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Callose formation in injured cells of the vegetative and generative thallus of *Chara vulgaris* L. Absence of callose in the process of cytodifferentiation

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Abstract

In an alga *Chara vulgaris* L. the processes of differentiation of vegetative system cells of the thallus, and initiation and development of generative organs are not associated with callose formation. It was demonstrated that damage to any of the somatic cells and also generative and nongenerative cells of the antheridium and oogonium are capable of callose formation independently of their developmental stage. The localisation and thickness of these layers depend on the way the cells are injured and on their size. The protective role of callose in such cells may consist, beside strengthening the damaged walls, in protection of the symplast by formation of callose deposits on the walls with plasmodesmata; it may also consist in increasing the water potential of the cells. Experiments in which callose deposition was provoked by pressing of the cells or damage leading to a sudden increase of the water potential of the extracellular environment suggest that a sudden increase of tension in the cells may be a factor triggering the "callose effect".

Key words: *callose, cell injury, water potential, cytodifferentiation, Chara vulgaris L.*

INTRODUCTION

Callose synthesis and deposition on cell walls have been proved in cells of both the vegetative and the generative system in lower and in higher plants (cf. Gabara 1977). The common occurrence of this process and the fact that callose appears very soon and usually does not remain as a permanent structural component suggest the important and specific role of this polysac-

charide in the functioning of plant organisms. In spite of accumulation of numerous observations, the role of callose is not clear in most cases, and the mechanism of initiation of its synthesis is unknown. In general the appearance of callose is connected with: a) protection of the symplast in case of damage of the cell, b) the process of cytodifferentiation. Investigations on the role of callose in cell differentiation and dedifferentiation processes are difficult because of the rapidity of its synthesis as compared with the relatively slow penetration of fixatives. Thus, in most cases this synthesis cannot be ruled out in the course of preparation, as a reaction to injury. These difficulties can be overcome in some cases by rapid killing of the cells by means of low temperature (Fulcher et al. 1976, Geuns-Longly and Waterkeyn 1976) or by the choice of the material through which the fixative penetrates quickly.

The aim of the present study was to investigate the participation of callose in the processes of cell differentiation in the alga *Chara vulgaris*. In this material fixatives penetrate readily into the thallus and kill the cells within several seconds. Therefore, this alga seems to be a convenient model for studying role of callose in the process of cell differentiation. It is characterised by a great diversity of the vegetative system of the thallus and also by a complex structure of generative organs. These differences concerned the size of the cells, the nuclear DNA content, number of nuclei, the functions of plastids etc.

MATERIAL AND METHODS

The investigations were performed on generatively mature thalli of *Chara vulgaris* L. and on plants not forming reproductive organs. The material was taken from ponds in the city of Łódź and cultured under laboratory conditions (L:D = 14:10, 4000 lux, 20-23°C, pH 7.2) in water from these basins.

The thalli were fixed in an ethanol-acetic acid mixture (3:1 v/v 30 min) or glutaraldehyde (3%, phosphate buffer 0.0125 M, pH 7.2, 60 min).

Injury was obtained by pressing various parts of the thallus with a coverslip or by pressing definite sites on the cell wall with a dissecting needle. The effect of the following operations on the antheridia was verified: a) detachment of these organs from the thallus, b) tearing of the antheridial envelope, c) crushing of these organs and squeezing of the cells out of the antheridium through the torn envelope.

Callose presence was revealed by its yellow-green fluorescence with aniline blue (Currier 1957) (Zeiss fluorescence microscope, HBO 50 lamp, exciting filtre UG_1, barrier filtre BG_3) and staining with resorcin blue (Eschrich 1954).

RESULTS

CALLOSE IN THE FIXED MATERIAL

In the thallus forming or not reproductive organs fixed with the ethanol-acetic acid mixture or glutaraldehyde callose was searched for with particular attention to: a) the apical bud of the thallus in which division of the apical cells and later unequal divisions condition the formation of various types of cells building nodes, internodes and lateral branches, b) initial cells of the antheridium, oogonium and rhizoids and c) the successive stages of development of generative organs – oogonia and antheridia. As regards antheridia, it was checked whether callose formation is connected with a) formation of initial cells of antheridial filaments arising by way of budding of capitular cells, b) synchronously occuring mitotic divisions determining growth of these filaments, c) lose of division capacity by the cells which have reached the stage of spermatids and d) transformation of spermatids to spermatozoids.

In material cultured under conditions excluding mechanical damage of the thallus and then fixed, the method applied did not indicate the presence of callose either in the vegetative system of the thallus or in the reproductive and nonreproductive cells of the generative organs (Figs. 1-3).

CALLOSE FORMATION AS THE EFFECT OF CELL INJURY

The thallus vegetative system

Most sensitive proved the cells of the apical bud (Fig. 4) (callose appeared even after delicate compression), lateral buds (Fig. 6), young internodes and "stem" nodes (Figs. 5 and 8). Somewhat less sensitive were "leaves", thorns (Fig. 5) and rhizoids (Figs. 11-13). In the node and internode cells of the fully developed part of the thallus, callose appeared after relatively strong pressure (Figs. 7 and 9).

Local damage produced by a dissecting needle in the internodes of the "stem" lateral branches (Figs. 7, 9 and 10) and of the rhizoids (Figs. 11-13) caused formation of callose patches. In the cortical cells of the "stem" internode and lateral branches and in the cells of the cortex-lacking internodes of the lateral branches, the chloroplasts are located at the cell walls (Figs. 9a and 10a). In these cells in the initial phase of callose formation, its deposition is observed in the form of a network corresponding to the spaces between the chloroplasts. As the amount of callose increases the whole surface of the patch is covered; and the network pattern remains only on the edges (Figs. 9 and 10). The size and shape of the callose patches in the rhizoids is conditioned by the degree of damage to the wall (Figs. 11 and 12). Rhizoids were also found in which the patches were distributed at regular intervals along the cells (Fig. 13). No plugs were noted cutting off the injured part of the rhizoid (Figs. 11 and 11a).

After pressing the cells with the coverslip callose was deposited on the transverse walls joining the cells along axis of the "stem" and lateral branches. Callose deposits occupied the whole surface of the wall (Figs. 4, 6 and 8) or only its part. Partial covering of the walls was more frequent in large cells, for instance in walls joining the cells of the lateral branches (Figs. 14-17), the thorns with the internode cortex, the internode cells with cells of the nodes (Fig. 5) etc.

Comparison of the callose deposits formed by cells of larger or smaller size showed the existence of a distinct dependence between the cell size and the amount of callose formed. Large cells formed much thicker callose layer as compared with that produced by cells of smaller dimensions.

Oogonia

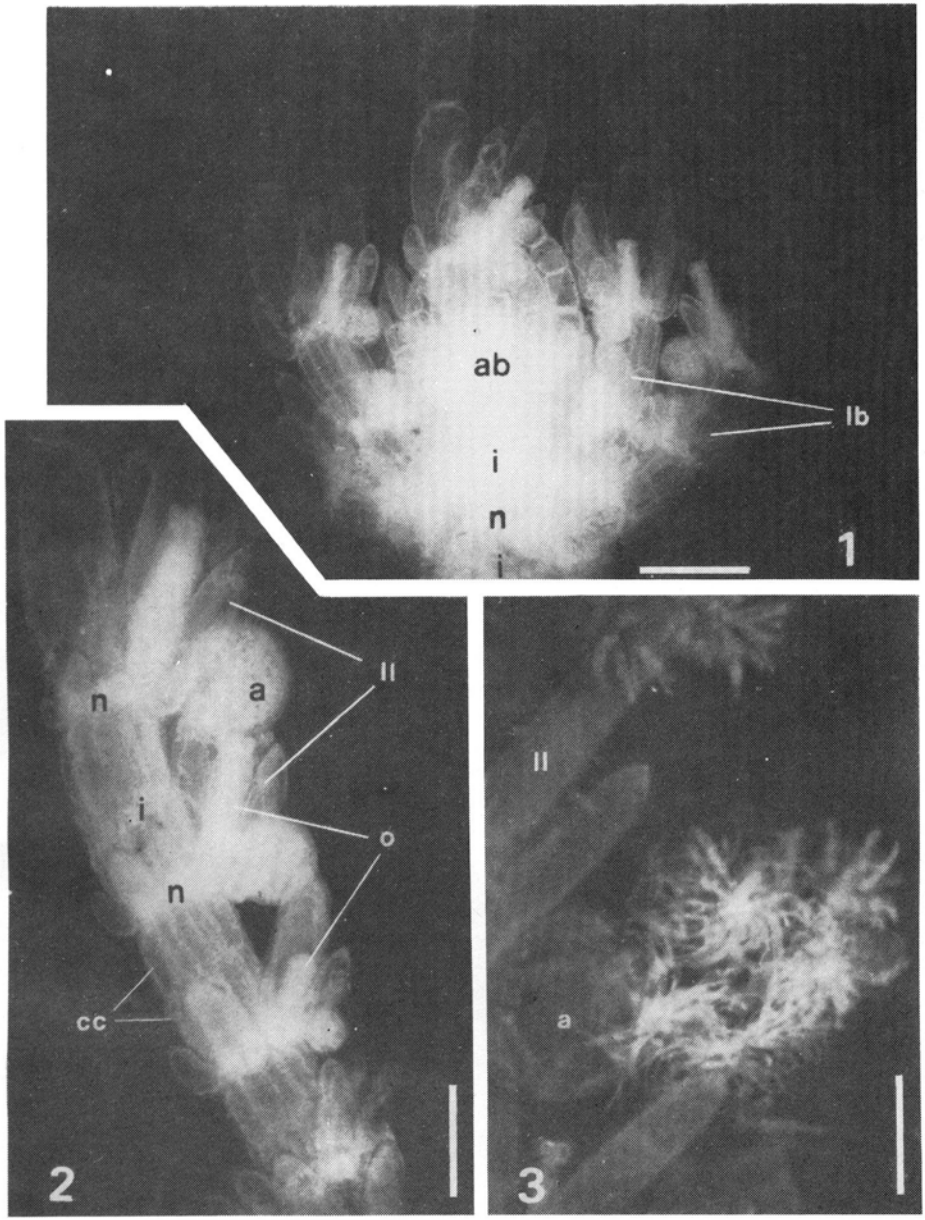
All cells, notwithstanding their development stage, that is from the initial cell of the oogonium to the stage of maturity were capable of callose formation. More sensitive to injury were cells at the early stage of oogonium development. The site of callose deposition were, similarly as in the vegetative part of the thallus the walls joining the cells along these organs and also those joining the cells of nodes of lateral branches. The presence of callose was noted in the wall between the initial cells of an oogonium and the node cells. In the 2-cell stage delicate pressure upon the external cell caused callose deposition on the wall connecting it with the cell caving into its interior (Fig. 18). In later development stages damage resulted in callose deposition in the walls connecting the crown cells with the oogonium cortex (Figs. 19 and 20) and also in the walls between the cortical cells and the basal cell. Strong pressure with a dissecting needle of even mature oogonia caused the appearance of callose in the lateral walls of the oogonium cortex and in the lateral wall of the egg cell (Fig. 21).

Antheridia

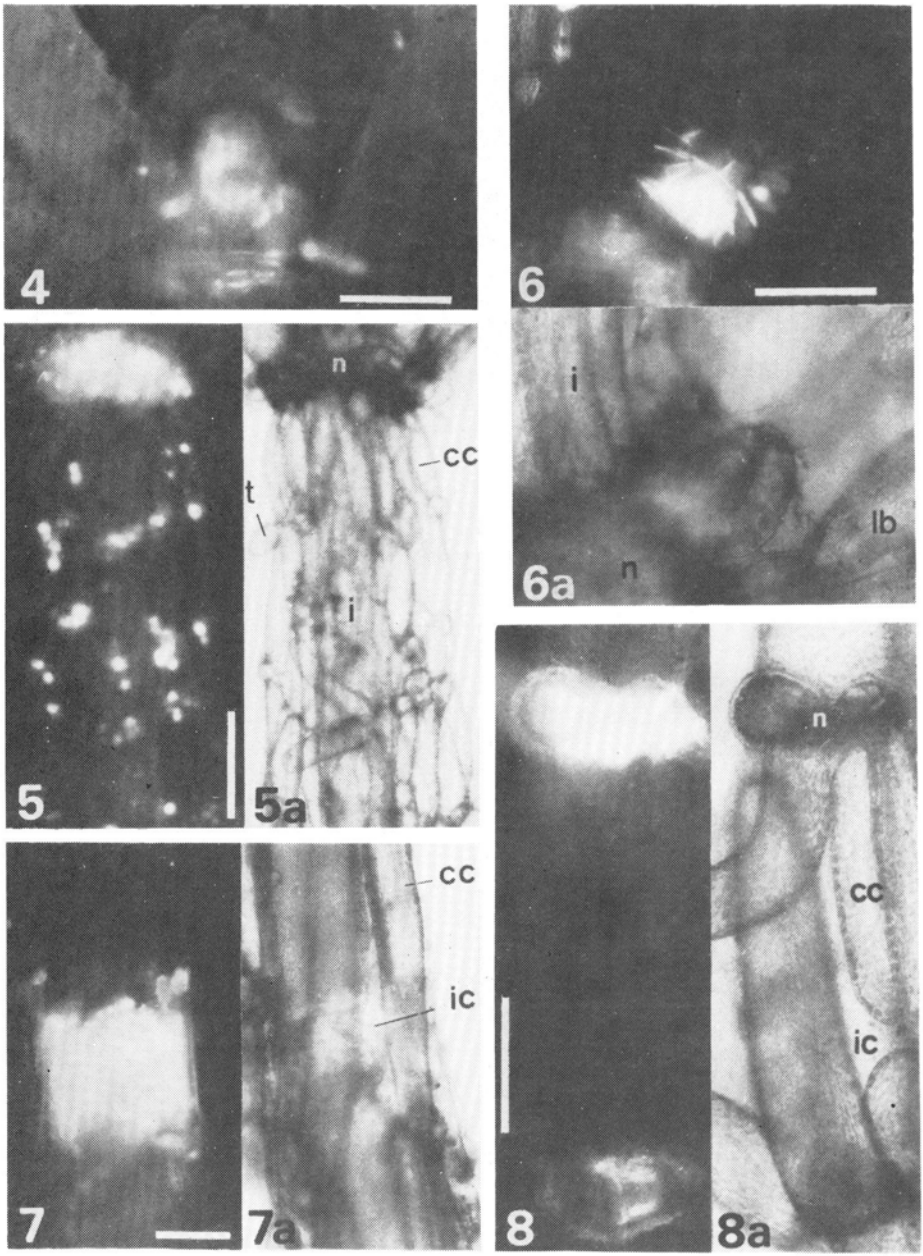
Damage of antheridia at successive developmental stages (that is from initial cell to the state of maturity) showed that both generative and nongenerative cells can synthesise callose.

In early stages of development in which the antheridium consists of barely several cells, squashing of external cells led to callose deposition in the wall connecting them with the internally lying cells. In the 2-cell stage delicate pressure on the external cell caused callose deposition in the wall connecting this cell with the one which is to become the basal cell (Fig. 22) and in the wall connecting the basal cell with the node cells (Fig. 18).

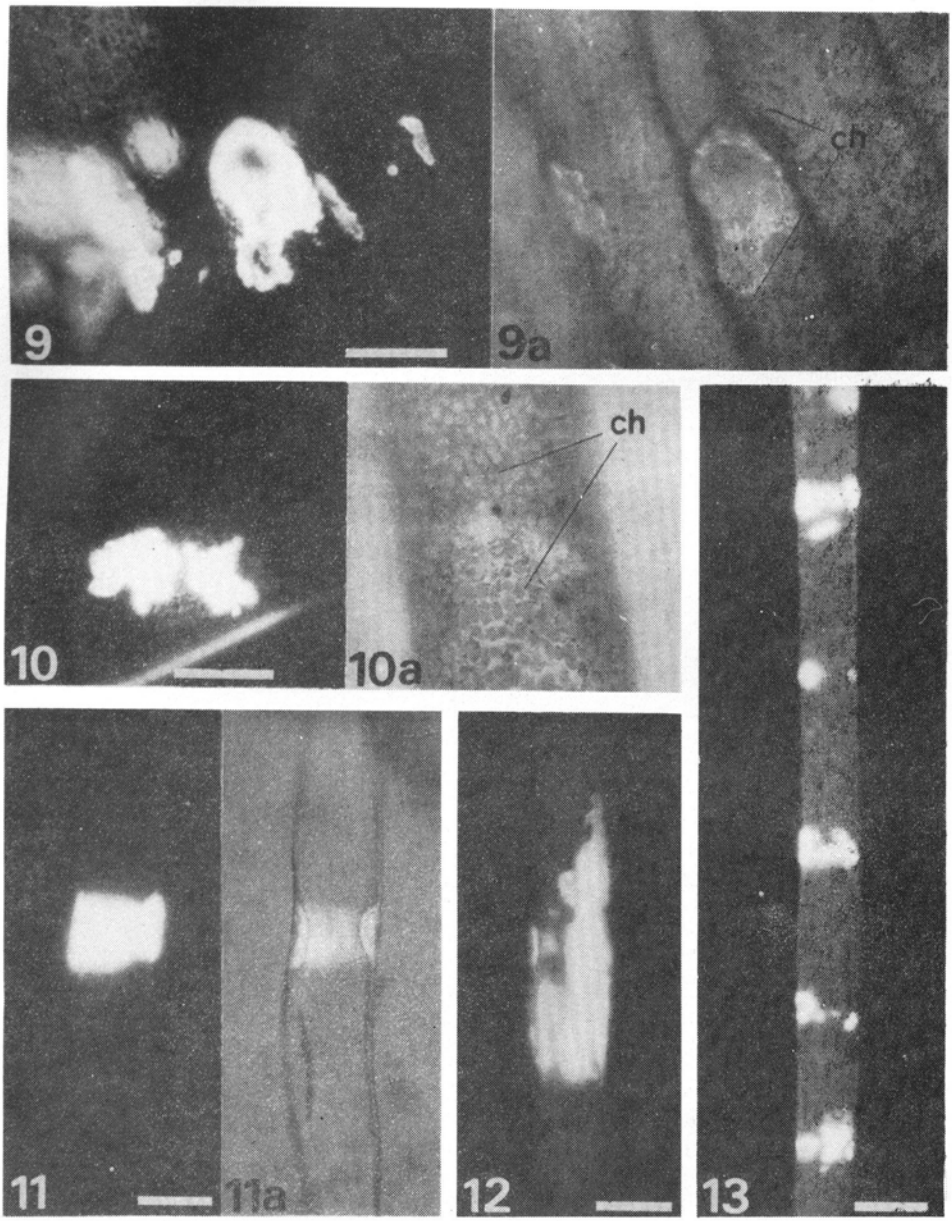
Crushing of formed antheridium causes breaking off of the manubria from the shield cells surrounding the interior of the antheridium. Owing to this, the manubrium together with the capitular cells and antheridial filaments flow out



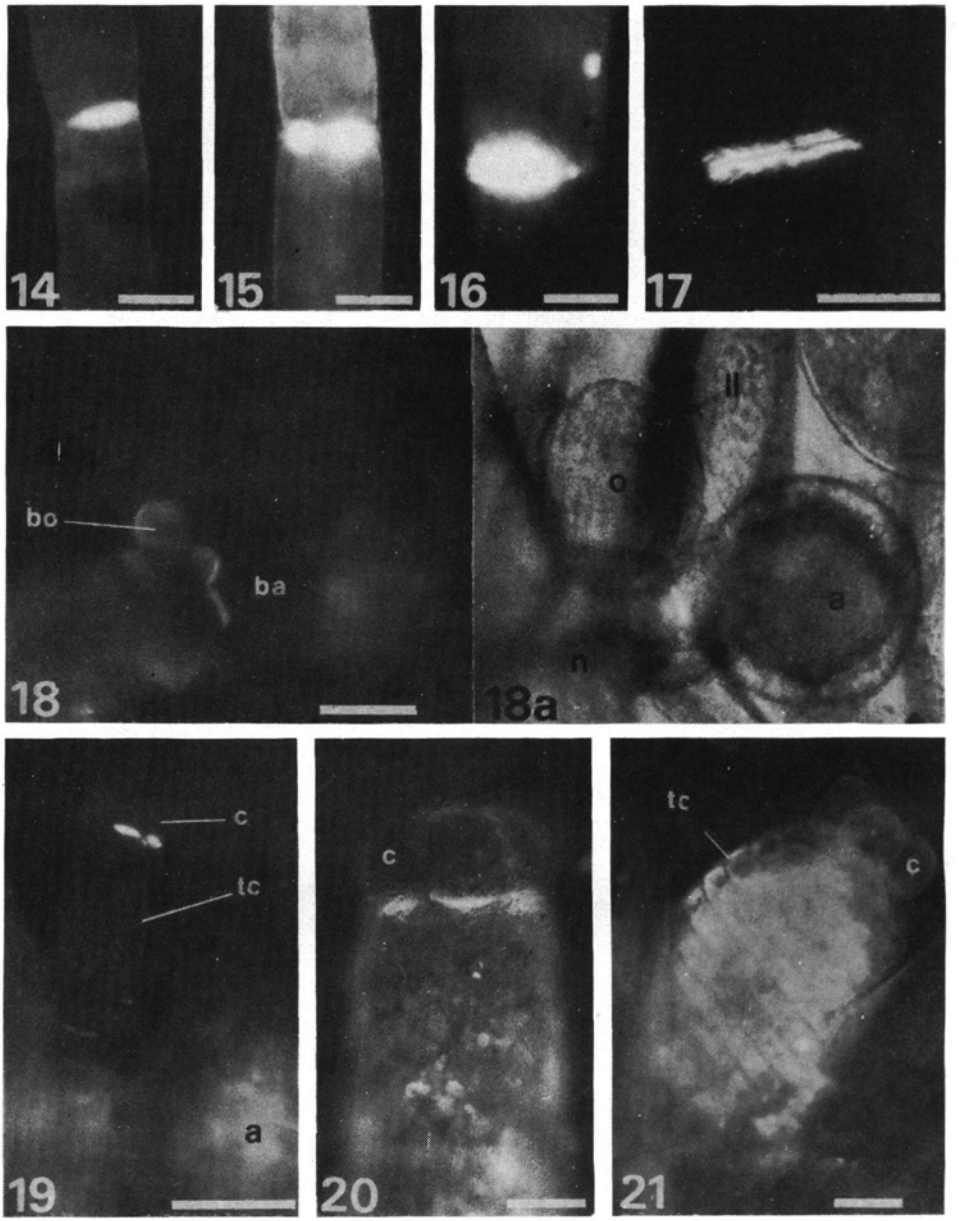
Figs. 1-3. Material fixed in ethanol-acetic acid mixture. Lack of callose fluorescence after aniline blue staining. Bars = 0.5 mm, × 29. Fig. 1. Apical part of thallus with apical bud and with lateral branches bearing generative organs at early developmental stages. Fig. 2. Lateral branch with generative organs at advanced developmental stage. Fig. 3. Fragment of lateral branch with antheridial filaments, capitular cells and manubria squeezed out of an antheridium. n – node, i – internode, ab – apical bud, a – antheridium, o – oogonium, lb – lateral branch, ll – leaflet, cc – cortical cells



Figs. 4-8a. Thallus cells from the vegetative system (damaged). Fig. 4. Apical bud of thallus. Bar = 50 μm, × 300. Figs. 5 and 5a. Node and internode of the upper part of the "stem". Bar = 100 μm, × 140. Figs. 6 and 6a. Lateral bud. Bar = 100 μm, × 160. Figs. 7 and 7a. Internode of lower part of the "stem". Bar = 100 μm, × 90. Figs. 8 and 8a. Internode and lateral branch nodes. Bar = 100 μm, × 180. Figs. 5a, 6a, 7a and 8a. Specimens photographed without excitation filter. n — node, i — internode, ic — internodal cell, cc — cortical cell, t — thorn, lb — lateral branch



Figs. 9-13. Thallus cells from vegetative system (damaged). Figs. 9 and 9a. Cortical cells of "stem" internode. Bar = 100 μm, × 130. Figs. 10 and 10a. Fragment of lateral branch cell without cortex. Bar = 100 μm, × 130. Figs. 11-13. Fragments of rhizoid cells. Bar = 100 μm, × 165. Figs. 9a, 10a and 11a. Specimens photographed without excitation filter. ch – chloroplast



Figs. 14-17. Cell walls joining lateral branch cells without cortex (damaged). Figs. 14-16. Bars = 100 µm, × 100. Fig. 17. Bar = 100 µm, × 165. Figs. 18-21. Generative organs (damaged). Figs. 18 and 18a. Antheridium and oogonium at early developmental stage. Bar = 50 µm, × 260. Figs. 19-21. Oogonia in earlier and later stages of development. Fig. 19. Bar = 100 µm, × 160. Fig. 20. Bar = 50 µm, × 210. Fig. 21. Bar = 100 µm, × 90. Fig. 18a. Specimen photographed without excitation filter. n — node, a — antheridium, ba — basal cell of antheridium, o — oogonium, bo — basal cell of oogonium, ll — leaflet, c — crown cells, tc — tube cells

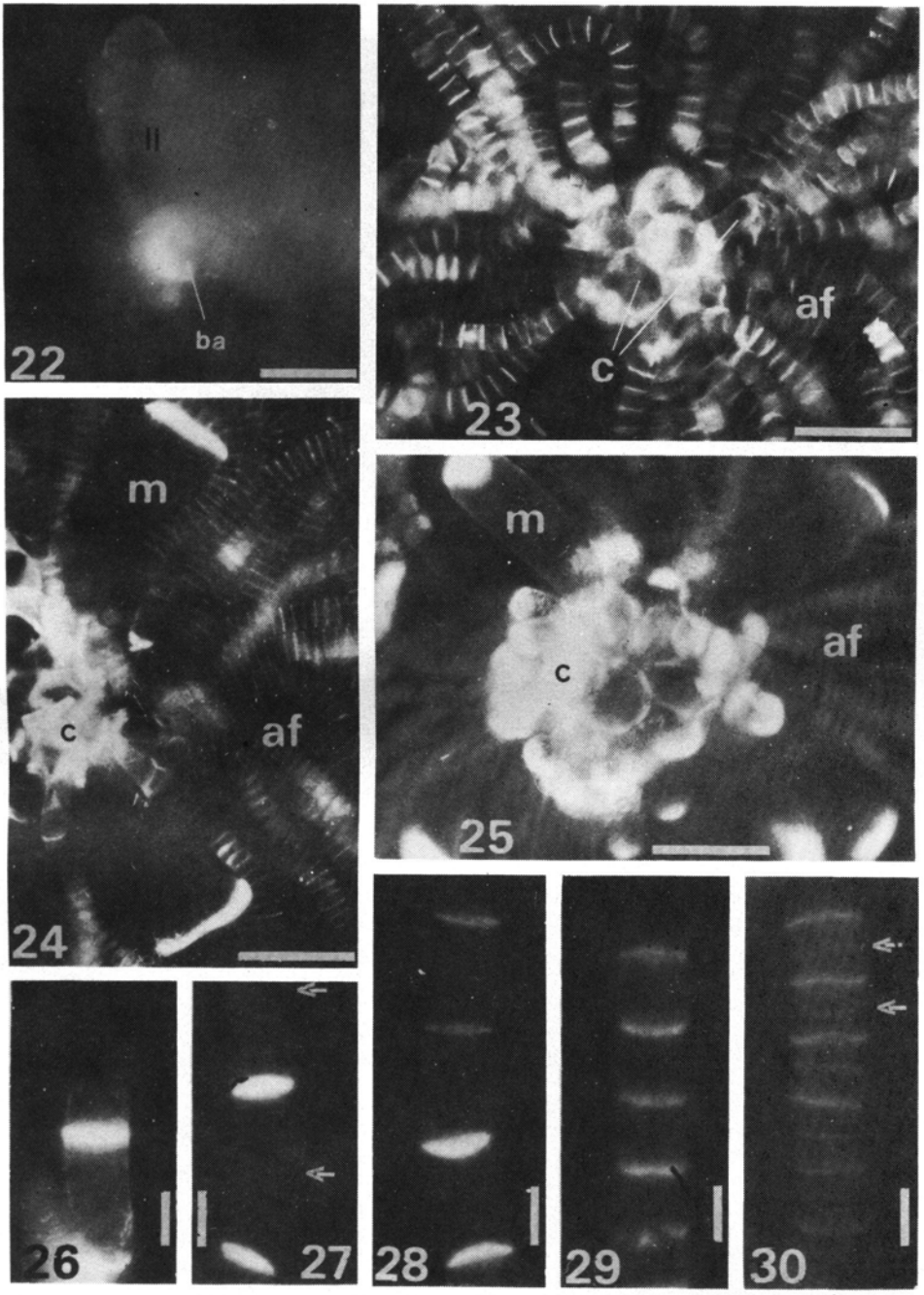


Fig. 22. Antheridium at 2-cell developmental stage (damaged). Bar = 50 μm, × 260. Figs. 23-30. Cells squeezed out of antheridium. Figs. 23-25. Bars = 50 μm, × 320. Figs. 26-30. Bars = 10 μm, × 750. Fig. 23. At the stage of 32-cell antheridial filament. Fig. 24. At spermatid stage. Fig. 25. At advanced stage of spermatozoid formation. Fig. 26. 2-Cell filament. Fig. 27. 4-Cell filament. Cells at early interphase. Fig. 28. 8-Cell filament. Asynchronous course of cell cycles. Fig. 29. 16-Cell filament. Cells with synchronous course of cell cycles. Fig. 30. 32-Cell filament. Cells at early interphase. ba – basal cell of antheridium, ll – leaflet, af – antheridial filaments, c – capitular cells, m – manubrium. Arrows point to the walls between sister cells

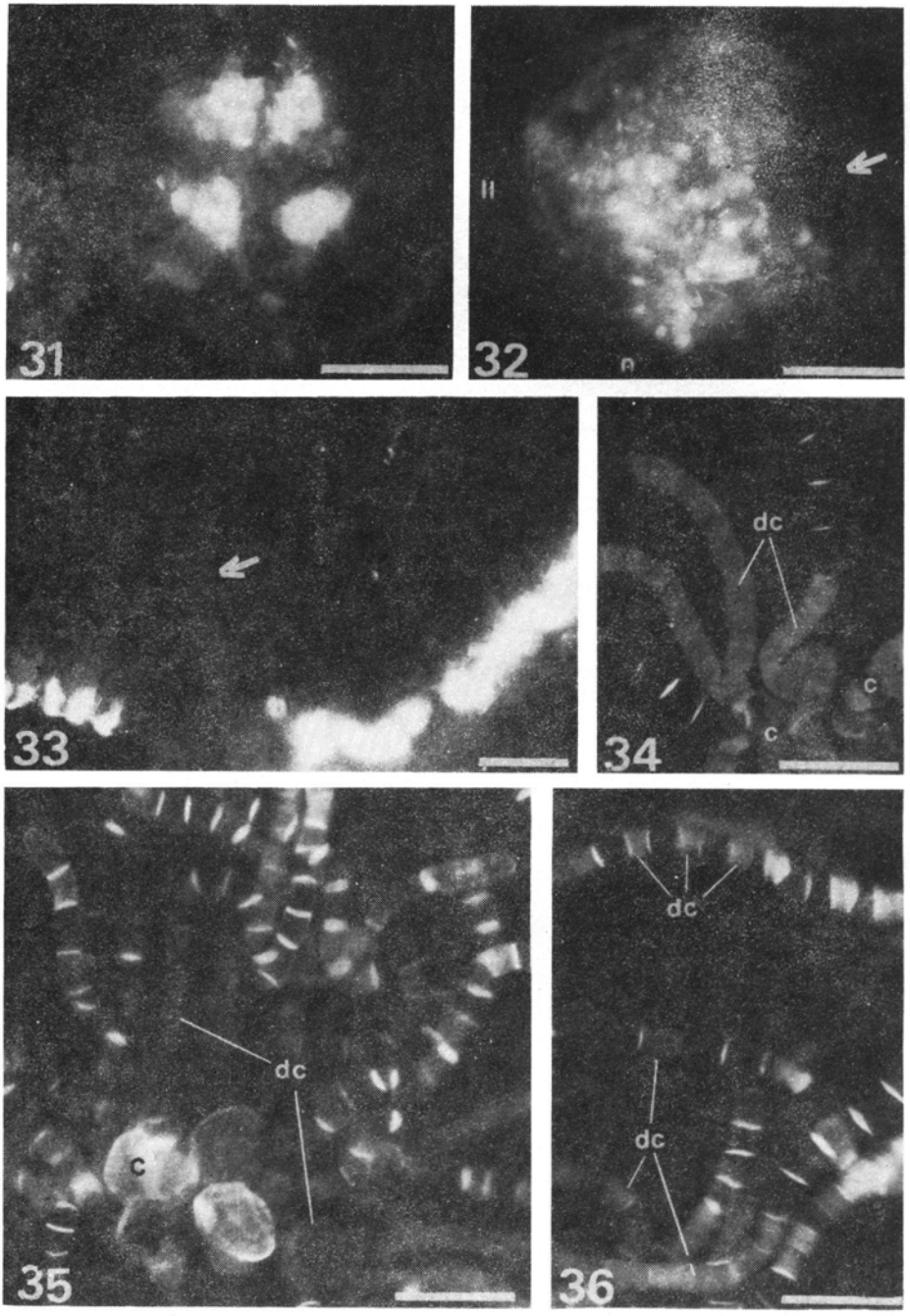


Fig. 31. Antheridium separated from thallus. Bar = 100 μm, × 170. Fig. 32. Antheridium after breaking of the shield cells. Arrow points to the place of breaking. Bar = 100 μm, × 170. Fig. 33. Shield cells after squashing antheridium. Arrow indicates cell walls not separated from each other. Bar = 50 μm, × 240. Figs. 34-36. Dying cells of antheridial filament about 20 min. after being squeezed out of antheridium. Bars = 50 μm, × 320 c – capitular cells, dc – dead cells, n – node, ll – leaflet

of the torn antheridium. In order to determine the callose formation capacity of these cells antheridia were crushed at the stage of initial cell formation of the antheridial filaments, antheridia in successive stages of development of these filaments to the stage of spermatids, and antheridia in the stage of spermatozoid formation. The manubrium and capitular cells were capable of callose formation throughout the whole period of spermatogenesis up to the moment of antheridium disintegration and release of mature spermatozoids (Figs. 23-25). Cells of the antheridial filaments lost this capacity in the advanced stage of spermatid transformation to spermatozoids – at the moment of shrinking of the protoplast and its detachment from the cell walls (Fig. 25).

In manubria, capitular cells and in the antheridial filaments the only place of callose deposition were the walls adjoining the cells. Callose deposits were not observed on the walls facing the intra-antheridial space. Thus, the manubrial cells formed thick callose layers on the wall joining them with the shield and capitular cells (Figs. 24 and 25). The capitular cells of the 1st, 2nd and 3rd order form a compact group localised in the central part of the antheridium. Callose deposition was observed on the walls joining these cells with one another and with those of the manubrium and antheridial filaments (Figs. 24 and 25). In the antheridial filament cells callose deposition was observed on the transverse walls adjoining the cells (Figs. 23-30). In the antheridial filaments with a synchronous course of cell cycle the thickness of the layers formed by all cells was similar (Fig. 29). A smaller amount of callose was deposited between sister cells in the initial part of interphase as compared with that deposited on the walls joining the mother cells (Figs. 27 and 30). In asynchronous filaments, that is those in which cells of one part of the filament differ in the stage of the cell cycle from those of the remaining part of the filament, differences in the thickness of the callose layer were noted (Fig. 28). The shield cells formed thick callose layers on the walls joining them with the neighbouring shield cells. This effect was only observed when these cells were separated from one another during crushing of the antheridium (Fig. 33).

Wide differences in the dimensions of antheridial cells allowed to follow the relation between the amount of deposited callose and the cell size. The largest shield cells form distinctly thicker layers of callose than the manubrial cells. The deposits of the latter were thicker than those of capitular cells and those of the capitular ones were larger that the deposits of relatively small cells such as those of the antheridial filaments. The size of the cells diminishes in the course of successive development phases of the filaments (Olszewska and Godlewski 1972). The amount of callose formed by the cells in the successive developmental stages of the filament from the 1-cell stage over 2-, 4-, 8-, 16-, 32- up to the 64-cell stage gradually diminished (Figs. 26-30, 23 and 24).

Callose formation in manubria and capitular cells and those of the antheridial filaments was also provoked without their squeezing out of the

antheridium. Delicate tearing away of the antheridium which causes destruction of the basal cell joining the node with the capitular cells inside the antheridium caused callose formation by the capitular cells and, with a certain delay, by those of the antheridial filaments and the manubrial ones (Fig. 31). Tearing of the shield cells without detaching the antheridium from the thallus triggered the "callose effect", first in the antheridial filament cells and the externally lying capitular ones (Fig. 32).

Living manubria, capitular and antheridial filament cells pressed out of the antheridium in distilled water or in an aniline blue solution, die within 15-30 min. The dead cells could be distinguished from living ones on the basis of the change in the protoplast structure and loss of turgidity. Dying of cells was accompanied by disappearance of deposits giving the fluorescence with aniline blue characteristic of callose. This process occured mostly in all filament cells. Disappearance of fluorescence was also noted in cells of parts of the filaments or filaments in which between dead cells living ones still remained (Figs. 34-36). Fading of fluorescence several minutes after the injury was also observed in the vegetative cells of the thallus. In the latter cells this disappearance mostly was not connected with symptoms indicating the dying of cells.

DISCUSSION

Among the mechanisms producing a change in the functioning of the genome in the period of initiation of cellular differentiation and dedifferentiation the possibility of periodical isolation of the cells is considered. Many authors point to callose deposits which on the cell walls might fulfil this isolating function. These sugestions refer mainly to the periodical appearance of callose in sporo- and gametogenesis of many sporulating and seed plant species (cf. Gabara 1977, Rodkiewicz 1981, Rodkiewicz et al. 1982). In somatic cells, apart from the known effect of callose appearance as reaction to injury (cf. Gabara 1977), formation of this polysaccharide may be considered in connection with the resistance of plants to infection with pathogenic fungi (Beckman et al. 1982, Hinch and Clarke 1982, De Leeuw 1985), reduced permeability of the seed coat to water (Bhalla and Slattery 1984). Callose was also found in the period of cell plate formation and the period preceding mineralisation and lignification etc. of cell walls (cf. Gabara 1977). The quoted papers point to the diversity of situations in which callose deposition was observed. In spite of accumulation of numerous observations, the role of callose is still under discussion. However investigations in which it has been demonstrated that cellose deposits do not function as a molecular sieve (cf. Gabara 1977) speak against the above mentioned concept of "a callose barrier". The controversy concerning the role of callose is enhanced by

difficulty in unequivocal exclusion of the possibility of its appearance as a reaction to injury.

In the present study the readily fixed thallus of the alga *Chara vulgaris* was investigated. Analysis of the extensive material comprising differently developed plants did not demonstrate the presence of callose in any type of cells of the vegetative system of the thallus. Formation of this polysaccharide was not associated with initiation, development and maturation of generative organs. No callose has been found in the cell plate of dividing cells of the antheridial filaments, although it has been found beyond doubt in the cell plate of angiosperms (Fulcher et al. 1976, Geuns-Longly and Waterkeyn 1976).

Induction of yellow-green fluorescence with aniline blue is considered as specific of callose (Eschrich and Currier 1964, Smith and McCully 1978). This method, however, does not reveal its form bound with proteins (Reynolds and Dashek 1976). Damage of the cells causes deposition on the cell walls of a substance with fluorescence characteristic of callose. It seems but little probable that one type of callose would be associated with cytodifferentiation and another would appear as the result of injury to cells. The negative result of search for callose in fixed material is not due to the fixation procedure since the fixatives applied preserved callose in other plants. Thus the investigations performed, seem to contradict the participation of this polysaccharide in processes connected with cell differentiation in *Chara vulgaris*. Injury of cells from both the vegetative and generative parts of the thallus causes formation of callose deposits. Disappearance of these layers after a certain time or with death of the cells is the evidence, that in *Chara* all cells posses not only enzymes participating in synthesis, but also those decomposing callose. Localisation of the callose layers depends on the cell type and the way in which they are injured. Local damage of the cells caused formation of callose patches at the site where the wall was damaged. In the cells of the internode cortex and cortex-free ones of the lateral branches protoplasts remain in permanent localisation close to the cell walls and are stabilised by actin filaments (Kersey and Wessells 1976). In these cells a network pattern was observed in the initial phase of callose patch formation, which corresponded to the interchloroplast spaces. This way of callose deposition suggests its transport in vesicles formed by the Golgi apparatus or endoplasmic reticulum (cf. Gabara 1977), and is the evidence against callose synthesis in the periplasmic space (Arsanto and Coulon 1975). For translocation of the vesicles chloroplasts would be a mechanical obstacle.

Pressing with a coverslip of various parts of the *Chara* thallus led to callose deposition only on the walls which according to numerous observations (Pickett-Heaps 1967, 1968, Fischer et al. 1974, Kwiatkowska and Maszewski 1985, 1986) contain plasmodesmata. Localisation of callose deposits induced by pressure on whole cells corresponds to the pattern of plasmodesmatal connections in the thallus. These observations confirm the

generally accepted view, according to which callose blocks the plasmodesmata and thus isolates the symplast from the injured part of the plant (cf. Gabara 1977).

Acceptance of such an interpretation of the role of callose in the reaction to damage of *Chara vulgaris* cells does not explain however: a) formation of frequently thick deposits on only part of the walls containing plasmodesmata; b) the wide differences in deposit thickness in cells in the same situation, for instance those in the antheridium interior; c) appearance of callose deposits when the cells were not subjected directly to injury, for instance cells of the antheridium interior after delicate detachment of these organs from the thallus or tearing of the antheridium cover.

In the antheridia of *Chara* a gradient of water potentials may be expected as in higher plants. This potential is probably lower in the cells of the antheridium interior, that is the antheridial filaments, capitular and manubrial cells as compared with that of the antheridial mucilage, whereas the potential of the latter is lower than that of the environmental water. Crushing of the shield cells forming the envelope of the antheridium leads to a sudden rise of the water potential of the environment of the internal cells of the antheridium. Saccharides are known to play an essential role in regulation of cell water potentials. The occurence in this situation of the "callose effect", consisting in glucose polymerisation and callose formation would lead to a rise of the cell water potential and to a reduction of the difference in these potentials. A similar interpretation is suggested by the observation of the chronology of the callose formation process in the cells inside the antheridium after detachment of these organs from the thallus, and also callose formation in all the cells pressed out of the antheridium, although only part of them were exposed to mechanical damage. On the other hand, callose formation was not observed in advanced stages of spermatid transformation to spermatozoids – these cells lose their turgidity and sensitivity to change of the environmental water potential as the shrinking of protoplast begins. The reduction of the amount of callose in the sieve elements with increasing distance from the site of the injury (Northcote and Wooding 1965) seems to confirm the suggestion that its synthesis may serve for diminishing the difference in the water potentials between the interior of the cells and their environment.

In in vitro tissue cultures the amount of callose formed by sieve elements was larger when saccharose concentration in the medium was higher (Młodzianowski and Szweykowska 1966, Aloni 1980). The different size of callose deposits in the injured cells of *Chara* may suggest varying saccharide concentration in the cells. The distinct dependence between the thickness of the callose layers and the cell dimensions supports the participation of callose in regulation of the water potential.

Experiment, in which callose deposition was provoked by crushing of the

cells or injury leading to a sudden rise of the water potential of the extracellular environment suggest, that a sudden rise of tension in the cells may be a factor triggering the "callose effect".

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REFERENCES

Aloni R., 1980. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta 150: 255-263.

Arsanto J. P., Coulon J., 1975. Application des méthodes cytochimique et radio-autographique de détection ultrastructurale des polysaccharides à l'étude de la différentiation des plateaux criblés du métaphloème caulinaire de deux Cucurbitacées voisines (*Ecballium elaterium* R. et *Cucurbita pepo* L.). C. R. Acad. Sci. Paris 280: 601-604.

Beckman C. H., Mueller W. C., Tessier B. J., Harrison N. A., 1982. Recognition and callose deposition in response to vascular infection in *Fusarium* wilt-resistant or succeptible tomato plants. Physiol. Plant Pathol. 20: 1-10.

Bhalla P. L., Slattery H. D., 1984. Callose deposits make clover seeds impermeable to water. Ann. Bot. 53: 125-128.

Currier H. B., 1957. Callose substance in plant cells. Amer. J. Bot. 44: 478-488.

De Leeuw G. T. N., 1985. Deposition of lignin, suberin and callose in relation to the restriction of infection by *Botrytis cinerea* in ghost spots of tomato fruits. Phytopath. Z. 112: 143-152.

Eschrich W., 1954. Ein Beitrag zur Kenntnis der Kallose. Planta 44: 532-542.

Eschrich W., Currier H. B., 1964. Identification of callose by its diachrome and fluorochrome reactions. Stain. Technol. 39: 303-307.

Fischer R. A., Dainty J., Tyree M. T., 1974. A quantitative investigation of symplasmic transport in *Chara corallina*. I. Ultrastructure of the nodal complex cell walls. Can. J. Bot. 52: 1209-1214.

Fulcher R. G., Mc Cully M. E., Setterfield G., Sutherland J., 1976. β-1,3-glucans may be associated with cell plate formation during cytokinesis. Can. J. Bot. 54: 539-542.

Gabara B., 1977. Kaloza w komórkach roślinnych w świetle nowych badań. Post. Biol. Kom. 4: 279-302.

Geuns-Longly B., Waterkeyn L., 1976. Les étapes callosiques de la plaque cellulaire dans la mitose somatique chez *Hyacinthus orientalis* L. C. R. Acad. Sc. Paris 283: 761-763.

Hinch J. M., Clarke A. E., 1982. Callose formation in *Zea mays* as a response to infection with *Phytophthora cinnamoni*. Physiol. Plant Pathol. 21: 113-124.

Kersey Y. M., Wessells N. K., 1976. Localization of actin filaments in internodal cells of *Characean algae*. A scanning and transmission electron microscope study. J. Cell. Biol. 68: 264-275.

Kwiatkowska M., Maszewski J., 1985. Changes in ultrastructure of plasmodesmata during spermatogenesis in *Chara vulgaris* L. Planta 166: 46-50.

Kwiatkowska M., Maszewski J., 1986. Changes in the occurrence and ultrastructure of plasmodesmata in antheridia of *Chara vulgaris* L. during different stages of spermatogenesis. Protoplasma 132: 179-188.

Młodzianowski F., Szweykowska A., 1966. Histogenesis in the tissue culture of carrot

(*Daucus carota* L.). III. Distribution of callose. Bull. Soc. Amis Sci. Poznań, Série D: 3-14.

Northcote D. H., Wooding F. B. P., 1965. Development of sieve tubes in *Acer pseudoplatanus*. Proc. Roy. Soc. B 163: 524-537.

Olszewska M. J., Godlewski M., 1972. An autoradiographic study of the nucleic acids and protein synthesis during the cell cycle of synchronously dividing antheridial filaments in *Chara vulgaris* L. Folia Histochem. Cytochem. 10: 245-256.

Pickett-Heaps J. D., 1967. Ultrastructure and differentiation in *Chara* sp. I. Vegetative cells. Aust. J. Biol. Sci. 20: 539-551.

Pickett-Heaps J. D., 1968. Ultrastructure and differentiation in *Chara* sp. III. Formation of the antheridium. Aust. J. Biol. Sci. 21: 255-274.

Reynolds J. D., Dashek W. V., 1976. Cytochemical analysis of callose localization in *Lilium longiflorum* pollen tubes. Ann. Bot. 40: 409-416.

Rodkiewicz B. 1981. Megasporogenesis in angiosperms (a retrospect). I. Callose distribution. Folia Soc. Sci. Lublin 23 biol. 2: 109-114.

Rodkiewicz B., Kudlicka K., Kuraś M., 1982. Callose and osmiophilic material deposition into walls of developing megagametophyte in *Stellaria*. Acta Soc. Bot. Pol. 51: 353-359.

Smith M. M., McCully M. E., 1978. Enhancing aniline blue fluorescent staining of cell wall structures. Stain Technol. 53: 79-85.

Tworzenie kalozy w uszkadzanych komórkach systemu wegetatywnego i generatywnego plechy Chara vulgaris L. Brak kalozy w procesie cytodyferencjacji

Streszczenie

U ramienicy *Chara vulgaris* L. procesom deferencjacji komórek systemu wegetatywnego plechy, a także inicjacji i rozwojowi organów generatywnych nie towarzyszy tworzenie kalozy. Uszkadzanie komórek wykazało, że wszystkie komórki somatyczne, a także komórki rozrodcze i nierozrodcze plemni i lęgni, niezależnie od stadium rozwoju, są zdolne do tworzenia pokładów kalozowych. Lokalizacja i rozmiary tych pokładów są zależne od sposobu uszkadzania i rozmiarów komórek. Ochronna rola kalozy w uszkadzanych komórkach oprócz tworzenia łat kalozowych wzmacniających uszkodzoną ścianę, czy też ochrony symplastu przez tworzenie pokładów kalozowych na ścianach z plasmodesmami, może także polegać na podwyższeniu potencjału wodnego komórek. Doświadczenia, w których tworzenie pokładów kalozowych prowokowano przez ugniatanie komórek lub przez uszkodzenia prowadzące do nagłego podwyższenia potencjału wodnego środowiska zewnątrzkomórkowego sugerują, że nagłe podwyższenie ciśnienia w komórkach może być czynnikiem wyzwalającym „reakcję kalozową".