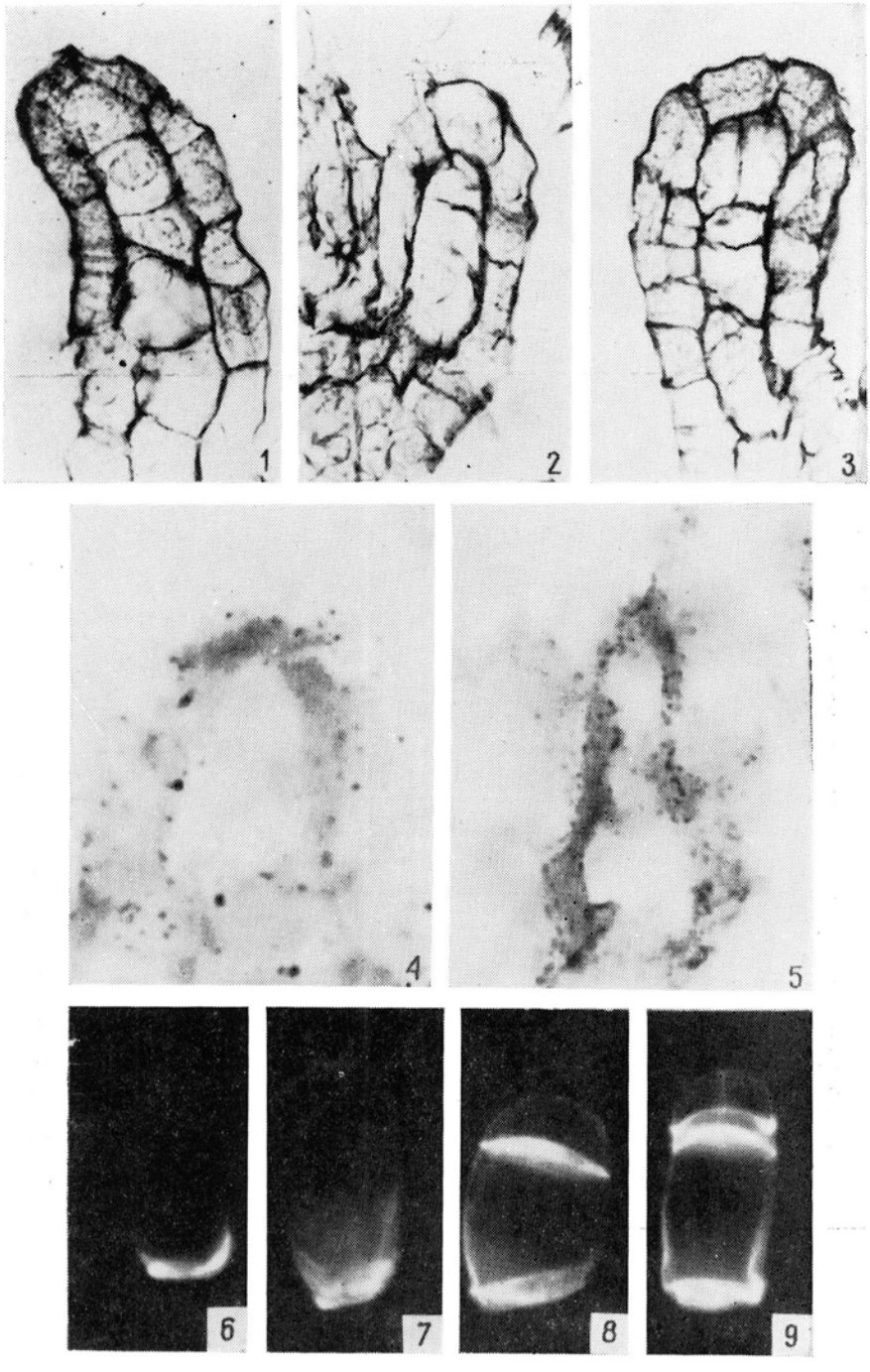

Plate II

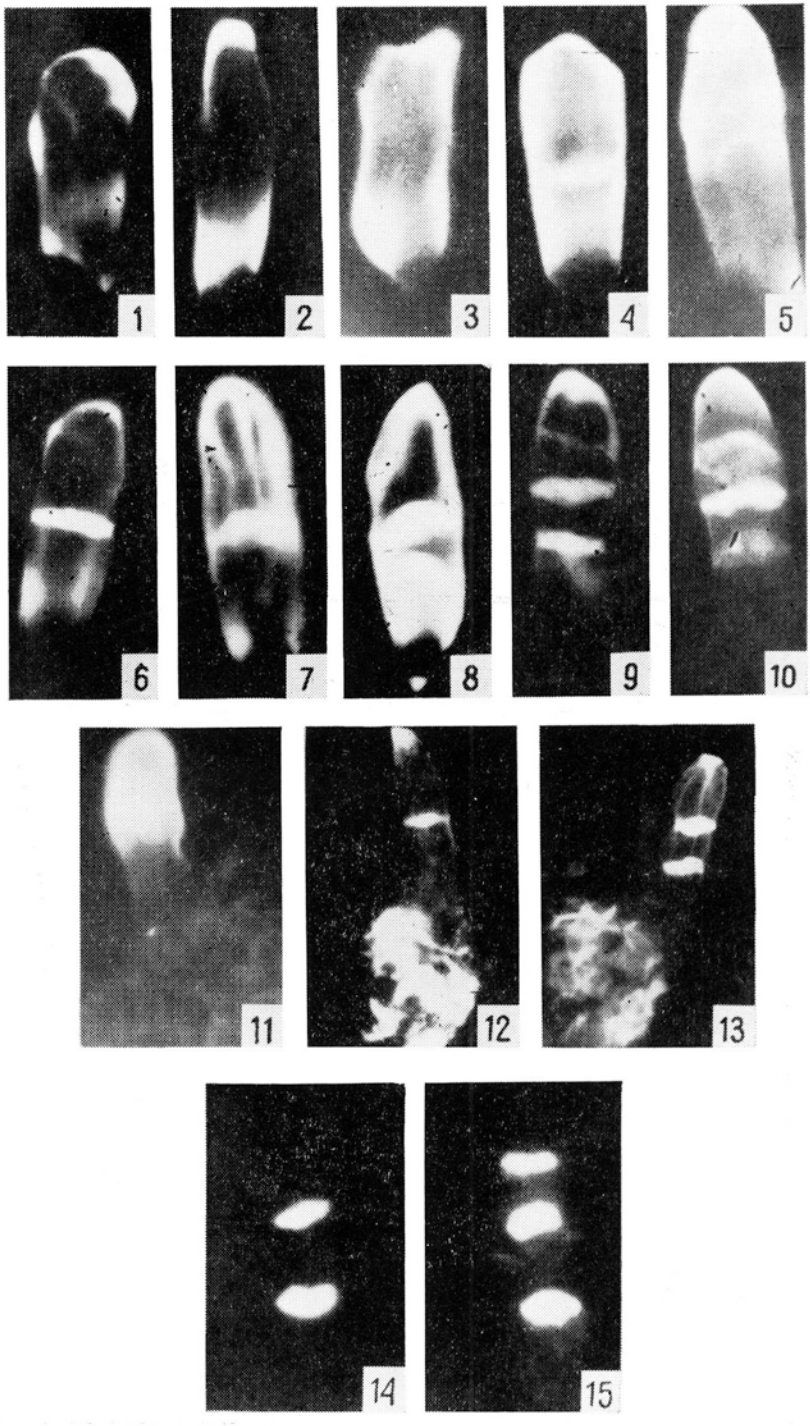

Plate III

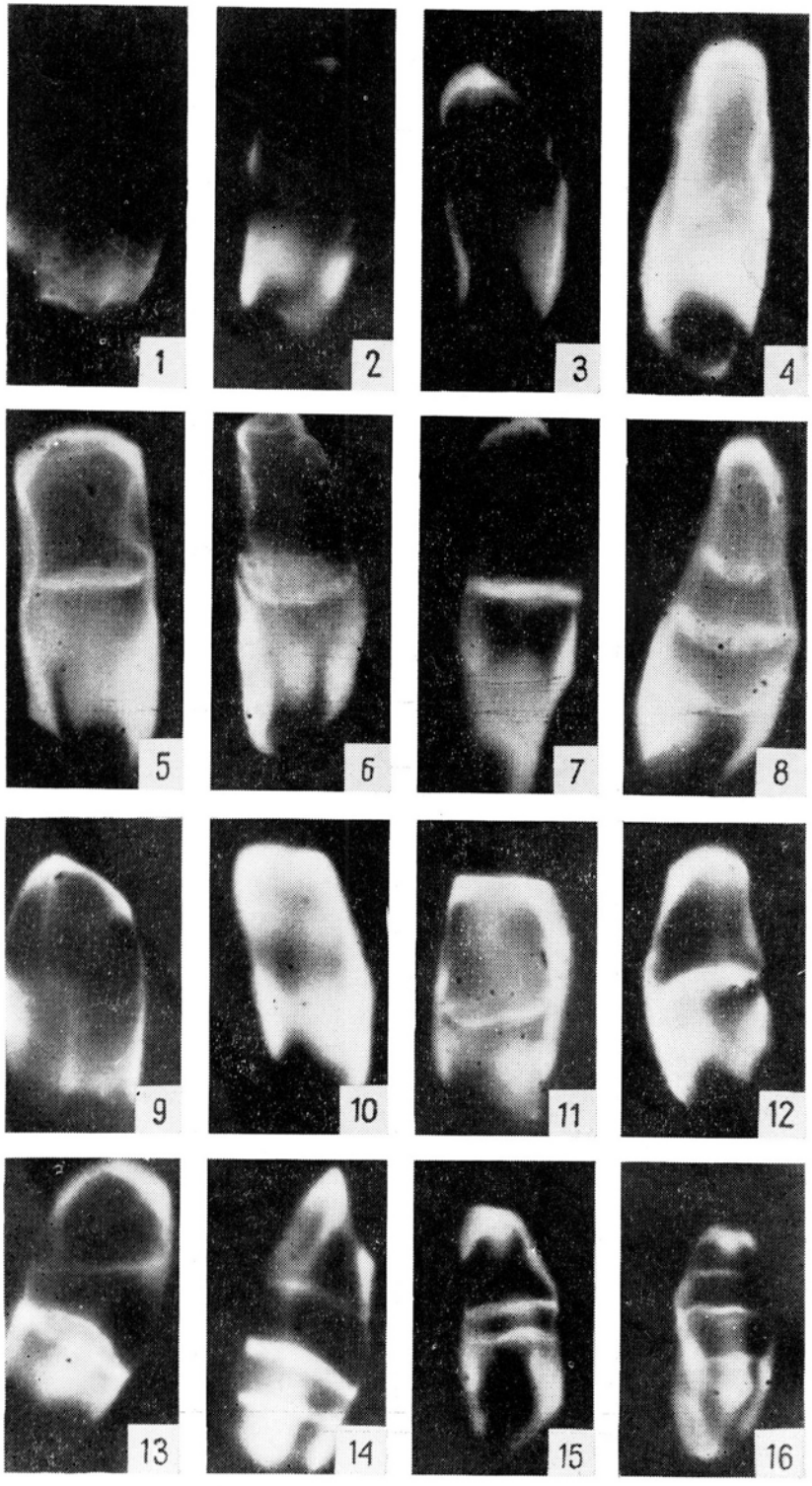

Plate IV

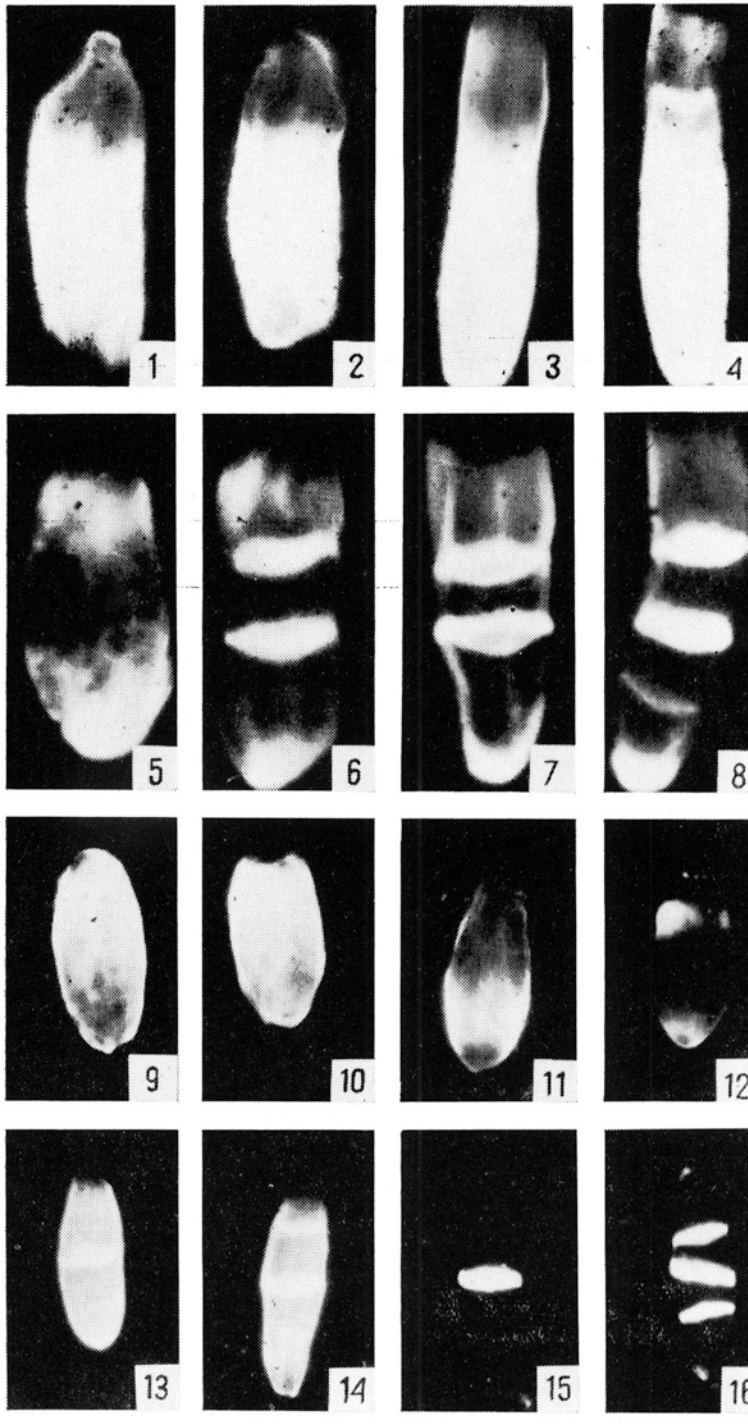

Plate V