Rape embryogenesis. IV. Appearance and disappearance of starch during embryo development

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Abstract

Starch appears first in the suspensor of the proembryo with two-cell apical part. It is observed in the embryo proper from the octant stage. At first it is visible in all the embryo cells in the form of minute transient grains which disappear during cell divisions. But the columella mother cells and their derivatives have persistent large grains. When the embryo turns green in the heart stage a gradual accumulation of storage starch begins and lasts to the end of embryogenesis. Storage starch grains appear first in the outer cortex layers of the hypocotyl where the largest grains are to be found later, and afterwards in all the other tissues. Starch is usually absent in the frequently dividing cells, but even there it appears in the form of minute grains after the end of cell divisions. Disappearance of starch starts when the intensive green colour of the seed coat begins to fade. The first to disappear are the smallest granules in the regions where they were noted latest. In the embryo axis the starch grains remain deposited longest in dermatogen and cortex cells in the lower hypocotyl part. They are visible there, still when the seed turns brown. In black seeds starch may be only found in the columella the cells of which throughout embryogenesis contain amyloplasts filled with starch. These grains disappear completely at the time when the seeds become dry.

INTRODUCTION

"Embryogenesis can be viewed as a preparation for successful germination" (Dure 1975). Rape seeds, like those of other *Cruciferae*, are endospermless. Storage materials are accumulated in the whole embryo. The literature supplies no precise data concerning the localisation and appearance of storage materials in the developing embryo. Usually the appearance of storage materials is described in relation to the embryo development in time. Kavetskaya (1960), while investigating the dynamics of storage materials

accumulation in the rape embryo states that starch present in the egg cell disappears after fertilisation. It reappears in the heart embryo 12 days after anthesis. The greatest amount of starch is noted after 25 days, when the embryo is most intensively green. After 30 days when the seed integument turns brown the starch content decreases. In mature seeds starch is rare and during their storage it disappears. The course of appearance and depletion of starch is similar in *Raphanus sativus* (Joffe 1957): 12 days after pollination the embryo turns green. At this time cotyledon formation starts in the embryo. Fourteen days after pollination starch is deposited in the embryo—first in the root and then in other tissues. On the 18th-20th day starch begins to disappear in the outer layer cells of the embryo and lipid droplets appear. During seed maturation the whole starch is transformed to oil.

Starch depletion during seed ripening has been observed in many oleraceous plants. It is completely substituted by oil in the seeds of mustard, sunflower, poppy, flax, peanut (Svieschnikova 1954) and many others.

When the development of the embryo is known (Tykarska 1976, 1979), the course of deposition of storage materials can be followed in it.

The paper presents the results concerning transient starch deposition in the embryo. The course of accumulation of the final storage materials, i.e. lipid and protein bodies will be described in a forthcoming publication.

MATERIAL AND METHODS

The investigations were performed on winter rape (*Brassica napus* L. cv. Górczańska). The embryos in successive stages of development and ripening (Tykarska 1980) were fixed in chrome-aceto-formalin (CrAF—0.5:1:20, 0.5:0.5:10 or 0.25:0.5:10) and in chromo-formalin (CrF—0.5:5 in 1% CaCl_2). After embedding in paraffin the material was cut into 0.5 μm sections and stained by the PAS method or Lugol's solution.

RESULTS

Minute starch grains which disappear after fertilisation are present in the egg cell (Fig. 1). Starch reappears first in the colourless proembryo in the suspensor cells. It was noted for the first time in the cells of the suspensor of the proembryo with a two-cell apical part (Fig. 2). Usually starch grains are absent in the first haustorial suspensor cell stuck in the micropylar part of the ovule.

Starch is found in the proembryo body first at the octant stage (Fig. 3). The starch grains are minute and present in small numbers in nearly all the cells. Large grains are only found in the suspensor and in the hypophysis differentiated from the latter and its derivatives, that is the intermediate layer mother cells (iec) and the columella.

GLOBULAR STAGE

In very young globular embryos minute starch grains occur in the whole dermatogen and in the central part of the upper layer (Fig. 4). Starch appears somewhat later in the lower layer (Fig. 5), but it rapidly disappears in the upper layer developing at this time (Tykarska 1979, 1980). In older stages the starch content increases. Minute starch grains continue to be found in the late globular embryo in the dermatogen and periblem and plerome initial cells. The starch grains in the suspensor cells and the hypophysis derivatives are much larger than in the embryo body cells (Fig. 6). At this time chloroplasts develop in the nuclear endosperm surrounding the embryo (Fig. 7). In the period of symmetry change, that is the transition stage between the globular and heart ones the embryo also begins to turn green.

HEART STAGE

In this stage the embryo acquires a strong green colouring. From now on a zone can be distinguished in the embryo in which starch grains not only are continuously present, but increase in size. This is the area of the periblem at the boundary of the hypocotyl and root (Fig. 9—left side of embryo). At the same time the starch grains enlarge in the columella cells of the heart embryo. Large starch grains now form in the lateral parts of the root cap (Fig. 5).

TORPEDO STAGE

In the period of embryo axis elongation growth the area of starch occurrence becomes wider. Large grains appear mainly in the outer layers of the hypocotyl cortex (especially under the cotyledons) and at the root base. The grains in the central cylinder and cortex initials are minute and less numerous. Starch grains are seldom found in the apical meristem of the shoot, in the root part of the central cylinder (Figs. 12, 13) and in frequently dividing cells (e.g. those of the cortex cell layers in the neighbourhood of the central cylinder).

PERIOD OF GROWING UP OF THE EMBRYO

There is frequently much less starch in the dermatogen and outer periblem layers of the curving embryo axis on the concave side than on the opposite one (Figs. 11, 12).

Over the whole period of growing up of the embryo the amount of starch increases in the elongating embryo axis. Starch is found in all dermatogen cells and in nearly the whole periblem up to the apical part of the

root. In its lower part starch grains are scarce and minute, and in the higher part they are larger and more numerous. The starch grains in the central cylinder are small and less numerous, grouped mainly in the hypocotyl. They are also found in the cotyledons above the forking of the bundles under the plumule (Figs. 14, 15). In frequently dividing cells starch continues to be scarce.

In the end phase of embryogenesis starch may be detected in nearly all the embryo cells. It is absent as yet in the procambium strands running to the cotyledons (Fig. 15). It is present, however, in the differentiating metaxylem cells (Fig. 18).

END OF EMBRYOGENESIS

In the period of the last cell divisions an enhanced starch accumulation is observed (Fig. 16). Starch synthesis still occurs for a certain time after the end of cell divisions (Fig. 17), when the embryo is still intensively green. In this last phase of starch storage its minute grains appear in the whole root apical meristem. They may even be found in the meristematic centre of the shoot apex, the final shaping of which takes place in the latest phase of embryogenesis (Tykarska 1979) as well as in the procambium strands entering the cotyledons (Fig. 22).

The greatest agglomeration of large starch grains is always found in the columella and the adjacent cells of lateral parts of the root cap. Starch is present in the whole cortex and cotyledons, but the largest grains are found in the lower part of the hypocotyl (Fig. 17). The grain size decreases gradually in the root periblem in the direction towards the root apex and so does their number, but they are present in all cells, the initial ones included. The starch grains in the central cylinder are small, numerous in the hypocotyl part and mainly accumulated in the metaxylem vessels mother cells in the root part (Fig. 12)

SEED RIPENING

During the change of colour of the seed coat from green to brown the starch grains begin to disappear. The beginning of this process can be noticed when the seed coat becomes paler. (At the same time the embryo axis is decolorised). First to disappear are the minute grains in the procambium strands and those in the apical shoot and root meristems. In the latter the grains still remain in the cortex initial, cells of the intermediate layer and those of the cap (Fig. 19). Starch disappearance in the embryo axis progresses basipetally from the root apex, and centrifugally in the cortex. In the root cap starch disappearance advances from the root base towards the columella.

The process of starch disappearance is at first very rapid. In the green-brown seed there practically is no starch left in the root. Minute grains may be sometimes found in the periblem initial cells. Neither is there any starch

Figs. 1-4. Occurrence of starch in the course of rape embryo development, CrAF, x 1500
 Fig. 1—Egg cell. Fig. 2—Proembryo cells with two-cell apical part. Fig. 3—Proembryo octants. Fig. 4—Cells of embryo proper. Minute grains are visible in the lower two cells on the left and in the para-axial cells of the upper layer, large grains, in the suspensor cells and those derived from the hypophysis

Figs. 5-7. Starch in the globular stage. CrAF, Figs. 5 and 6—PAS, Fig. 7—iron haematoxylin, x 800

Fig. 5—Scarce starch grains in early globular stage. Fig. 6—Older globular embryo.
Fig. 7 Chloroplasts in nuclear endosperm

Figs. 8 and 9. Starch in rape embryo development. CrAF, PAS, x 800
 Fig. 8—Starch grains in cells of lower part of embryo (root apex) in transition stage.
 Fig. 9—First grains of "storage" starch in cells at the hypocotyl and root boundary in heart stage

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Figs. 10 and 11. Starch in torpedo stage of rape embryo. CrAF, PAS, x 200

Fig. 10—In primary cortex around embryo axis. Fig. 11—Starch grains appear only on the outer side of curved embryo axis

Figs. 12 and 13. Root apices of torpedo embryos, CrAF, PAS, x 400
Fig. 12—Starch grains in root cap and cortex cells on the outer side of curved embryo axis and in root apex. Fig. 13—Starch grains in dermatogen and cortex cells and in pericycle on both sides of axis and in cap

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Figs. 14 and 15. Starch in the upper layer of the embryo in end stages of embryogenesis. CrAF, PAS, x 500

Fig. 14—Fragment of growing up embryo with 5-layer columella. Fig. 15—Shoot apex of embryo towards the end of embryogenesis

Figs. 16 and 17. Starch in axes of grown up embryos from green seeds. CrAF, PAS, x 80
Fig. 16—End phase of embryogenesis. Fig. 17—Grown up embryo

Figs. 18 and 19. Starch in root apices of ripening embryos. CrF, PAS. x 500
 Fig. 18—from green rape seed, CrF. Fig. 19—from green-brown rape seed, PAS

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Fig. 20. Starch in embryo axis from green-brown rape seed. CrF. PAS. x 80

Figs. 21 and 22. Starch in shoot apexes of embryos during seed ripening. CrF. PAS x 500
 Fig. 21—Fragment of embryo in initial phase of seed turning brown. Fig. 22—Fragment of
 embryo from green-brown seed

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Figs. 23 and 24. Starch in the columella in end phase of seed ripening. x 500
 Fig. 23—Root apex of embryo from brown seed, CrAF. Fig. 24—Root apex of embryo
 from ripe black seed, CrF

in the apical part of the shoot and in the forking of the bundles to the cotyledons (Fig. 22). The number of grains decreases in the hypocotyl plerome, and so does part of the large grains in the periblem. In the brown-green seed starch grains can only be detected in the dermatogen and periblem of the lower part of the hypocotyl (in the distended part of the embryo axis) and in the outer cap layers close to the columella which continues to be filled with starch (Fig. 20). When the seed becomes brown there still are large starch grains in the columella (Fig. 23). In the dermatogen and periblem of the hypocotyl only the last single grains are now seen.

In the seed changing colour to black starch grains begin to disappear from the columella (Fig. 24), during its drying they disappear completely.

DISCUSSION

Starch is present in the rape embryo as an intermediate storage material. As found earlier (Kavetskaya 1960, Frey-Wyssling et al. 1963), not only in rape, but in other oleraceous plants (Svieschnikova 1954, Joffe 1957) it is a precursor of the embryo storage lipids.

In the rape embryo the first starch grains appear in the suspensor cells of proembryos with a two-cell apical part. They have been observed earlier in the egg cell. Schulz and Jensen (1968) noted starch in the electron microscope in the colourless plastids of the proembryo apical cell in *Capsella bursa-pastoris*. These grains disappeared during cell division. These small stores of starch in the rape and *Capsella* proembryos served no doubt as energy material for the cell division processes. In the further rape embryo development starch is seldom present in the frequently dividing cells, and its grains are minute.

Norton and Harris (1975) report that the main requirement for precursors for synthesis of storage materials in rape must be covered from substances supplied by the seed coat and shoot. They do not refer to photosynthesis in the embryo. The rape embryo, however, begins to turn green as early as the transitional stage and as soon as its colour becomes intensive (beginning with the heart stage) starch is deposited in the cells of the embryo axis. It not only does not disappear during cell divisions, but the grains become larger and larger throughout embryogenesis. The disappearance of this starch only starts when the green coloring of the embryo axis begins to fade, that is in the period of seed ripening. It seems that, in the synthesis of this starch, products of *in situ* photosynthesis by the embryo's own chloroplast may take part, beside precursors supplied from without. This is, therefore, the second kind of starch in the rape embryo, which may be called storage starch, although its presence in the embryo is also of transient character.

Starch storage runs a similar course in *Raphanus* (Joffe 1957). It appears when the embryo turns green and is gone together with loss of this colour-

ing. After hydrolysis this starch changes to oil which is present in ripe seeds. Joffe (l.c.) reports that in *Raphanus* starch disappearance starts in the endosperm and the outer cell layer of the embryo. It results, however, from my investigations that in rape first to disappear are the smallest grains most of which occur in the central cylinder and in the inner cell layers of cortex. Probably, however, starch hydrolysis begins simultaneously in the whole embryo (as indicated by unpublished studies on the cell ultrastructure of the ripening rape embryo). Therefore, the small grains formed latest disappear first. Owing to this, two zones may be distinguished in which the oldest grains are visible for the longest time: the outer cortex layers of the lower hypocotyl part and the columella.

On the basis of observation of starch presence in various stages of embryo development, its flitting appearance and disappearance in the initial phase of embryogenesis, maximal accumulation in the grown up embryo of the green seed and drastic depletion during seed ripening the following conclusions may be advanced:

1. There are two kinds of starch in the embryo: transient, utilised as energy material for cell division, and storage starch used for the synthesis of the final storage material of the embryo, that is lipid and proteins.

2. Intermittently appearing starch grains occurring in the colourless embryo are derived from materials supplied from without via the suspensor and the whole surface of the embryo.

3. Storage starch arises when the embryo turns green. In its synthesis may, therefore, participate products of synthesis of the embryo's own chloroplasts.

4. Storage starch grains are deposited in partly differentiated cells which seldom divide.

5. Two zones may be distinguished in the embryo where starch grains appear earliest in the embryo axis and persist longest: a) the outer cell layers of periblem in the lower part of the hypocotyl at the boundary with the root, and b) the columella in which starch is present ever since this part has differentiated. The starch in columella cells is accumulated in amyloplasts, usually in the form of several grains, and disappears completely at the stage of seed drying.

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Embriogeneza rzepaku. IV. Występowanie skrobi w czasie rozwoju zarodka

Streszczenie

Skrobia pojawia się w suspensorze prazarodka z dwukomórkową częścią apikalną. W zarodku obserwuje się ją od stadium oktantów. Początkowo występuje w postaci nielicznych drobnych ziaren we wszystkich komórkach zarodka i zanika w czasie podziałów komórkowych. Jednak komórki macierzyste kolumelli i ich pochodne mają nie zanikające, duże ziarna skrobi. Po zazielenieniu się zarodka, w stadium sercowatym, rozpoczyna się stopniowe nagromadzanie się skrobi zapasowej, trwające aż do końca embriogenezy. Najpierw pojawiają się ziarna skrobi zapasowej w zewnętrznych warstwach kory pierwotnej hypokotyla, później w innych tkankach. W osi zarodka największe ziarna skrobi zapasowej znajdują się w korze pierwotnej hypokotyli. W czasie rozwoju zarodka zwykle brak jest skrobi w komórkach często dzielących się, tj. w warstwach kory przyległych do walca osiowego oraz w wierzchołkach wzrostu pędu i korzenia. Jednak nawet w centrum merystematycznym pędu bardzo drobne ziarna skrobi pojawiają się na krótko w kulminacyjnym okresie magazynowania skrobi, po zakończeniu podziałów komórkowych. Zanikanie skrobi rozpoczyna się wtedy, kiedy zaczyna słabnąć intensywnie zielone zabarwienie łupiny nasiennej. Najpierw zanikają ziarna najdrobniejsze, tam gdzie pojawiły się najpóźniej. W osi zarodka najdłużej utrzymują się ziarna skrobi w komórkach dermatogenu i kory pierwotnej hypokryli. Są one tam widoczne (szczególnie w dolnej części hypokotyli) jeszcze w brązowym nasieniu. Natomiast w nasionach czarnych skrobię można znaleźć już tylko w kolumelli, której komórki przez całą embriogenezę zawierały amyloplasty wypełnione ziarnami skrobi. Te ziarna zanikają całkowicie dopiero w czasie wysychania nasion.