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# Rape embryogenesis

## I. The proembryo development

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Abstract

The development of the proembryo of rape *Brassica napus* L. from the zygote to the young embryo proper is described. A number of regularities were found in the direction, succession, and distribution of segmental and differentiating divisions of the proembryo. The direction of the divisions seems to be determined by the direction of growth and the shape of the cells. The term — young embryo proper is proposed to denote the globular embryo which already possesses separate plerome and periblem mother-cells and mother-cells of the *iec* layer and of columella. The body of the embryo proper is derived from the apical cell *ca* which arose from the first division of the zygote and from the hypophysis — the only suspensor cell which closes the spheroid of the embryo. The development of the *Brassica napus* L. proembryo follows the sub-archetype *Capsella bursa-pastoris* in the IV megarchetype of Soueges.

## INTRODUCTION

In the present work the development of the proembryo of rape from the zygote to the young globular embryo proper was investigated. Some regularities were found in the direction, succession and distribution of segmental and differential divisions of the proembryo.

The author was prompted to study this subject by results of research of M. K u r a ś which were carried out in the Group for Plant Morphogenesis of the Institute of Botany, Warsaw University. K u r a ś proved that in different stages of development of the embryonal rape root, on median longitudinal sections, the distinctly outlined transversal boundaries between the cells families i.e. the groups of cells of common origin would

be distinguished in the cortex. In the neighbouring layesr of cells these boundaries usually lie at different levels. Nevertheless, in some parts of the root they converge, forming a common boundary across several layers, and sometimes across the whole cortex. The boundaries, running across the whole cortex are the boundaries of segments separated from initials. In the dermatogen there are analogous segments. K u r a ś (1974) examined the course of growth of the apical root meristem during germination of the seeds, comparing the homologous segments of the dermatogen.

## MATERIAL AND METHODS

Investigations were carried out on two varieties of rape (*Brassica napus* L.): Młochowska (spring variety) and Górczańska (winter variety) cultivated in the Institute of Plant Breeding and Aclimatization, Radzików, Poland.

No differences in the embryo development between both varieties were found, except some single cases of intensified growth of the suspensor and rootcap in the winter variety.

The material was fixed in CrAF — 0.5-0.5-20, embedded by the paraffin method, and cut on a rotary microtome into 7.5 μm and 5 μm sections.

The slides were stained with Ehrlich hematoxylin, iron hematoxylin, toluidine blue, alcian blue, safranin, fast green and the PAS method.

The schematic drawings were made on the basis of microscope observations and photographs taken in a Zetopan (Reichert) Microscope with an Exacta-Varex camera.

## OBSERVATIONS

### 1. First division of zygote

The zygote is an elongated and distinctly polarly differentiated cell. The nucleus lies in the strongly basophilic dense cytoplasm at the apical end. The cytoplasm in the basal part is weakly basophilic and vacuolated.

The first zygote division is a differential division. The zygote divides transversally, cutting off at finger-like elongated basal cell *cb* and a small semispherical apical cell *ca* (Fig. 1 : *1*). The cytoplasm of the latter is strongly basophilic (Photo. 1). Form this cell the "body of the proembryo" (C r e t é, 1963) will be formed. The basal cell *cb* is several times long as than the apical one (Fig. 1 : *1—4*). It has a weakly basophilic vacuolated cytoplasm and a large nucleus. From this cell the suspensor, and hypophysis, will be formed. The cell wall between *ca* and *cb* will be termed the *"O boundary"* in the further description. It is easy to identify

in all stages of embryo development. The length of the two-celled proembryo is at first 44 μm. Before the next division the proembryo elongates to about 100 μm (Fig. 1 : *1*—*4*). This takes place mainly at the cost

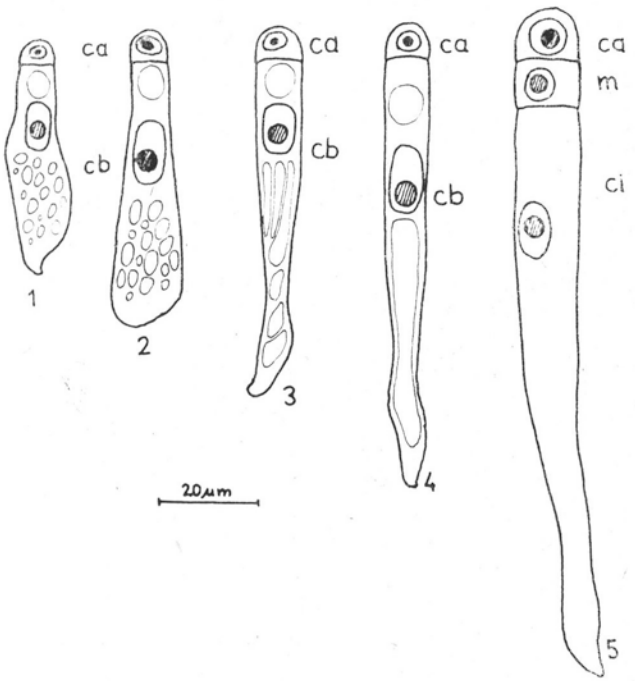



Fig. 1. First division of zygote

of the basal cell. Its small vacuoles in the micropylar part fuse forming a single large vacuole (Photo. 1). The apical cell enlarges too. Its length increases from 4—5 to 7—7.5 μm and the width from 6—7 to 7—8 μm. (Fig. 1 : *2*—*4*).

The next transversal proebryo division appears in the basal cell *cb*, dividing it into *m* and *ci* (Fig. 1 : *5*, Photo. 2). This division is also unequal and differential. The cell *ci* is very elongated (in tree-celled proembryo about 130 μm long — Fig. 1 : *5* — 110 μm falls to *ci*). The massive end of cell *ci*, of the shape of a foot or horse hoof (Fig. 1 : *3*—*5*; Fig. 2 : *2*; Fig. 3 : *2*, *5*) sticks in micropylar part of the ovule (Photo. 6). In the further development of the proembryo divisions of cell *ci* were not observed.

## 2. Formation of quadrants

During the already described changes in the basal cell the apical one still enlarges, but its height never exceeds the width (in the preparation shown in Fig. 1 : *5* this cell is 10 μm high and 12 μm wide). That is why

the first division of cell *ca* runs always in vertical direction (Fig. 2 : *1*, *2*).

In the stage of two-celled *ca* the suspensor usually consists of three

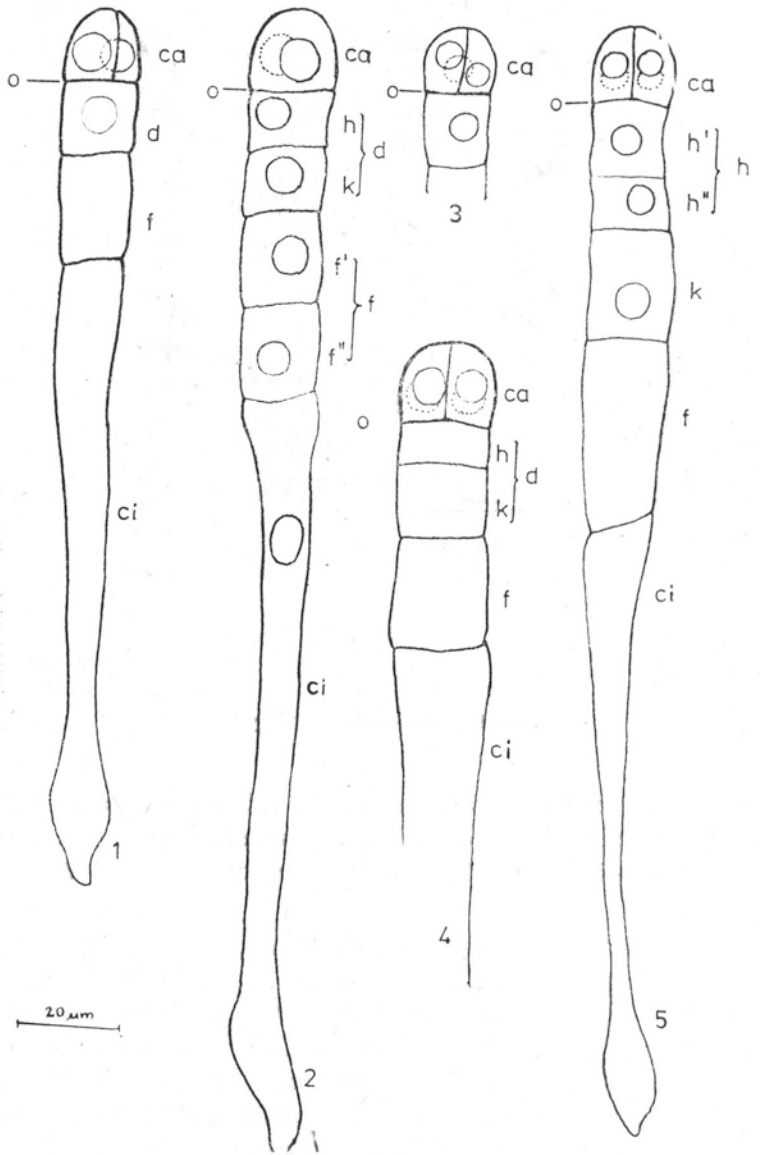



Fig. 2. Formation of quadrants

cells, as the result of division of *m* to *d* and *f* (Fig. 2 : *1*). Still in the same stage the acceleration of development of *cb* can lead to the formation of a 5-celled suspensor by the division of *d* and *f* (Fig. 2 : *2*).

All the cells of the proembryo enlarge continnously and the cell *ci*

elongates still more. The dome-like apical part *ca* which now consists of two cells begins to grow upward and soon its height is equal in size to its base, i.e. with to the *O* boundary (Fig. 2 : *1* : *O* boundary is 14 μm long, *ca* height is 14 μm). Then both cells start to swell (Fig. 2). Their exterior walls bulge outward in such a way that *ca* is widest not at its base, but half way up its height (Fig. 2 : *3* height *ca* 13 μm, width at the base 14 μm, maximum width 15 μm). Owing to such a shape of the apical part, the next division also occurs always in vertical direction (Fig. 2 : *3*—*5*). Four identical quadrants are formed. The division of both *ca* sister cells may be non-simultaneous (Fig. 2 : *3*). The contact line of four quadrants will be called the main (central) axis of the embryo in the fur[illegible]r description.

After the quadrants are formed, the extenal walls [illegible] the cells grow quicker than the central axis, so the *O* boundary run[illegible]g up till now in one plane curves, and the suspensor cell adjacen[illegible] *ca* penetrates somewhat into the proembryo's body (Fig. 2 : *4*, *5* ar[illegible] to. 3).

In this stage the suspensor usually consist[illegible]r (Fig. 2 : *4*), sometimes of five cells (Fig. 2 : *5*). In the devel[illegible]f the suspensor the speeded up division of the cells lying at t[illegible]part is characteristic. Therefore, the suspensor may already [illegible]ve cells before the quadrant-stage (Fig. 2 : *2*).

## 3. Formation of octants

Before divisions of the quadrants into octants, their height increases considerably and during the divisions is nearly twice their width. Their average height is 17 μm, their width at the base 8.75 μm and at the widest place 9.5 μm (Fig. *3* : *1*—*4*).

The quadrants divide transversally. This division segregates every quadrant into two equal cells, probably of the same volume, but different in shape and position. The cells usually divide simultaneously (Fig. 3 : *2*, *3*). Acceleration (Fig. 3 : *1*) or retardation (Fig. 3 : *3*, *4*) of division of one of the cells may occur. In this way two layers of different octants *1* and *1'* (Photo. 4) with dissimilar destination, arise. The cotyledonary region and embryo stem tip will be formed from layer *1*, and the hypocotyl and radicule from layer *1'*. The boundary between them, marked *O'*, is formed by the transversal walls of quadrants. It does not lie strictly in one plane, because these walls are always slightly shifted in relation to one another in the adjacent quadrants (Fig. 3 : *2* and 4 : *1*—*3*).

During the formation of octants the suspensor may still consist of four cells (Fig. 3 : *2*, *5*) as it did in the quadrant-stage. But in the final phase of the octant-stage the mother-cell of hypophysis *h'*, that is the fourth

generation of *cb* separates (Fig. 3 : *6*). The suspensor then usually consists of six cells (Fig. 3 : *4*).

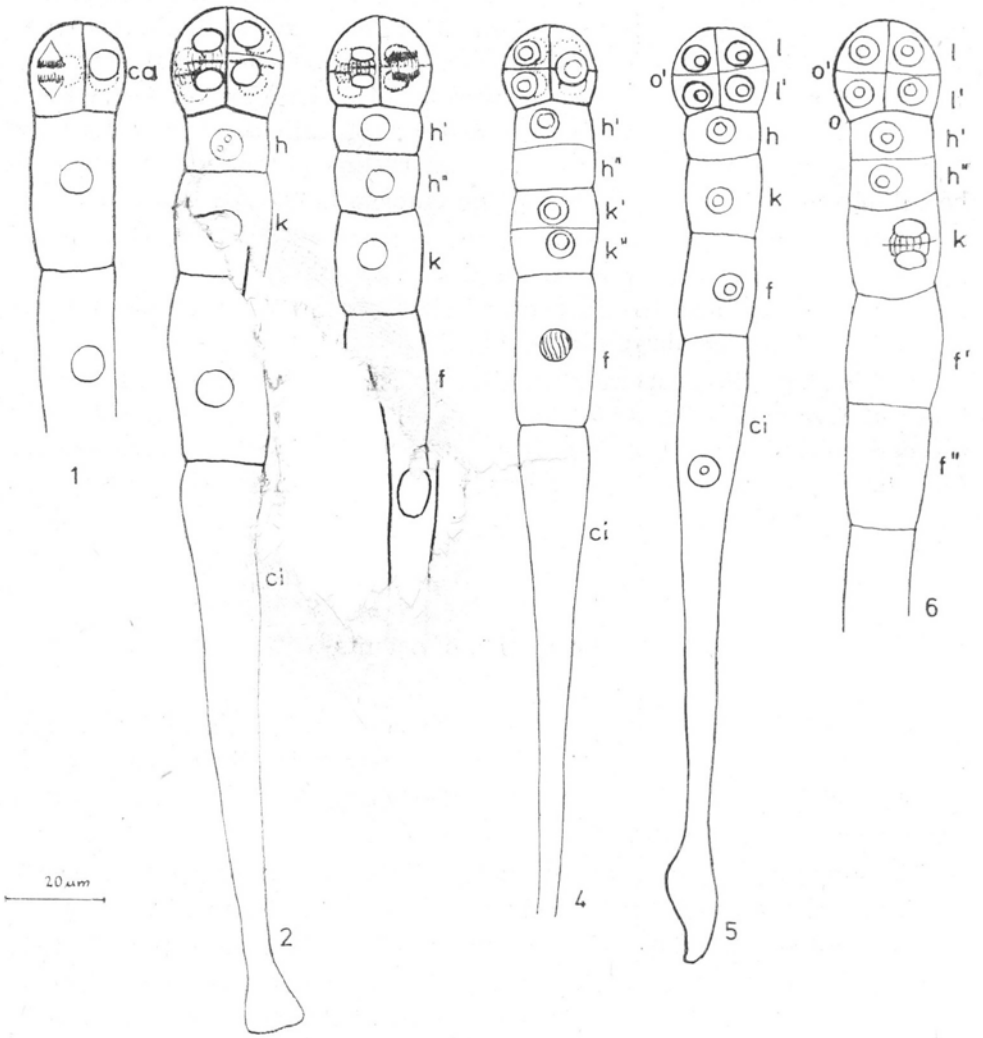
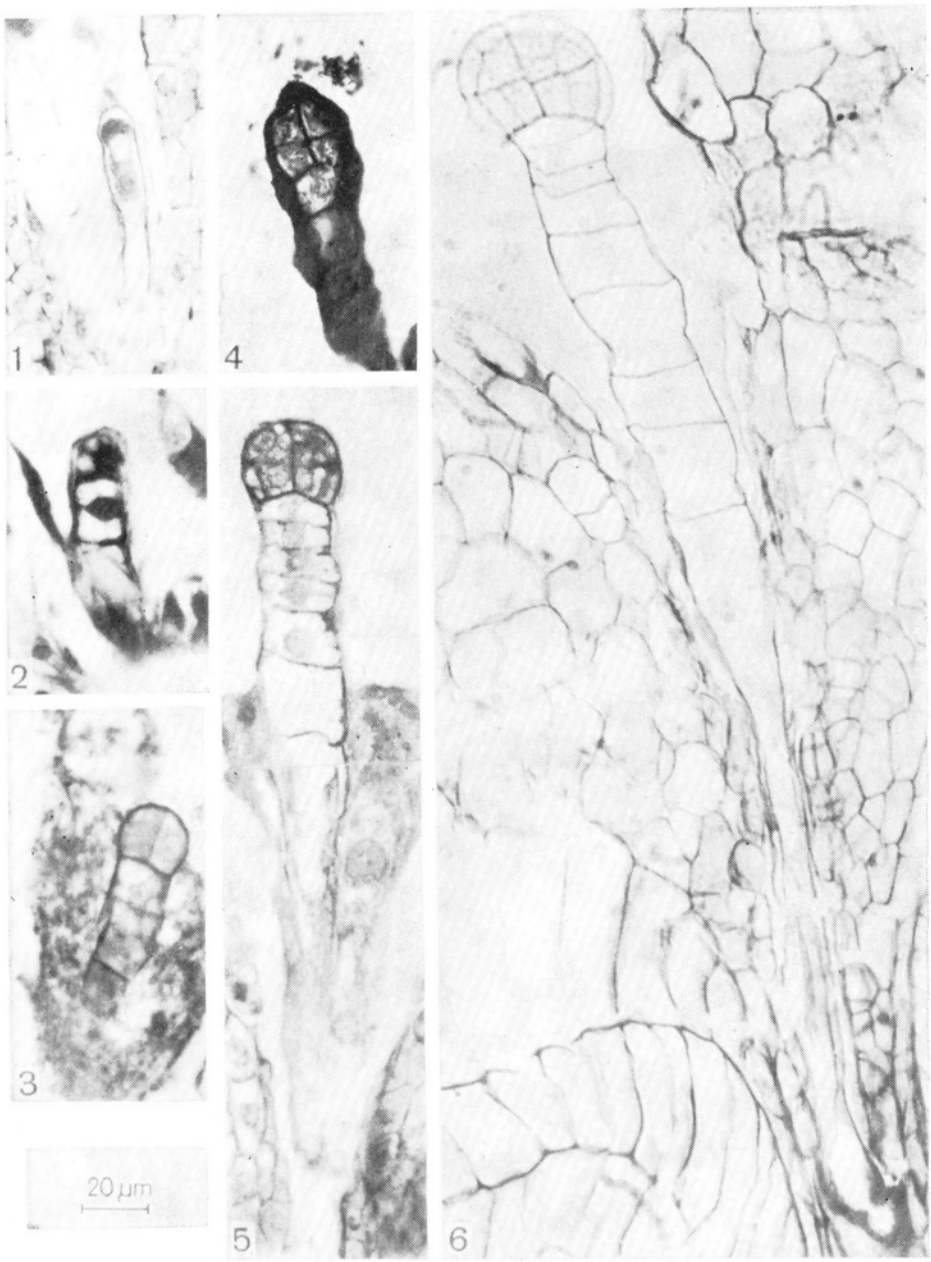



Fig. 3. Formation of octants

## 4. Differentiation of dermatogen and inner cells in octants

The apical region of the proembryo consisting of octants grows in radial direction and gradually becomes rounded (Fig. 3 : *2*—*6*). If it were closed with an arc at the bottom all the inner walls would be of nearly the same

Plate I




The development of the proembryo of rape (× 500)

Photo. 1. 2-celled proembryo, stained with Ehrlich hematoxylin. Photo. 2. 3-celled proembryo, stained with Ehrlich hematoxylin. Photo. 3. Quadrant stage, stained with PAS. Photo. 4. Octants stage, stained with PAS. Photo. 5. Proembryo with differentiated dermatogen, stained with Ehrlich hematoxylin. Photo. 6. Early globular embryo, stained with fast green.

length (in Fig. 4 : 2 three upper partition walls are 12 μm long and the axis of layer 1'—10 μm). The octants always divide parallely to the surface. In this way the dermatogen is formed (Photo. 5).

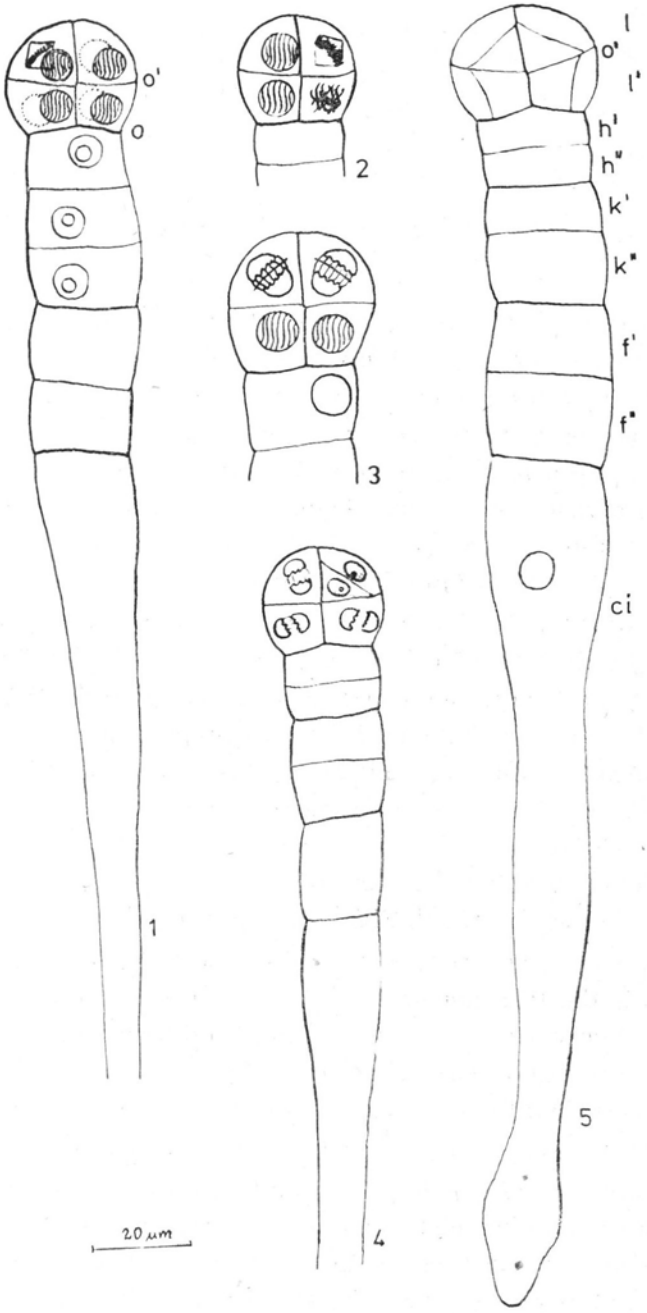



Fig. 4. Differentiation of dermatogen

The divisions of nuclei in the octants, leading to the formations of dermatogen, do not usually begin at the same time (Fig. 4 : *1*—*4*). However synchronization of the sister-cells divisions (Fig. 4 : *1*—*2*) or acceleration of the divisions in the upper layer of the proembryo (Fig. 4 : *1*—*4*) can be observed.

In this stage the suspensor usually consists of 7 cells (Photo. 5, Fig. 4 : 5).

## 5. Formation of hypophysis and further development of suspensor

The formation of hypophysis is preceded by the division of dermatogen cells. The development of the upper proembryo layer *1* usually outdistances (in 7 of 8 examined cases) the division in the lower layer *1'* (Fig. 5). This is connected with the somewhat quicker growth of layer *1* in this stage. In the proembryos with differentiated dermatogen, but before further cell division in the uper layer the average heigths of both layers are: $l = 11.6$ μm, $l' = 8.4$ μm, and after the first division of dermatogen or the inner cells $l = 13.3$ μm, $l = 7.7$ μm.

Most frequently the development of the upper layer (in 8 out 10 cases) begins by the division of dermatogen (Fig. 5 : *1*—*3*; Fig. 5 : *6* — division of dermatogen after division of inner cell). This is connected with a rather quick surface growth of layer *l*. The embryo grows not only along its axis, but also in equatorial plane. In the sixteen-celled "body of the proembryo", i.e. after differentiation of dermatogen its average width is 26.9 μm, and after the division of one of the dermatogen cells in *l* it is 30.0 μm. Sometimes the divisions of dermatogen and the inner cells of layer *l* may by synchronized. This refers to the sister-cells (Fig. 5 : *3*).

The hypophysis $h'_1$ appears after the first division of dermatogen (Fig. 5 : *2*—*4*), it is the fifth generation of the basal cell *cb* and most often the eighth cell of suspensor (Fig. 5 : *4*). When dividing periclinally, it gives rise to the mother-cells of two layers of cells of the embryo: to the central binding cells *iec* and the initials of the columella of the rootcap *co*.

After the hypophysis is formed the suspensor does not grow so quickly as before, but is does not lose the ability to divide. In the globular and heart stage the number of suspensor cells increases to 9, 10 or 11 and in the Górczańska variety even up to 14. The elongating suspensor begins to displace the embryo deep into the endosperm and probably supplies the nurient substances to the embryo from the micropylar part of the ovule.

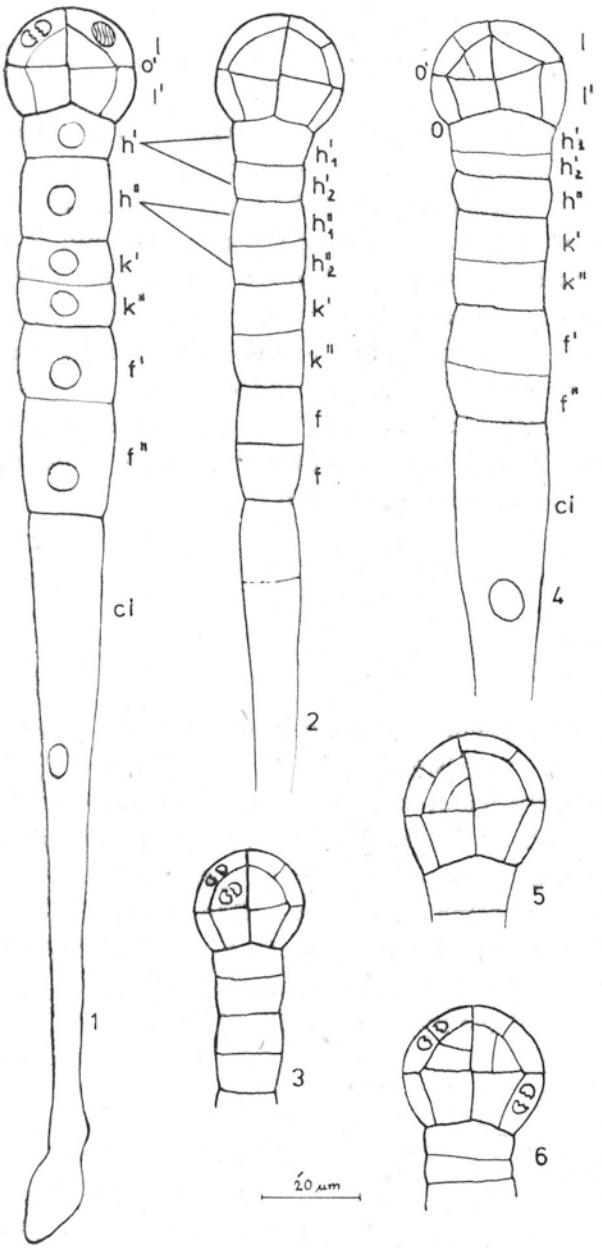



Fig. 5. First divisions in the 16-celled proembryo

## 6. Further development of the proembryo and formation of the embryo proper

### 6.1. Upper layer

The inner derivatives of octants enlarge before division. This is manifested by the considerable growth of, the proembryo in breath and by the fact, that the average height of the inner cells of the layer *l* is smaller than their width. The corresponding sizes are 11.2 μm and 13.3 μm. That is why the first cell division of layer *l* is usually vertical, i.e. more or less parallel to the central axis of the embryo (Fig. 5 : *3, 4, 6*). Sometimes it is horizontal, i.e. perpendicular to the embryo axis, when the hight of the cell exceeds its width (Fig. 5 : *6* and 6 : *3*), and oblique, i.e. parallel to the plane when both walls are of the same length (Fig. 5 : *5* height and width = 10 μm). Fourty two cases of vertical, nine of horizontal and three of oblique division have been observed.

### 6.2. Lower layer

After the first division of the inner cells of layer *l* (in extreme cases — before — Fig. 6 : *1*) divisions appear in the *l'* layer. The inner cells of layer *l'* are slightly wider than long. Their average height is 8 μm and their average width 8.6 μm. That is why the first division is always periclinal, forming outward the primary periblem cell- *per*, and inwards — the primary plerome cell — *pl* (Fig. 6 : *4*, Photo. 8). Then, the dermatogen divides anticlinally (Fig. 6 : *4, 5*). But the opposite may also occur (Fig. 5 : *6* and 6 : *5*). The dermatogen mostly divides after the periclinal division of the hypophysis (Fig. 6 : *6*).

### 6.3. Hypophysis

Soon after the differentiation of the periblem and plerome (Fig. 6 : *6*) or after the first anticlinal division of dermatogen (Fig. 6 : *4, 5*) in layer *l'* the hypophysis stuck in the "body of the proembryo" cuts off at the top a lenticular cell, which shuts off the inner cells of the embryo at the bottom. From this cell the *iec* layer commonly considered as the initial

layer of the embryo root cortex, is formed. The layer of initial cells of the columella *co* forms from the lower derivative of the hypophysis.

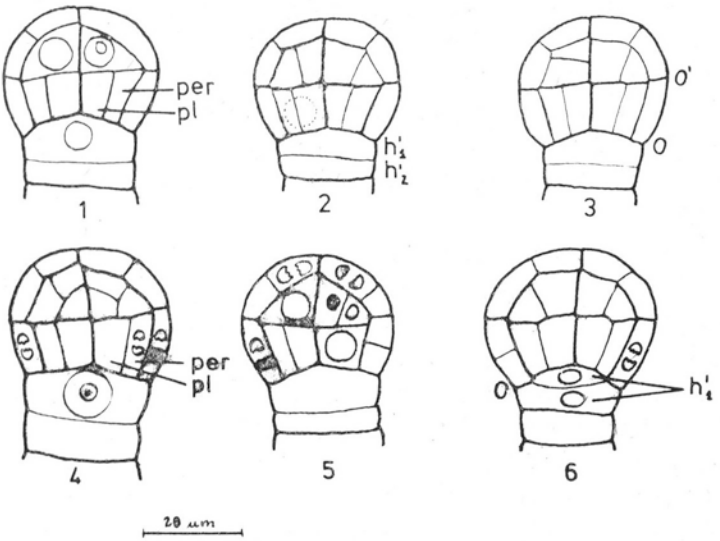



Fig. 6. Division of hypophysis

## DISCUSSION AND CONCLUSIONS

The development of the rape proembryo is similar to that of the *Capsella bursa-pastoris* proembryo (Hanstein, 1870; Coulter and Chamberlein, 1903; M. Schaffner, 1906; Souèges, 1916; Schnarf, 1929) and others *Cruciferae,* qualified by Souèges (1939) to IV megarchetype with the formula

$$ca = pvt + pco + phy + icc$$
$$cb = iec + co + s$$

Souèges (1919) suggested that the essential features differentiating the development of the embryo in various representatives of *Cruciferae* can appear in the development of the suspensor and in the origin of the hypophysis. Lebéque (1952) confirmed this in his widespread comparative studies including twenty genera of nearly all *Cruciferae* tribes. From his work he drew the following conclusions:

— the suspensor in the *Cruciferae* family is fusiform in shape, but in some species it tends to hypertrophy,
— the moment of differentiation of the hypophysis is a stable feature of the species,
— the first division of the hypophysis in *Cruciferae* is usually horizontal, but sometimes it may be oblique and even vertical.

In *Brassica napus* the hypophysis is the fifth generation of the *cb* cell (chapter 6.3). In the genus *Brassica* the hypophysis divides horizontally. The basal suspensor cell is characteristicaly elongated and widened at its end. This was found by Pearson in his studies (1933, quoted after Johansen 1950) in *Brassica oleracea*, Ahuja and Bhaduri (1956) in *Brassica campestris*, Ilina (1962) in *Brassica juncea*, and Kavetskaya (1958) in *Brassica napus*. Kavetskaya devvoted special attention to the development of the ovule, the endosperm, the integuments and the structure of the seed, but she treated quite perfunctorily the development of the embryo.

When following the development of the rape proembryo we observe that the quick elongation of the suspensor is characteristic for this period. Owing to this the "body of the proembryo" is displaced deep into the endosperm.

The stages of proembryo development differ considerably. But in each stage the direction of growth and the shape of the cells seem to decide on the direction of their division:

— the domelike widened at the bottom apical cell *ca* divide verticaly only, giving two cells similary widened and this implies the next vertical division if the apical part,
— the transversal division of quadrants into octants is preceded by the increase of the quadrants height,
— the periclinal division of octants leading to the formation of dermatogen is preceded by radial growth,
— the appearance of divisions in the layer *l* before the division in layer *l'* is preceded by the quicker growth of layer *l*,
— the periclinal divisions of the inner cells of layer *l'*, i.e. the formation of primary plerome and periblem cells, are preceded by the more intensive growth in width as compared with that in height of this layer,
— the hypophysis which sticks in the "body of the proembryo" divides always in a characteristic way, cutting off the lenticular cell *iec* on the side facing the embryo.

It follows then, that almost all the proembryo divisions are strictly determined. The identification of the segmental boundaries, formed in the course of these divisions is easy. The young globular embryo developed in this way forms a closed sphere and comprises the initials of all the histogens of the embryo axis (Fig. 6 : *6* and Photo. 6).

That is why it would seem justified to treat this stage as the first stage of the embryo proper. Cretè (1963) after Souèges (1932, 1936) refers this transition to the change of symmetry to bilateral.

This work has been accomplished in the Department of Plant Anatomy and Cytology, Institute of Botany, Warsaw University.

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## *Embriogeneza rzepaku*

### *I. Rozwój prazarodka*

Streszczenie

Opisano rozwój prazarodka rzepaku — *Brassica napus* L., od zygoty do ukształtowania się młodego zarodka właściwego. Stwierdzono duże prawidłowości w kierunku, następstwie i rozmieszczeniu podziałów segmentacyjnych i różnicujących

prazarodka. Kierunek podziałów zdeterminowany jest sposobem wzrostu i kształtem komórek. Terminem „młody zarodek właściwy" określono zarodek globularny, posiadający już wyodrębnione komórki macierzyste pleromu i peryblemu oraz komórki macierzyste piętra *iec* i kolumelli. Ciało młodego zarodka właściwego pochodzi z komórki apikalnej *ca* powstałej w wyniku pierwszego podziału zygoty i z hypofizy — jedynej komórki suspensora, która zamyka kulę zarodka. Rozwój prazarodka *Brassica napus* L. przebiega zatem zgodnie z subarchetypem *Capsella bursa-pastoris* w IV megarchetypie Souéges.