ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 4: 539-563
1986

Activation of rape (*Brassica napus L.*) embryo during seed germination. IV. Germinating embryo. The first zones of mitoses, starch and DNA synthesis and their expansion pattern

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(Received: January 14, 1986. Accepted: April 17, 1986)

Abstract

The rape radicle is completely covered by the root cap. The successive lateral cell layers of the root cap are terminated by T-forming walls in the dermatogen layer in a statistically constant position. T-walls in dermatogen were utilized for delimitation of successive root sectors on longitudinal microtome sections at the succeeding germination stages. The length and the cell number of the corresponding sectors were studied and the starch and DNA synthesis sites as well as cell divisions localized. All these processes are initiated in a constant sequence and in specific embryo zones. The first symptom of activation of the embryo is starch synthesis. It begins in two centres: in the apical part of the radicle columella and in the hypocotyl dermatogen it moves deep into the cortex and in both directions along the columella the activation of starch synthesis shifts basipetally into the whole columella and the initial centre as well as the lateral parts of the root cap. From the hypocotyl dermatogen it moves into the cortex and in both directions along the embryo axis. In the root dermatogen and periblem the activation zone is first located in the basal sector and, then, gradually, in the lower ones. Just before germination the basal and apical zones of activation meet. Starch can then, be found throughout the root. During starch synthesis, in the basal part of the radicle, DNA synthesis and cell growth begin. In the root sectors which have already begun to grow and synthesize DNA, cell divisions start. The boundary of the dividing cells zone shifts acropetally at some distance above the lower boundary of DNA synthesis and the zone of cell premitotic growth. The acropetal shift of the mitotic activation zones can be described as wave expansion. Before the first mitotic wave reaches the promeristem it is followed by at least three acropetal waves, arising in the already

activated basal sectors, and then the mitoses are asynchronised. Mitotic activation of the root cap is partly independent of the acropetal wave of cell activation and results from the expanding weaker basipetal wave.

Key words: rape, germination, mitose zones, starch synthesis, DNA synthesis

INTRODUCTION

The present paper is based on detailed descriptions of embryogenesis and morphology of the mature rape embryo (Tykarska 1976, 1979, 1980, 1982, Kuraś 1978, 1980, Kuraś and Teleżyński 1978). The extraordinarily regular structure of the rape embryo enabled receiving through information on the pattern of embryo activation and growth distribution in germinating seeds.

MATERIAL AND METHODS

Winter rape seeds (*Brassica napus* L, var. *oleifera*, cv. Górczański) were received from the Plant Breeding Institute, Radzików. In the end phase of ripening the plants were covered with a transparent foil roof to prevent seed germination inside the silique. The germination capacity of seeds was 100%.

Germinating embryos were prepared out from seeds placed hilum downwards on wet filter paper extended over an almost vertically oriented glass plate in a moist chamber at 21°C, in darkness. Due to such an orientation, the embryo axes grew straight. The embryo axes from dry and germinating seeds with 1, 5, 7, 10 mm roots (including radicale and part of hypocotyl outside the seed), as well as 30 mm long seedlings were taken 24, 33, 37, 41 and 57 hrs after the start of imbibition. They were fixed in chromacetoformalin (CrAF, percentual ratio 0.5-1-20), uranylacetate and formalin (UF, percentual ratio 0.2-10) or in an ethanol, acetic acid, formalin, chloroform mixture (FAAC, 5:1:1:1 volume ratio). The material was embedded in paraffin and cut into 5 µm thick longitudinal sections. On the medial longitudinal sections stained with safranin and fast green (Sass 1940), were measured: 1) the length of the epicotyl, hypocotyl and radicle of the dry embryo and 24 hrs after beginning of imbibition; 2) the length and the cell number in dermatogen sectors after 24-57 hrs of imbibition was counted. T-forming walls in the dermatogen layer were recognized as the boundaries of dermatogen sectors (Kuraś 1978). The medial slices of 10 embryo axes were studied in every stage examined. The average lengths of the sectors and

the number of cells within particular sectors were compared in the successive stages. The results were elaborated with the use of Student's t test. Two significance levels were applied: 0.05 = significant and 0.01 = highly significant.

Mitotic activity was evaluated on the basis of the number of mitoses and number of divided cells forming complexes arising after one, two and three cell division cycles.

Starch was detected using the PAS reaction (Mc Manus 1948).

DNA synthesis was detected by the autoradiographic method. Seeds germinating for 6 to 36 hrs were incubated for 6 hrs in ^{3}H-thymidine (5 μCi · cm^{-3}, spec. act. 120 mCi per mM). Slides were covered with stripping film — Kodak AR-10. After 3-week exposure at 4°C, the autoradiograms were stained with alcian blue (Beneš 1969).

RESULTS

GROWTH OF GERMINATING EMBRYO AXIS

The length of the dry-seed embryo and of its parts, the epicotyl, hypocotyl and embryonic root (the natural developmental boundaries were recognized in the cell arrangement on the longitudinal slices (Kuraś 1978)), were compared with the corresponding parts after 24 hrs of germination. From the comparison presented in Table 1 it can be concluded that:

Table 1

The lengths of the embryo axis, hypocotyl, root and epicotyl during the first period of growth (arithmetical means from the measurements of 10 embryos)

	Dry embryo, µm	The embryo after 24 hrs of germination		
		length, µm	increase	
			µm	%
Embryo axis	2602	3169	567	21.8
Hypocotyl	1915	2409	494	25.8
Root	476	524	48	10.1
Epicotyl	211	236	25	11.8

1) The growth of the embryo axis is differentiated. The part growing fastest is the hypocotyl and embryonic root grows slowest. 2) Protrusion of the radicle is mainly the consequence of hypocotyl growth.

The hypocotyl inside the mature seed is curved. The length of the concave side constitutes 69 percent of that of the convex one, but just

Length and increase of dermatogen

Time, h	Root length, mm	Sector I			Sector II		
		length, µm	increase		length, µm	increase	
			µm	$\%_0 \cdot h^{-1}$		µm	$\%_0 \cdot h^{-1}$
0	dry embryo	237.1	—	—	86.5	—	—
24	1	277.6	40.4	0.7	97.6	11.1	0.5
33	5	410.1	132.5	5.3	138.7	41.1	4.7
37	7	557.4	147.3	8.9	246.0	107.3	19.3
41	10	—	—	—	313.6	67.6	6.8
57	30	—	—	—	—	—	—

after germination — 87 percent. The increase of length on the concave side is twice as much and the relative increase three times as much as that on the convex side. In the dry embryo the mean number of cells on both sides is almost identical (108 and 110) and does not increase during the first stage of germination. The growth of the hypocotyl and its straightening up result only from cell growth.

GROWTH ACTIVATION AND DISTRIBUTION IN THE EMBRYONIC ROOT

Initial growth of the embryonic root and growth distribution in roots after germination were examined by analysis of the dermatogen sectors. In the mature embryo there are four dermatogen sectors and a dermatocalyptrogen sector. From time to time the latter undergoes partition into a new sector of dermatogen, new layer of the root cap and new sector of dermatocalyptrogen. The length and a number of cells in the sectors of dermatogen and dermatocalyptrogen were estimated.

Embryonic root growth preceding its emergence 24 hrs after start of imbibition

After sowing of the seeds the embryonic root increases in 24 hrs by 10 per cent. The basal sector grows fastest. Its increase was 40 µm, which constitutes 2/3 of the increasing part of the whole root. The increase of the remaining sectors is slight and statistically negligible. The growth of the root cap is rather pronounced (Figs. 1-3). Consequently, it can be assumed that in the first stage of activation the embryonic root begins to grow in two zones; in the basal sector adjacent to the first growing hypocotyl and in the root cap. In the basal part of the first sector cell divisions begin at this stage, but the cell number does not increase so much as to be statistically significant.

Table 2

sectors during growth of embryonic root

Sector III			Sector IV			Sector V			Sector VI		
length, µm	increase		length, µm	increase		length, µm	increase		length, µm	increase	
	µm	%·h⁻¹		µm	%·h⁻¹		µm	%·h⁻¹		µm	%·h⁻¹
41.9	—	—	30.1	—	—	34.2	—	—	—	—	—
44.1	2.2	0.2	32.1	2.0	0.3	38.1	4.0	0.5	—	—	—
63.7	19.6	4.9	37.0	4.9	1.7	40.1	1.9	0.5	—	—	—
76.2	12.5	4.9	27.6	9.4	6.5	52.3	12.9	7.6	—	—	—
121.9	45.7	15.0	53.2	25.6	23.2	32.7	7.2	7.1	41.0	14.2	13.2
359.2	237.3	12.2	208.2	155.0	18.2	79.8	47.1	9.0	67.4	26.4	4.0

Radicle growth between 24 and 33 hrs after the start of imbibition

In comparison with the root 24 hrs after the beginning of imbibition, that studied after 33 hrs is 0.2 mm longer. The length of the root cap has also distinctly increased (Figs. 1-3). However, growth of the basal root

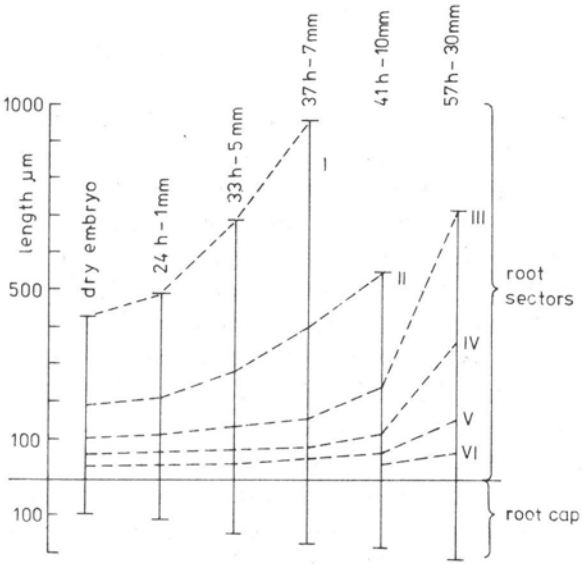

Fig. 3. Changes of dermatogen sector and columella lengths during germination

sector is still the strongest. Two sectors situated below also grow and their relative increase is almost the same as that of the first one (Table 2). Growths of the next sectors is statistically insignificant. It can, therefore be presumed that growth spreads from the root base towards its apical part.

Number of cells and its increase in dermatogen

Time, h	Root length, mm	Sector I			Sector II		
		cell number	increase		cell number	increase	
			cells	$\%\cdot h^{-1}$		cells	$\%\cdot h^{-1}$
0	dry embryo	27.9	—	—	9.9	—	—
24	1	29.4	1.5	0.2	9.7	0.2	—
33	5	44.7	15.3	5.8	14.4	4.7	5.4
37	7	45.5	0.8	0.4	28.6	14.2	24.6
41	10	—	—	—	33.4	4.8	4.2
57	30	—	—	—	—	—	—

In the upper two elongated dermatogen sectors the cell number has also increased, though it remains statistically unchanged in sector III, in spite of the fact that its length has also increased (Tables 2 and 3). Thus, it can be concluded that activated root cells first begin to grow and, then, divide. The increase of the cell number in sector I and the appearance of mitotic divisions in sector II are evidence that the mitotic activity also spreads acropetally.

Radicle growth between 33 and 37 hrs after the start of imbibition

At that time the root has considerably lengthened, mainly as a result of growth of the embryonic root body (95 per cent of the total increase), whereas the participation of the root cap is very slight (5 per cent). Root lengthening is mainly due to the three basal sectors, though sector II revealed the greatest relative increase (Table 2, Fig. 3). The cell number has remarkable grown only in sectors II and III (Table 3). It means that mitotic activity subsists in sector II and has appeared in sector III. The enlargement of these sectors resulted from divisions and growth of cells. However, at that time the cells in sector I ceased to divide and all entered the phase of elongation growth.

Simultaneously, periclinal divisions of the initial cells of the columella and dermatocalyptrogen occurred. This led to the formation of the 7th root cap layer and partition of the partition of the primary dermatocalyptrogen (A_1) into the fifth sector of dermatogen and secondary dermatocalyptrogen (A_2).

Thus, in the embryonic root 37 hrs after the beginning of imbibition two zones of cell divisions can be distinguished: anticlinal, in the root proper body, determining its longitudinal growth, the acropetal spread of the mitotic zone and perpetuation of the meristem and periclinal in the root cap leading to the formation of a new root cap cell layer and longitudinal growth of the columella.

Table 3

sectors during embryonic root growth

Sector III			Sector IV			Sector V			Sector VI		
cell number	increase		cell number	increase		cell number	increase		cell number	increase	
	cells	$\%\cdot h^{-1}$		cells	$\%\cdot h^{-1}$		cells	$\%\cdot h^{-1}$		cells	$\%\cdot h^{-1}$
4.6	—	—	2.6	—	—	3.0	—	—	—	—	—
4.4	0.2	—	2.6	—	—	3.3	0.3	—	—	—	—
4.2	0.2	0.5	2.4	0.2	0.8	3.3	—	—	—	—	—
8.5	4.3	25.6	2.2	0.2	2.1	4.5	1.2	9.1	—	—	—
10.7	2.2	6.5	3.6	1.4	15.9	2.0	—	—	3.1	0.6	6.0
31.8	21.1	12.3	19.6	16.0	27.8	5.2	3.2	10.0	5.2	2.1	4.2

Radicle growth between 37 and 41 hrs after the start of imbibition

The upper boundary of sector I of dermatogen ceases to be discernible, those of the remaining sectors are still visible. Sector A_1 is partitioned into the fifth sector of dermatogen and secondary sector of dermatocalyptrogen — A_2. The boundary between these sectors changes subsequently as the result of additional periclinal divisions of apical cells in dermatogen sector V. All the sectors have grown by this time. This fact indicates that the embryonic root has been fully activated. However, the greatest relative increase of length occured in the fourth sector (Table 2). In this sector, the cell number remarkably increased as well (Table 3). In comparison with the preceding stage, the zone of activation has shifted acropetally by one sector. However, in the remaining lower sectors the cells number has not changed yet. The convergence of the most intensive growth occurring at that time with the greatest increase of the number of cells indicates, that the growth rate of the activated part of the root is directly dependent on the mitotic activity, i.e. on the production of cells.

Radicle growth between 41 and 57 hrs after the start of imbibition

In this stage the dermatogen sectors I and II cannot no more be distinguished. In the apical part the boundaries of sector V were definitively established, whereas the sector of secondary dermatocalyptrogen (A_2) divides periclinally by T-forming walls, giving the eighth layer of the root cap and two still unestablished sectors: the sixth sector of dermatogen and the related dermatocalyptrogen one (A_3). Those two last sectors are treated as one sector VI (Tables 2 and 3).

Since the preceding interval sectors III, IV and V were markedly increased, but the greatest relative increase was noted in the sector IV (Table 2).

PLATE I

Figs. 1-2. Dermatogen sectors of the mature dry embryo root and 24 hrs after start of imbibition (1 mm long roots). I-V sector boundary, S_I-S_{IV} and A_I lengths of the successive sectors. CrAF. Safr.+fast green. ×300. Fig. 1 — One half of dry radicle. Fig. 2 — One half of radicle 24 hrs after start of imbibition

PLATE II

Figs. 4-10. Cell families in root cortex formed after the successive division cycles from quiescent embryo cells. Arrows mark the cell wall of the quiescent embryo. CrAF. Safr+fast green. ×400. Fig. 4 — Thick cell walls between quiescent embryo cells. Fig. 5 — 2-cell complexes formed after the first division cycle in the roots 33 hrs after start of imbibition, 5 mm long roots. Fig. 6 — 4-cell complexes after the second division cycle in the root 37 hrs after start of imbibition, 7 mm long roots. Fig. 7 — 6- and 7-cell families, formed as a result of third cell division cycle in the roots 41 hrs after start of imbibition, 10 mm long roots. Fig. 8 — 4-, 5- and 8-cell complexes localized close to each other in the root 41 hrs after start of imbibition, 10 mm long roots. Fig. 9 — Gradual reduction of cell families in acropetal direction of the root. Roots as in Figs. 7 and 8. Fig. 10 — 16-cell complexes after the fourth cell division cycle in the root 57 hrs after start of imbibition, 30 mm long seedlings

PLATE III

Figs. 11-12. First cell division and pairs of sister cells in the basal root part 24 hrs after start of imbibitions. 1 mm long roots. CrAF. Safr.+fast green. ×1400. Fig. 11 — First cell divisions in the dermatogen and subdermatogen just below the hypocotyl boundary. Fig. 12 — Acropetal direction of the first cell division extension in the dermatogen of sector I basal part

PLATE IV

Figs. 13-16. 2-cell complexes in the root 33 hrs after beginning of imbibition, 5 mm long roots. CrAF. Safr.+fast green. ×700. Fig. 13 — Upper boundary of 2-cell complexes zone at the border between the hypocotyl and root. Individed cells above this boundary (marked with x) are entering the elongation phase. Fig. 14 — Middle root zone with only 2-cell complexes occurring there. Figs. 15, 16 — Lower boundary of the 2-cell complexes zone. The undivided adjacent cells are marked with x

PLATE V

Fig. 18. Cell complexes of the root cortex 45 hrs after start of imbibition (15 mm long roots). CrAF. Safr.+fast green. ×400. O — undivided cells of apical root part, I — cells situated in subapical root part after 1st cell division cycle — covered by the 1st wave of mitoses, II — 3- and 4-cell complexes formed owing to second cell division cycle (zone of the 2nd wave of mitoses), III — 5- and 8-cell complexes formed as the result of third cell division cycle (zone of the 3rd wave of mitoses), e — beginning of elongation zone

PLATE VI

Figs. 19-21. Distribution of the first labelled cells within the embryo axis 12 hrs after start of imbibition (6 hrs H_2O+6 hrs ^{3}H-thymidine). UF. Alcian blue. ×400. Fig. 19 — The first labelled nuclei of the dermatogen and periblem cells in the lower hypocotyl part. Fig. 20 — A basal root part without any symptom of labelling. Fig. 21 — The first labelled nuclei in 1st and 2nd layer of root cap columella

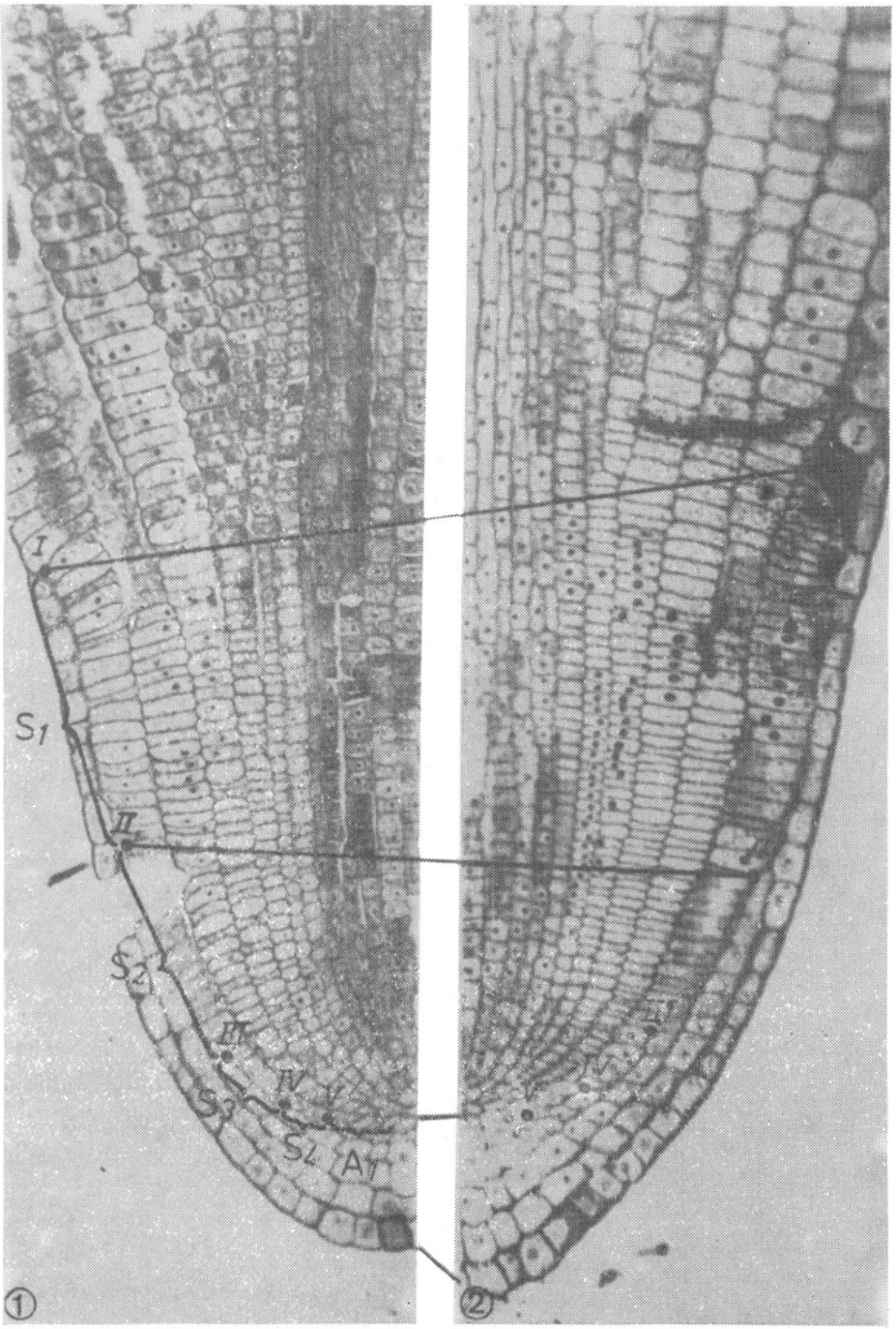
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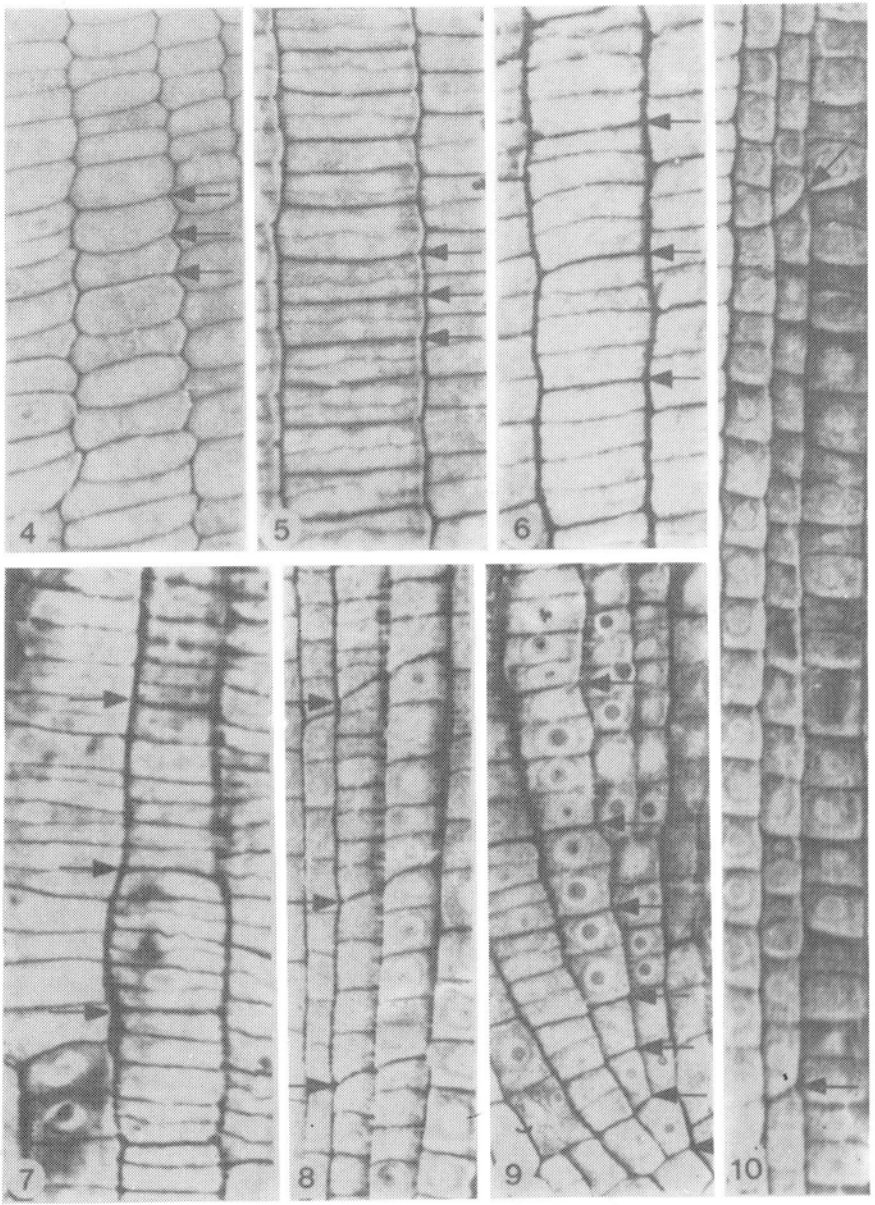
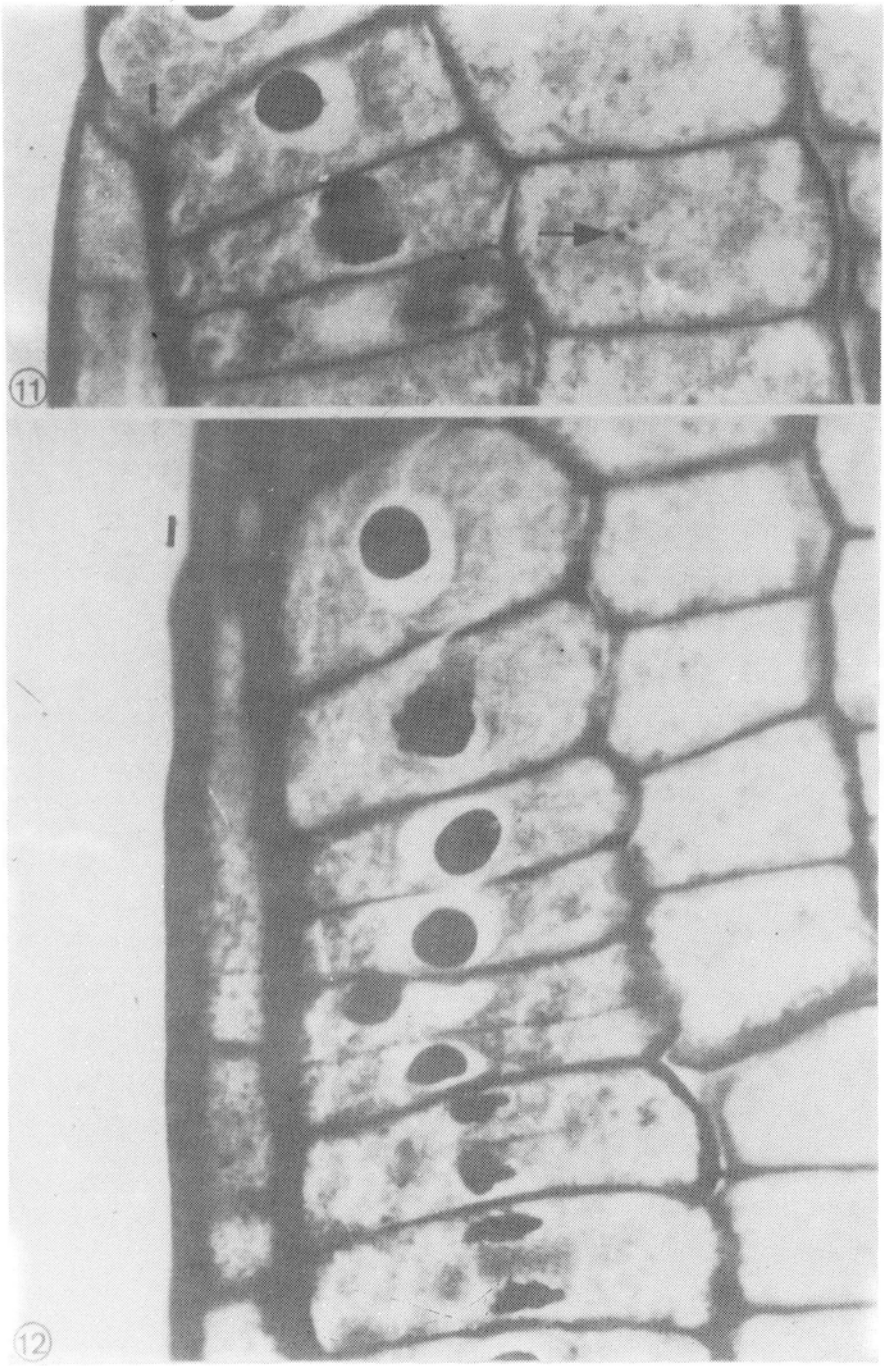
PLATE II

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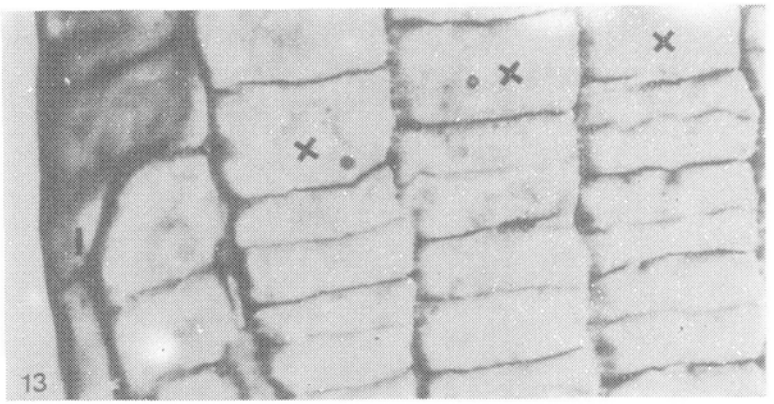
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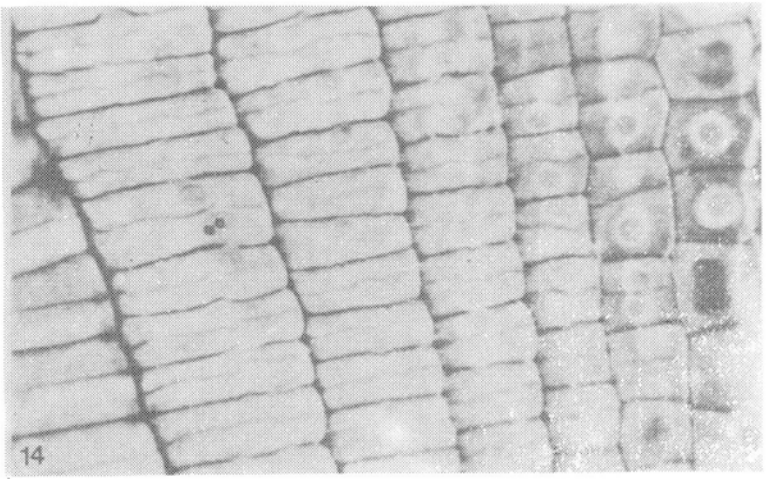
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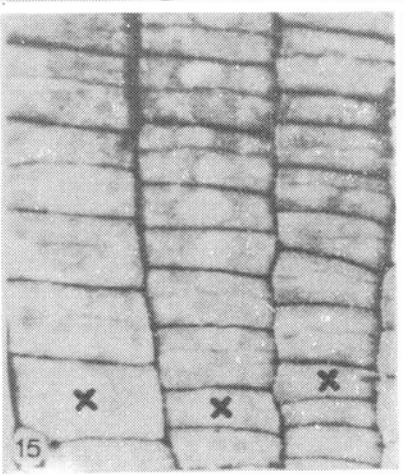
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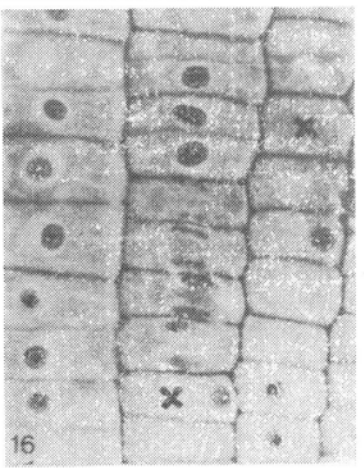
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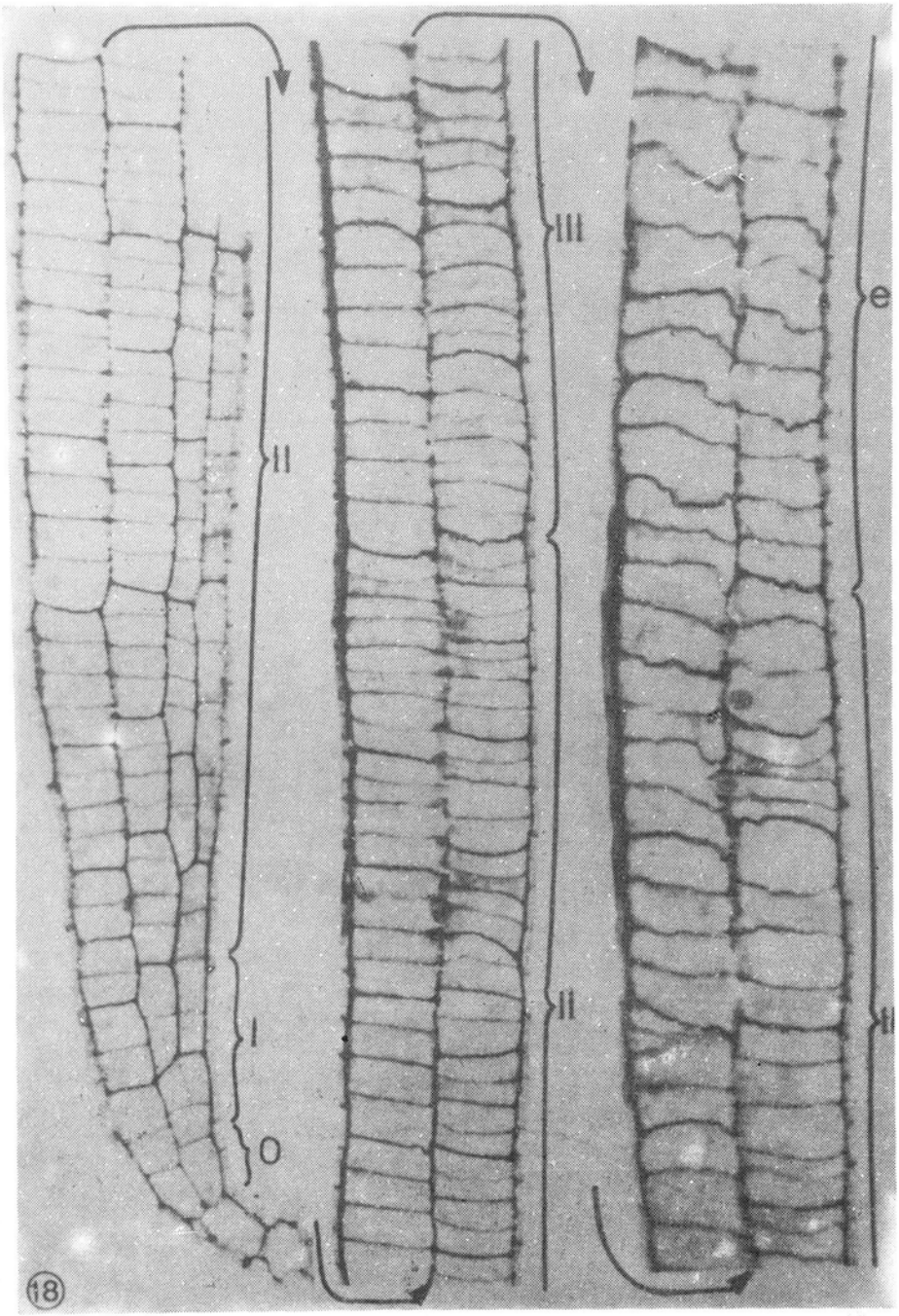
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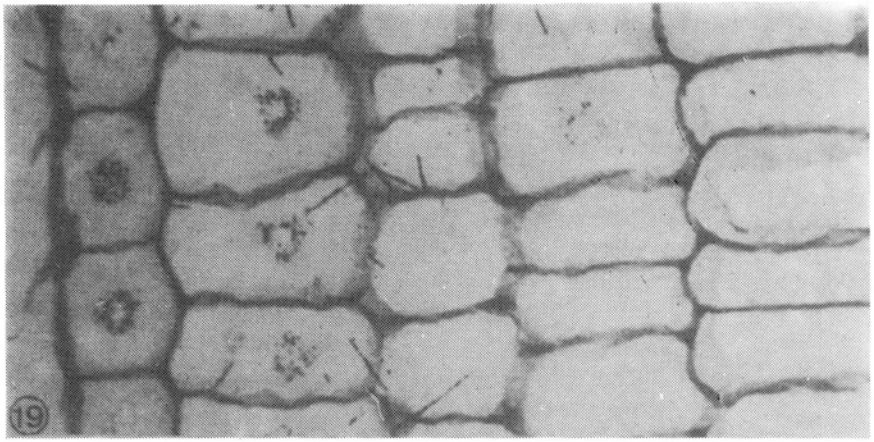
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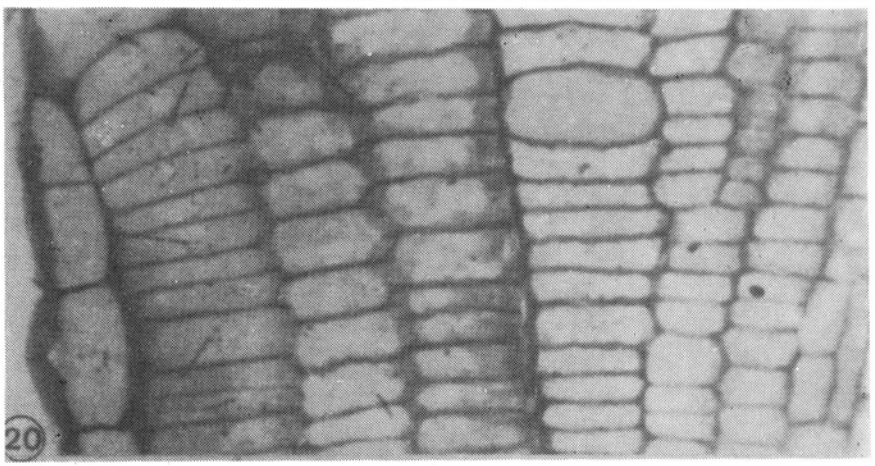
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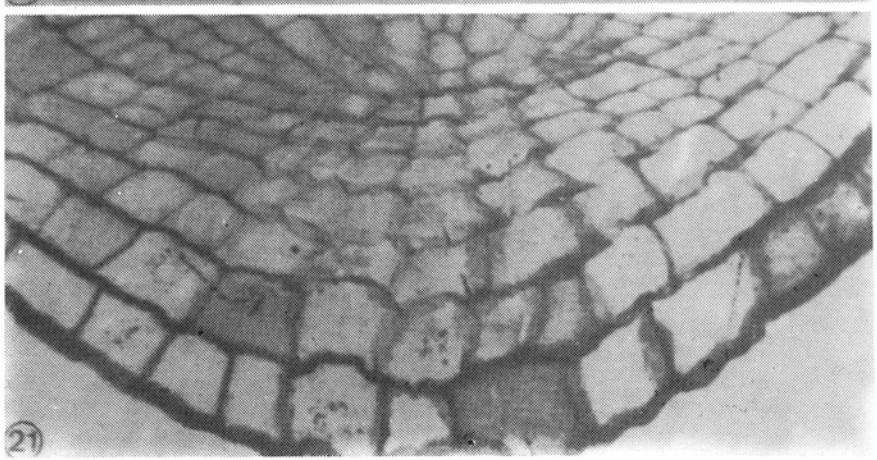
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The cell number also augmented (Table 3). In sector III the rate of cell number increase doubled after the fall in the preceding time interval ($12\%\cdot h^{-1}$ versus $6\%\cdot h^{-1}$). In sector V the cell number increase was for the first time the effect of cell division. In sector A_2 the changes of length and cell number were nonsignificant.

EXPANSION OF CELL DIVISION IN THE EMBRYONIC ROOT

The increase of cell number in the dermatogen sectors during germination is described in the previous chapter. Apart from that, cell divisions in the sectors of the outer three cell layers of periblem, corresponding to the dermatogen sectors, were studied. New cells formed during germination are surrounded by much thinner cell walls than the old cells arising during embryogenesis. This difference allows to identify not only pairs of sister cells, but as well larger complexes originating from the second, third and fourth cell division cycles (Figs. 4-10 and 18).

Localization of the first cell divisions

The first scarce mitoses appeared as well as newly formed cell pairs 24 hrs after the beginning of imbibition (1 mm long roots) in 10 out of 14 studied roots (Figs. 11 and 12). All of them were localized in dermatogen and outer periblem layers near the hypocotyl. They were most numerous in dermatogen (42), whereas their number gradually decreased in subdermatogen (22) and the remaining part of periblem (8). In dermatogen most mitotic cells were localized beside the pairs of sister cells. The new sister cells were situated more closely to the hypocotyl boundary than the dividing ones (Fig. 12). The subdermatogen cells always began to divide later than the adjacent dermatogen cells (Fig. 11). Hence, it can be concluded that mitotic activation is initiated in the basal part of the root dermatogen and shifts acropetally and centripetally.

Mitoses and pairs of sister cells appear on a much larger area 33 hrs after the start of imbibition (5 mm long roots) (Figs. 13-16), but they are still absent in the lower hypocotyl part (Fig. 13). However, their range of occurrence shifts distinctly towards the root apex (Figs. 15, 16 and 17). In 6 cases (out of 48 examined) it shifted towards the end of sector I and in 23 cases to sector II whereas in 19 cases it occupied at least half of sector III (Fig. 17). In the periblem the range of occurrence of mitoses was slightly smaller than in the dermatogen.

In roots fixed 37 hrs after the beginning of imbibition (7 mm long) mitoses occurred as far as sectors IV and V (10 cases out of 30), whereas after 41 hrs after the start of imbibition (10 mm long germs) mitoses reached

sector V (11 cases out of 30) and the secondary dermatocalyptrogen sector A_2 (12 cases out of 30).

In roots fixed 57 hrs after the start of imbibition (30 mm long seedlings) mitotic cells and/or pairs of new sister cells were present in the dermato-

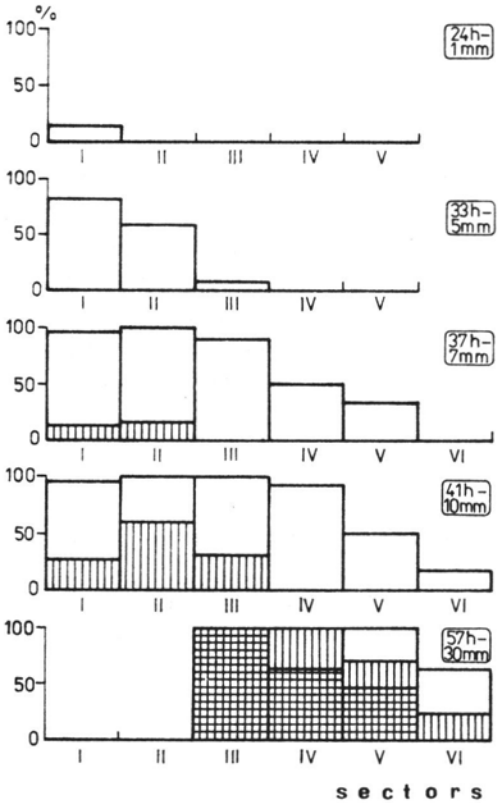


Fig. 17. Percentual graph of the divided cells in the sectors (calculated in relation to primary number of cells in the quiescent embryo sectors) after the first cell division cycle (clear figures), the second (figures hatched vertically) and the third one (figures hatched crosswise)

calyptrogen in all the roots examined. In some roots of 10 mm seedlings and in 1/3 roots of 30-mm seedlings the initial periblem and plerome cells also divided.

Cell complexes in the periblem sectors after the successive cell division cycles

In the three outer periblem cell layers of the successive sectors the cell complexes, i.e. the new cell families, formed during germination were examined. Pairs of sister cells were found formed after the first cell division (Fig. 5),

4-cell complexes (Fig. 6) which founded after the second cell division cycles as well as 8-cell complexes (Fig. 7) after three cell division cycles. Three-cell complexes were formed after the second cell division cycle had occured in one of the sister cells. Larger — 5-7-cell complexes resulted from the third cell division occurring only in some cells of the 4-cell complex.

The first mitoses and pairs of sister cells were discernible in the first root sector 24 hrs after the start of imbibition (Figs. 11, 12 and 17). The cells in the remaining sectors did not show any traces of mitotic activity at that time (Fig. 17).

In longer roots (fixed 33 and 37 hrs after the start of imbibition) the percentage of the first dividing cells in sector I rose to 97.5. Afterwards, while some lower situated cells in sector I undergo the second cell division cycle (Fig. 17), the upper basal cells of this sector enter the elongation phase. As the root grows, all the cells of periblem layers examined localized in lower sectors are gradually doubled (Fig. 17). Moreover some of the doubled cells underwent cell division again. The cell number was fully doubled in sector II 37 hrs after the start of imbibition, in sector III of the roots after 41 hrs as well as in sector IV and V after 57 hrs (Fig. 17). In those roots the lower mitotic zone boundary shifts to sectors V, VI and the dermatocalyptrogen cells, respectively. Doubling of the cell number is much delayed in relation to the appearance of the first mitoses. The first mitoses most frequently occur in the zone localized at least by two sectors further from the zone where full doubling of the cell number has been accomplished. Four-cell (as well as 3-cell) complexes marking the second cell division cycle appeared first in sector I 37 hrs after the start of imbibition and in the adjacent part of sector II. In older roots (41 hrs after the start of imbibition) twice divided cells appeared in sector III (Fig. 17).

In the roots of the oldest examined seedlings 57 hrs after the start of imbibition the cell complexes with twice divided cells are to be found in sectors IV, V and VI. However, in sector III almost all cells are divided for the third time (mainly 8-cell complexes). These complexes are slightly fewer in sectors IV and V (Fig. 17).

This regular increase of the cell number in the cell complexes results from the recurrent acropetal shifts of the mitotic zone. Mitoses are initiated in the basal root sector and the gradually spread toward the apex. In the meantime, when the radicle and the part of hypocotyl visible outside the seed, grew from 1 to 7 mm, the zone of the first mitotic cycle spread from sector I to V. In sector I the second cell cycle had started before all the cells divided for the first time. This was indicated by the appearance of 4-cell complexes besides 2-cell ones and some single cells. The second cell cycle starts probably in the cells which divided earliest. The zone of the second mitotic cycle is thus established. The lower boundary of this zone is

localized in the root sector where most cells have undergone the first division.

In roots 57 hrs after the start of imbibition (30 mm long seedlings) all the cells of sector III underwent three cell division cycles. The third cycle was also completed in some cells of sector IV, and in a lesser percentage of cells of sector V. In the sixth sector the third cycle has not been initiated yet. On the basis of comparison of the roots 41 and 57 hrs after the start of imbibition (10 mm long roots and 30 mm long seedlings) it can be concluded that the third division cycle may have begun in sector III when the first cycle was almost completed there, whereas the second was far advanced (Fig. 17).

PERICLINAL DIVISIONS IN THE DERMATOGEN AND DERMATOCALYPTROGEN

The lower boundary of the mitotic zone initiated in the basal part of the dermatogen and periblem shifts towards the apex of the growing embryonic root. At first (1 mm long root) all the divisions occurred in the anticlinal plane. When the mitotic zone reaches sector III (5 mm roots) periclinal divisions appear besides anticlinal ones. They are localized only in sectors III and IV of the dermatogen. The lowest cells of sector III divided periclinally or anticlinally, whereas the corresponding cells of sector IV were only periclinally divided. In longer roots (7 mm) the zone of anticlinal mitoses reached the dermatocalyptrogen, sector A_1 including its basal cells. The apical cells of this sector divide periclinally by T-forming walls partitioning the primary dermatocalyptrogen sector A_1 into secondary dermatocalyptrogen A_2 and the dermatogen sector V.

The apical cells of dermatogen sector V begin to divide periclinally in this stage, this leading to a decrease of the dermatogen cell number in sector V and to an increase of cell number in the secondary dermatocalyptrogen sector. Periclinal divisions of the apical cells of these sectors always preceded the anticlinal ones. However, no periclinal divisions appear in more basal sectors. In the roots 41 hrs after the start of imbibition (10 mm long), the initial cells of the columella have already divided periclinally. It means that the zone of the first mitotic cycle comprises now the whole dermatogen, dermatocalyptrogen and initials of the columella. In the cortex, however, the cells of which divide only anticlinally, the cell division zone approaches the initial centre.

THE FIRST DNA SYNTHESIS AND ITS EXPANSION

From the examination of the autoradiographic slides it may be concluded that DNA synthesis in embryos has already begun long before germination.

The first labelling was noticed in the embryos of seeds soaked for 6 hrs in water and then incubated for 6 hrs in a solution of ^{3}H-thymidine (6 hrs H_2O + 6 hrs ^{3}H-thymidine). Most frequently labelled were the nuclei of the outer cell layers in the lower hypocotyl part slightly above the boundary between the hypocotyl and root (Figs. 19 and 20). Generally, in the dermatogen on the hypocotyl convex side, the labelled cells were most numerous. In some embryos the subdermatogen cells and even deeper cortex cell layers were also labelled (Fig. 19). The zone of labelled nuclei was longest in the dermatogen, whereas in the periblem it diminished centripetally. In the plerome region, no labelling at all appeared. In the small number of embryos the outer root cap layers also underwent labelling. However, their labelling was much weaker than that of the cells in the hypocotyl (Fig. 21). This pattern of labelling, does not, however, correspond to the previously discussed one of distribution of the first mitoses, as during incubation in ^{3}H-thymidine, between 6 and 12 hrs of imbibition, those cell nuclei were labelled only, which do not divide. Therefore, it can be assumed that endomitotic DNA synthesis precedes the mitotic one in the germinating embryo.

In the 3 hrs older embryos (9 hrs H_2O + 6 hrs ^{3}H-thymidine) the zone of labelled nuclei increases together with intensification of the labelling (Figs. 22-24). The zone of labelling covers almost the whole lower hypocotyl part as well as the basal sector of the radicle dermatogen. In the radicle dermatogen cell nuclei labelling in the uppermost part of the first sector is most pronounced (Fig. 23), thus, exactly in the zone where, as described previously, the first mitoses will occur. The same pattern of labelling may be found in slightly older embryos — 12 hrs H_2O + 6 hrs ^{3}H-thymidine (Figs. 25-28). However, at this stage the zone of nuclear labelling is often shifted further acropetally. In the dermatogen it occupies the lower part of sector I and the upper one of sector II (Fig. 27). No labelled cells are present now in the upper part of the first sector, where they were found 3 hrs earlier (Fig. 26). Therefore, it may be suggested that these cells are already in phase G_2. In the periblem the upper labelled cell boundary is still higher than that in the dermatogen. This boundary is still higher in the pericycle. However, along the central part of the plerome only scarce cell nuclei were labelled (Fig. 28). In the labelled dermatogen zone an area of different degree of labelling can be distinguished. Most intensively labelled are cell nuclei in the middle of the labelled zone, whereas the intensity of labelling diminishes gradually in its lower and upper parts. It would seem that in the most intensively labelled nuclei all DNA synthesis occurred in the presence of labelled thymidine, whereas in weaker labelled cells situated higher synthesis must have ceased soon after the isotope had been administered. At the same time the nuclei localized lower begin to synthesize. Some nuclei in the labelled zone are not labelled at all. In these nuclei synthesis must have occured in the last phase of seed ripening. Thus, they

PLATE VII

Figs. 22-24. Distribution of labelling in the root 15 hrs after start of imbibition (9 hrs H_2O + 6 hrs ^{3}H-thymidine) UF. Alcian blue. × 400. Fig. 22 — Labelled nuclei in lower hypocotyl zone (compare intensity of labelling with that in Fig. 19). Fig. 23 — The first labelled nuclei of dermatogen cells of the basal root part. Fig. 24 — Intensively labelled nuclei of the hypocotyl inner cortex cell layers close to the root hypocotyl boundary

PLATE VIII

Figs. 25-28. Labelling of lower embryo axis part 21 hrs after start of imbibition (15 hrs H_2O + 6 hrs ^{3}H-thymidine). FAAC. Alcian blue. × 400. Fig. 25 — Endomitotic labelling of the dermatogen and periblem cell nuclei in lower hypocotyl part. Fig. 26 — Unlabelled basal root part just below the hypocotyl-root boundary in which DNA synthesis occurred 3 hrs earlier. Fig. 27 — Nuclei labelling in second sectors of dermatogen. Fig. 28 — Intensive labelling of cytoplasm below the zone of labelled nuclei shown in Fig. 27

PLATE IX

Figs. 29-31. Labelling of cell nuclei in the successive root zones 30 hrs after start of imbibition (24 hrs H_2O + 6 hrs ^{3}H-thymidine). UF. Alcian blue. × 400. Fig. 29 — Second labelling of root dermatogen nuclei just below the root-hypocotyl boundary (beginning of the second wave of DNA synthesis). Fig. 30 — Unlabelled cells localized below the zone of the labelled cells presented in Fig. 29. Fig. 31 — Promeristem of the root presented in Figs. 29 and 30. Labelled nuclei are undergoing the first DNA synthesis

PLATE X

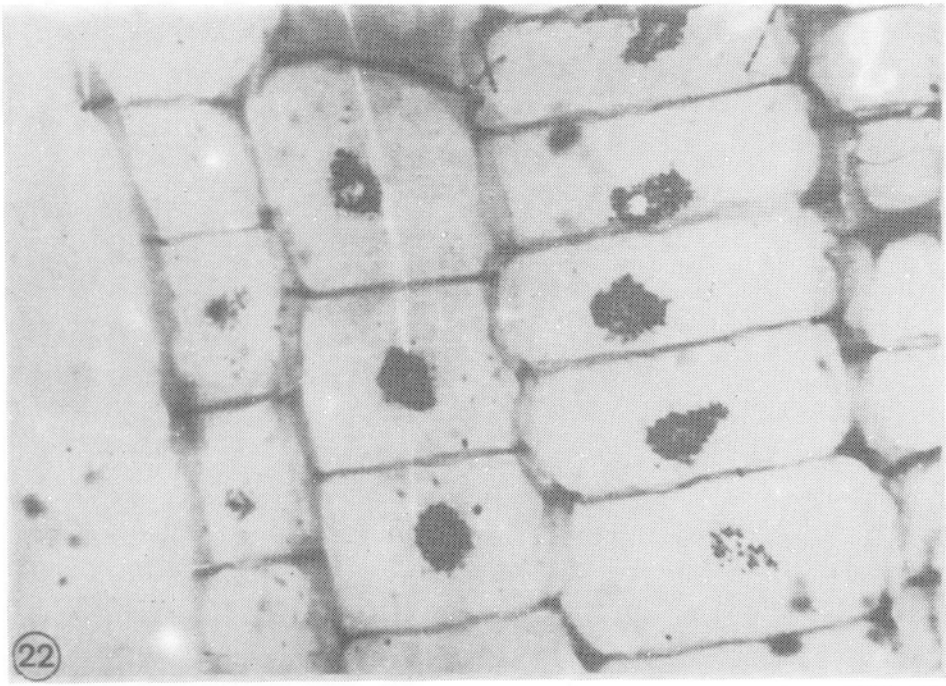
Figs. 32-35. Labelling of the apical root part, indicating gradual translocation of DNA synthesis zone. Figs. 32, 34 and 35 — UF. Fig. 33 — FAAC. All Figs. Alcian blue. × 350. Fig. 32 — Zone of labelled nuclei including the fourth dermatogen sector (24 hrs H_2O + 6 hrs ^{3}H-thymidine). Fig. 33 — Zone of labelled nuclei including the initial dermatocalyptrogen cells (27 hrs H_2O + 6 hrs ^{3}H-thymidine). Fig. 34 — Labelling of nuclei in the initial columella cells and cortex cells of sector IV. Large quiescent centre (30 hrs H_2O + 6 hrs ^{3}H-thymidine). Fig. 35 — The zone of labelled nuclei has reached the promeristem. The quiescent centre restricted to the intermediate cell layer between columella and plerome initials (36 hrs H_2O + 6 hrs ^{3}H-thymidine)

PLATE XI

Figs. 36-38. Starch in the apical root part during germination. CrAF. PAS. × 350. Fig. 36 — 3 hrs after the start of imbibition — starch in the cells of the second and third columella layers. Fig. 37 — 12 hrs after the start of imbibition — starch in the whole apical root cap part, its absence in the promeristem cells. Fig. 38 — 24 hrs after the start of imbibition — starch is present in the whole promeristem

PLATE XII

Figs. 39-44. Semi-schematic drawings of starch distribution in the roots after different periods of imbibition and germination. The time after start of imbibition is given. × 160. Fig. 39 — — 6 hrs — starch only in the columella. Fig. 40 — 9 hrs — starch outside columella in the lower hypocotyl part. Fig. 41 — 12 hrs — starch appears in the plerome cells and lateral root cap parts. Fig. 42 — 18 hrs — enlargement of the zones in which starch occurred formerly. Fig. 43 — 24 hrs — 1 mm long roots — maximum starch accumulation in whole root. Fig. 44 — 36 hrs — 5 mm long roots — almost complete disapearance of starch. Only statolit starch grains are present in the columella and endoderm cells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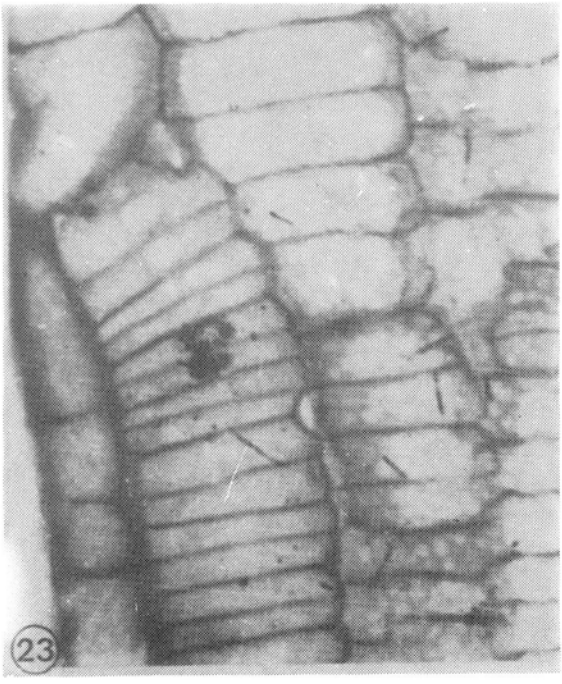
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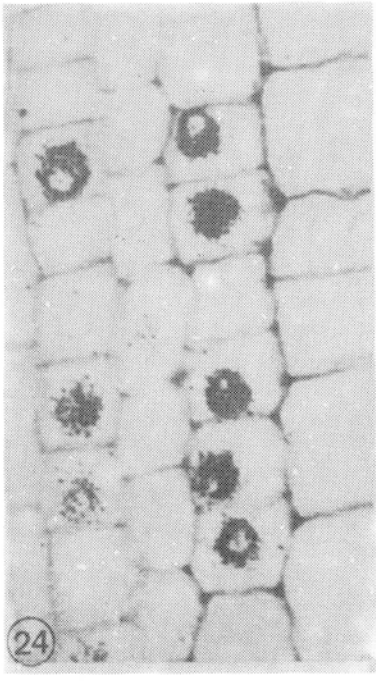
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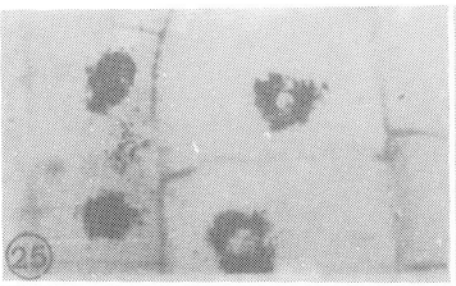
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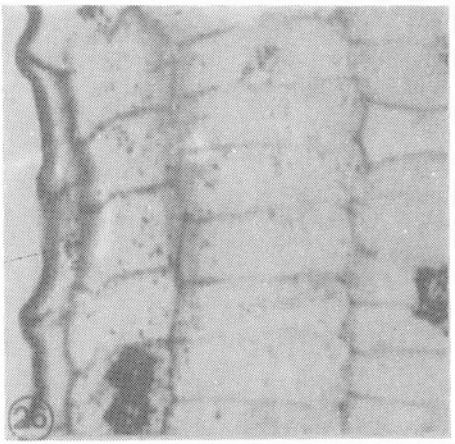
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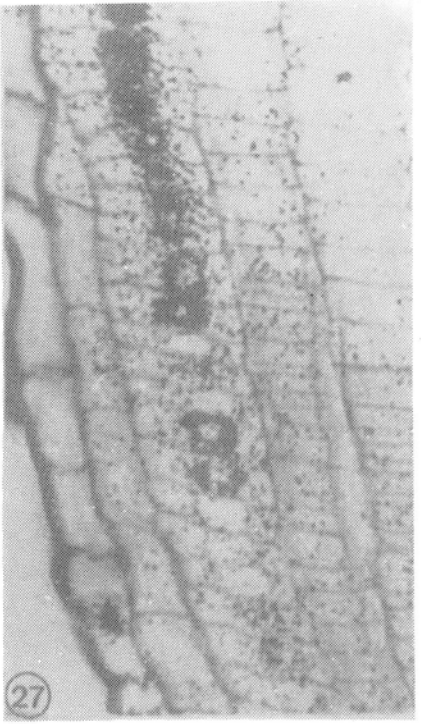
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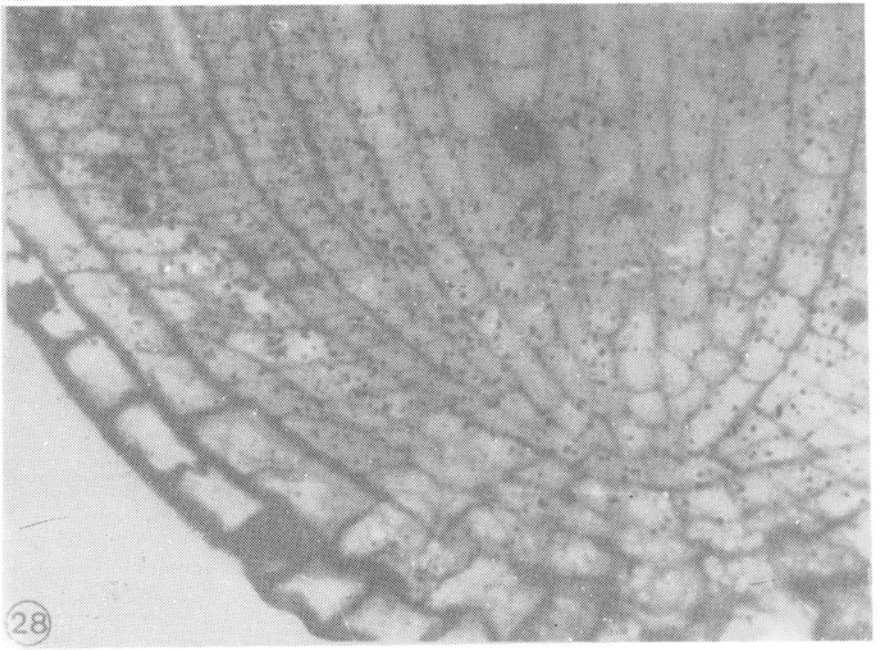
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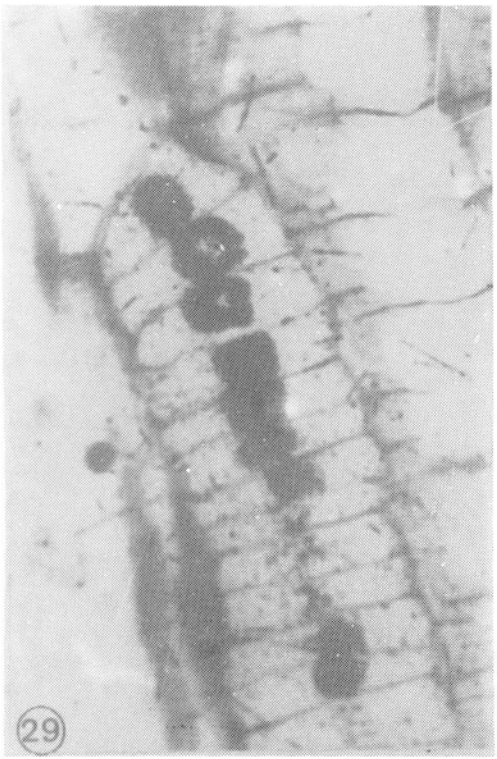
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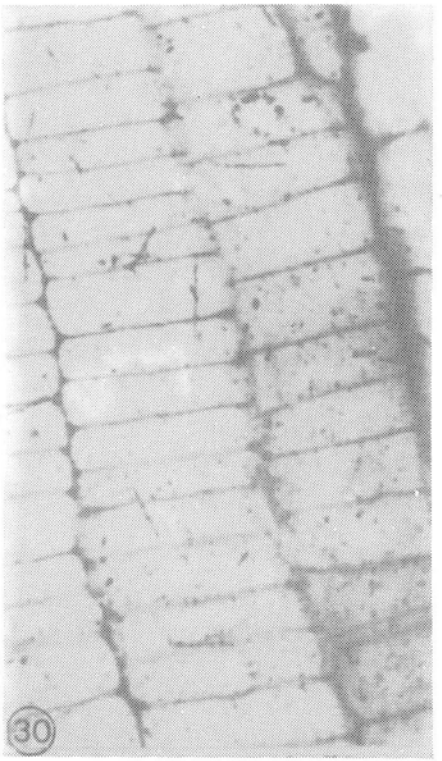
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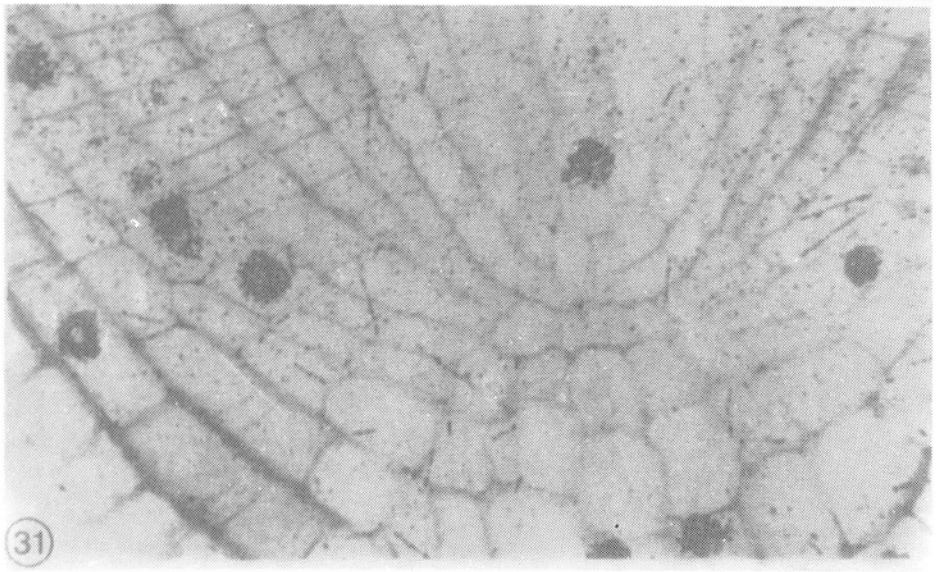
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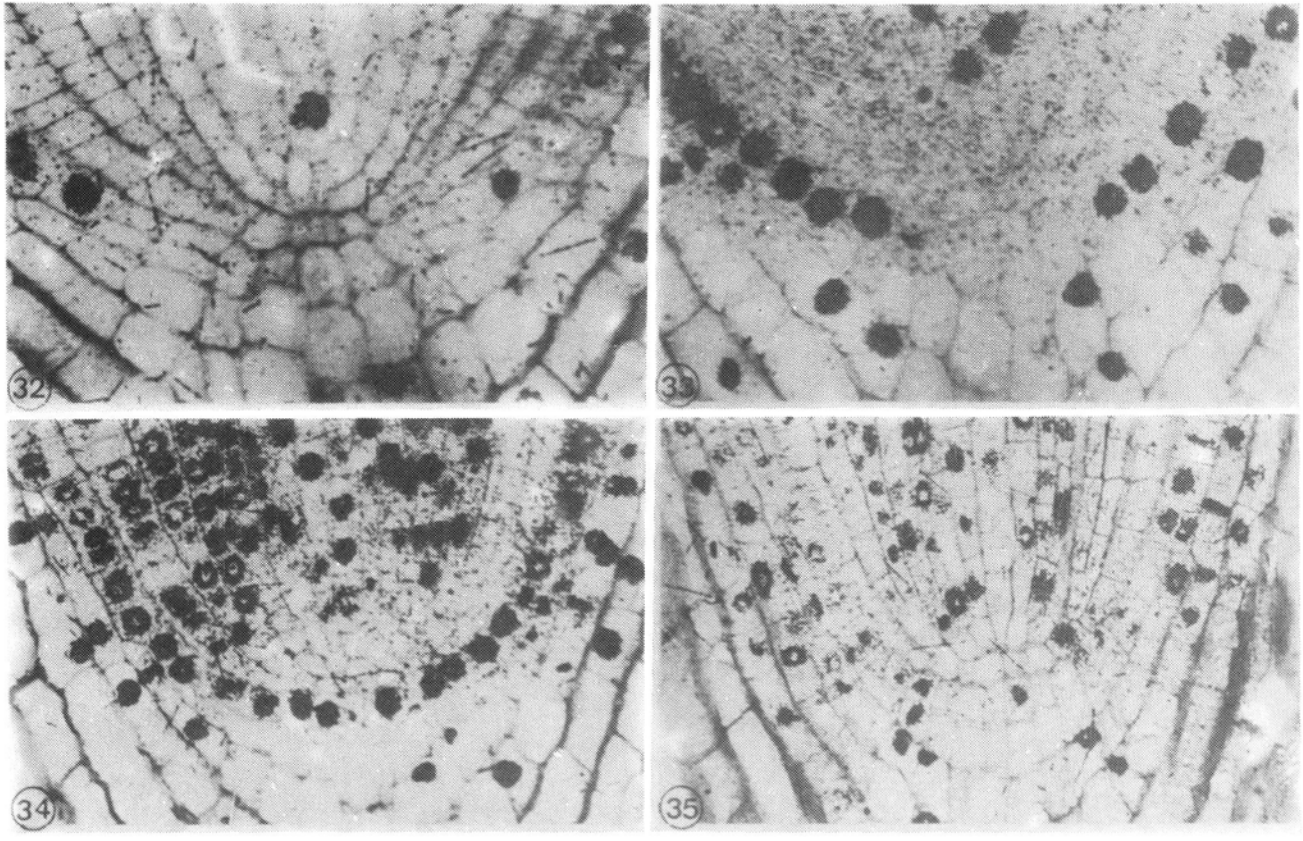
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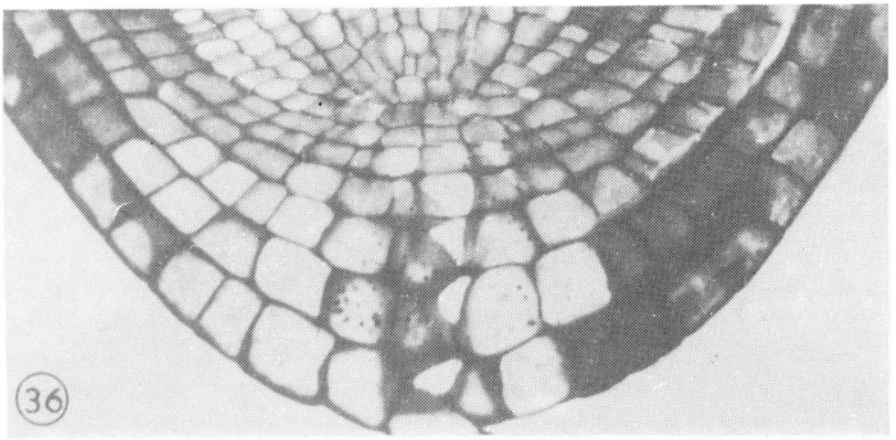
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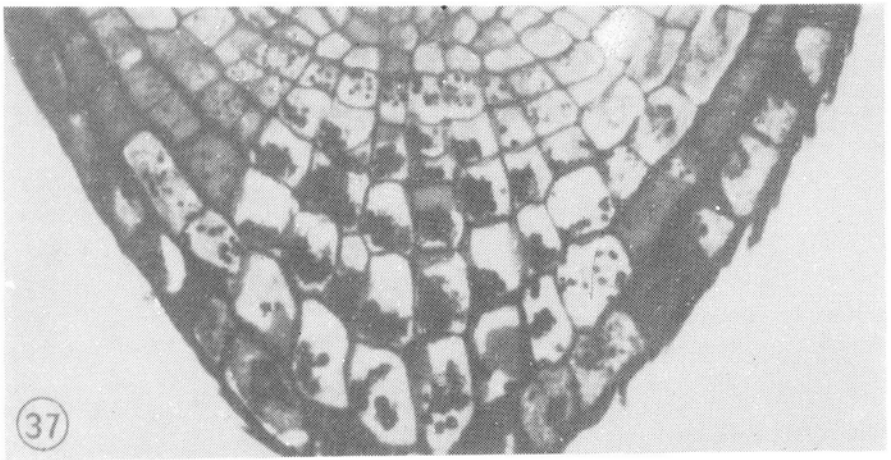
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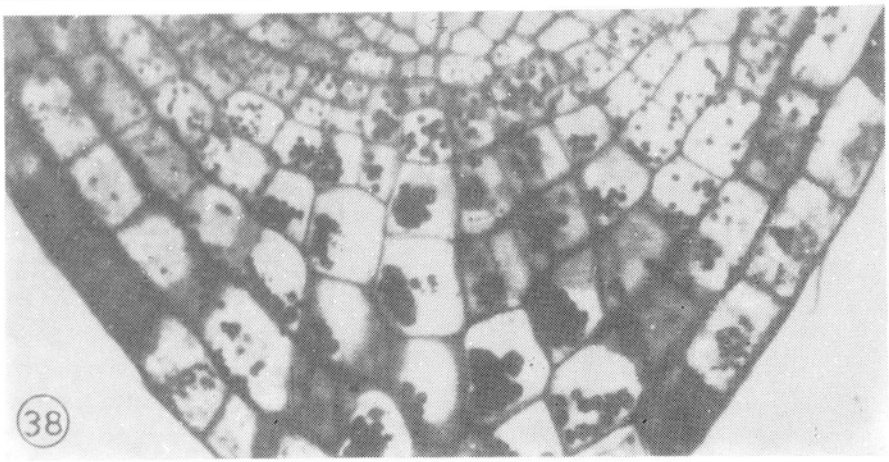
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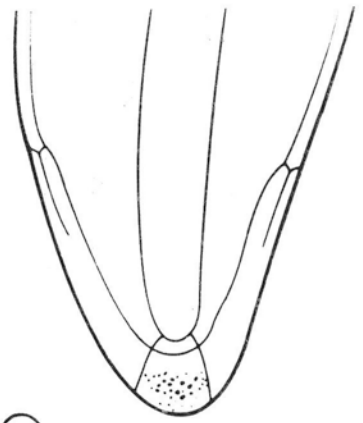

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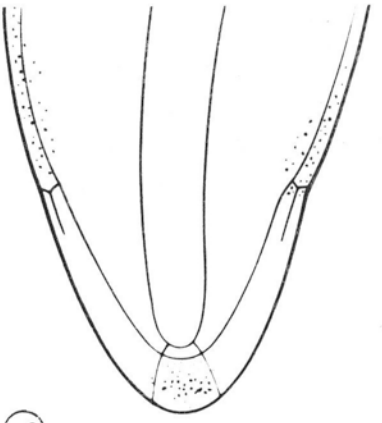

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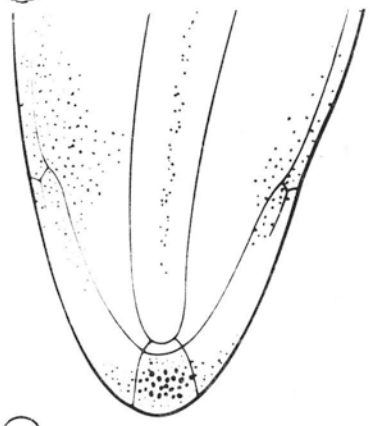

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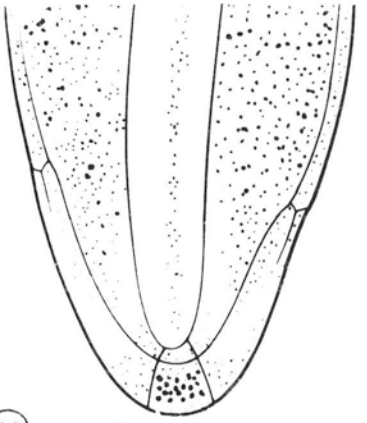

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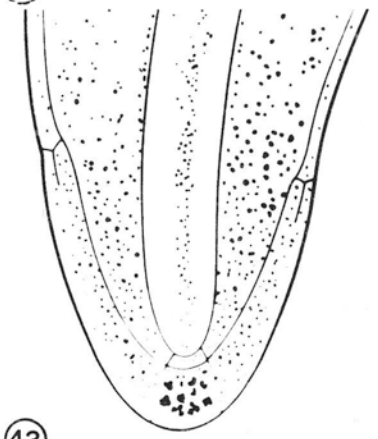

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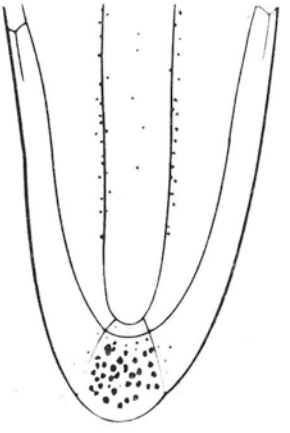

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underwent the phase of quiescence in phase G_2 not in G_1 as did most cells. Below the labelled nuclear zone there occur cells with distinct cytoplasmic labelling reflecting presumable mitochondrial DNA synthesis (Fig. 28).

In the roots just before germination (15 hrs H_2O + 6 hrs ^{3}H-thymidine) the dermatogen cells in the whole upper part of sector II as well as the slightly higher lying cells of the periblem and plerome are already labelled.

After 24 hrs the boundary of the labelled nuclei zone is still more depressed and appears in the dermatogen sector III and periblem sector II. In 3 hrs older roots this boundary was still more lowered; the labelled nuclei were in the dermatogen sector IV (Figs. 31 and 32) and periblem sector III. Above the labelled zone there is a zone of unlabelled cells (sector II and partly, sector I) (Fig. 30). It is significant that the continuous series of labelled cells occurs in the dermatogen sector I just below the hypocotyl (Fig. 29). In these cells the first DNA synthesis had been accomplished 18 hrs before. Therefore, it can be assumed that this labelling resulted from ^{3}H-thymidine incorporation during the second cell division cycle of these cells. Unlabelled cells situated between these two labelled zones, as may be presumed, either terminate the first mitotic cycle or begin the second one. In some roots of this length as well as in slightly longer ones (27 hrs H_2O + 6 hrs ^{3}H-thymidine) the zone of labelled dermatogen nuclei approaches the initial dermatocalyptrogen cells (Fig. 33), it does not, however, include the initial columella cells. In the periblem the zone of labelled nuclei is separated from the initials by some unlabelled cells. After further 3 hrs the initial columella cells also become labelled (Fig. 34). The cells of the remaining part of the initial centre and plerome are not labelled yet. All these cells from the area of a large quiescent centre. In further root development this area gradually diminishes as the zone of DNA synthesis shifts towards the apex. At the same time to the zone of the first DNA synthesis there approaches from above the zone of the second DNA synthesis. Finally, in roots fixed after 42 hrs (36 hrs H_2O + 6 hrs ^{3}H-thymidine) the wave of DNA synthesis has already attained the cortex and plerome initials restricting the zone of the quiescent centre to the intermediate cell layer (Fig. 35). Simultaneously, the zone of unlabelled cells between the zones of the first and second DNA synthesis have completely disappeared. The zone of the first synthesis is caught up by the second one, which in turn, is caught up by the third zone. Thus, as the initial centre cells enter the synthesis phase for the first time, the cells localized above them do this for the third time.

EXPANSION OF STARCH SYNTHESIS

Mature embryos do not contain any starch, within 3 hrs after soaking of the seeds, small starch grains begin to appear, first in the second, then in the third columella layer (Fig. 36). Then, the number and size of the grains increase and they appear around those columella layers (Figs. 37 and 39) as well as in the lower hypocotyl part — mainly in the dermatogen cells (Fig. 40). After 12 hrs starch grains are also present in the first root sector but the range of occurrence of the cells with starch is wider in the dermatogen than in the periblem (Fig. 41). At that time starch has already been synthesized in the whole columella and adjacent root cap cells (Fig. 37).

At the stage preceding germination, starch is already present in almost the whole root (Fig. 42). Most starch grains occur in the cells close to the hypocotyl boundary, whereas its amount diminishes acropetally. In the apical part of the periblem adjacent to the plerome there is no starch, but it is present in the cells of the intermediate layer and in the initial cortex cells.

In the outer layers of the lateral root cap parts it occurs at the level corresponding to the second dermatogen sector. However, in the inner root cap layers starch is localized only near the initial centre at the border of sector V. Thus, between the regions with starch there are regions without it — including the inner periblem layers the adjacent dermatogen sector (Fig. 42) and the inner layers of the root cap. Just after germination starch appears almost everywhere (Figs. 38 and 43), however, only some cells of the inner plerome, situated close to the initial centre, were without starch. At that time the amount of starch in the cells attains its maximum and during further root growth it disappears beginning from the zones undergoing elongation earliest. In the 5-mm stage starch is present only in the columella and endoderm cells (Fig. 44).

DISCUSSION

RAPE SEEDS AND THE METHOD OF HOMOLOGOUS SECTORS EXAMINATION

The choice of rape seeds was very important for studies on the activation of germinating seeds. Rape seeds, led to the state of full maturity under the transparent foil roof preventing their wetting in the siliques, are characterized by 100 per cent and simultaneous germination. The structure of the rape radicle and the ultrastructure of its cells in mature seeds was throughly examined (Kuraś 1978, 1980, 1984). Embryogenesis and structure of the mature embryo are well known (Tykarska 1976, 1979, 1980, Kuraś 1978, 1980), what allowed to establish the way of formation of the boundaries between

the roots, hypocotyl and epicotyl. The root cap of very regular structure was found to consist of the columella with its own initial cells and lateral cell layers protecting the surface of the whole embryonic root. Periclinal divisions of the initial dermatocalyptrogen cells allowed the successive lateral root cap cell layer to form very regularly. At the level of the upper boundary of the first root cap cell layer the anatomical structure of the embryo axis is distinctly changed. Therefore, it has been assumed that this is the level, where the boundary between the hypocotyl and root occurs. The upper boundaries of the successive deeper lying root cap cell layers were used for identification of the dermatogen and periblem sectors. In the radicle of the mature embryo there are four dermatogen sectors already established and a fifth apical dermatocalyptrogen sector (Kuraś 1978). The successive sectors formed during germination. In the growing roots of young seedlings the outer root cap layers are destroyed and hence the boundaries of the upper dermatogen sectors which at that time enter the phase of elongation cannot be recognized.

Comparative examination of homologous radicle sectors in successive germination stages led to the discovery of the regions where cell activation starts and of the wave-like spread of the latter in the radicle.

The distribution of root growth was studied by Sachs (1873) and present-day authors (e.g. Brumfield 1942, Goodwin and Stopka 1945, Hejnowicz 1956, 1959, Hejnowicz and Brodzki 1961) in fully active growing roots. By analysis of the homologous sectors it became possible to study growth distribution in germinating embryo roots.

EMERGENCE OF RADICLE

Rape radicle protrusion is due to cell elongation in the hypocotyl and the basal part of the radicle. Only after germination first mitoses appear in the basal radicle part. Without cell division there germinate also the seeds of broad bean (Wolf 1954), lettuce (Evenari et al. 1957, Haber and Luippold 1960, Fainbrun and Klein 1962), onion (Bryant 1969a, b) and probably those of many other plants. However, in cherry seed (Pollock and Olney 1959) and pine (Berlyn 1972) cell division start in the embryo axis before germination.

EXPANSION OF STARCH FORMATION AREA

The earliest symptom of rape embryo activation is starch formation. This process involves enzymatic transformation of storage lipids into saccharides, frequently taking part in many germinating seeds (Beevers 1961).

In the rape seeds, soon after the start of imbibition, starch grains appear in two strictly defined cell activation centres: in the dermatogen of the lower hypocotyl part and in the outer columella cells of the root cap. They are the regions where during embryogenesis starch is stored for the longest period (Tykarska 1982). The region of starch formation expends radially from the first of these centres to the periblem and plerome cell layers and along the hypocotyl and radicle. From the second centre, the region of starch occurrence expands basipetally onto the whole columella and adjacent lateral root cap parts and then to the promeristem. Just prior to germination starch fills all the embryonic cells and particularly, the embryonic root cells. At the moment of seed coat breakage the accumulated starch can be used for the intensified respiratory processes at that time and its presence may be a symptom of readiness to germination in oleiferous seeds, as for example in the stratified seeds described by Nikolaeva (1967), Villiers (1971), Dawidowicz-Grzegorzewska and Lewak (1978).

After germination starch disappears. This process gradually spreads onto the embryonic zones in the same sequence as when starch formation occurred in the initial period of germination. Thus, starch disappeared first from the hypocotyl cells at the boundary between the hypocotyl and root where cell elongation started. The starch disappearance zone expanded upwards and downwards along the embryo axis together with the expansion of the cell elongation zone. Starch subsisted longest in the root basal part as well as in the apical shoot meristem where elongation growth occurs latest. In the fully activated embryonic root starch remains only in the endoderm and columella cells.

ENDOMITOTIC DNA SYNTHESIS

Several hours after starch appearance cell growth and autoradiographically detected DNA synthesis begin. Both processes start exactly in the same two zones in which starch synthesis began, i.e. in the basal part of hypocotyl dermatogen and the apical columella part. It is significant that in these places the cells do not divide at all during germination. It can be assumed, therefore, that the labelling of these nuclei results from DNA endoreplication leading to endopolyploidy. Endoreplication in the permanent tissues was autoradiographically localized in the root of *Vicia faba* (Pelc and La Cour 1959), in the root cap of barley (Stein and Quastler 1963), in the hypocotyl of *Lactuca sativa* (Fainbrun and Klein 1962). The increase of DNA amount was also biochemically detected in the hypocotyl of *Lens culinaris* seedlings (Nitsau and Lang 1966) as well as in the hypocotyl of *Sinapis alba* seedlings (Capesius and Bopp 1970, 1974). The high endopolyploidy level reaching to almost 512C was also evaluated cytophoto-

metrically in the cells of various *Scilla* organs (Frish and Nagl 1979). From our experiments it may be concluded that DNA endoreplication beginning shortly after cell imbibition in the lower hypocotyl part spreads to the whole hypocotyl and continues throughout the whole experimental period i.e. 42 hrs. It may be associated with the fact that the *Brassicaceae* family is characterized by a low DNA content in 2C nuclei (*Raphanus sativus* = 1.25 pg) and DNA endoreplication in the hypocotyl may exceed 64C (Olszewska and Osiecka 1983).

In imbibed rape seeds, apart from the hypocotyl cells, also the columella and lateral root cap cells, which do not undergo any divisions, reveal intensive labelling. Similarly the columella cells of the *Zea* root cap may be in state 2C to 8C (Stein and Quastler 1963, Clowes 1968). The high polyploidy level may be closely related to the intensive elongation of root cap cells in the germinating embryo. According to Nagl (1978) as well as Frish and Nagl (1979) endoreplication in the hypocotyl and root cap cells leads to an increase of metabolic activity and intensification of growth.

The zone of DNA replication in the rape hypocotyl shifting downward reaches the radicle cells. In the radicle the first nuclei are labelled in the basal part of the dermatogen sector I, thus, exactly in the place where later the first mitoses will occur.

MITOTIC DNA SYNTHESIS

The relationship between DNA synthesis and mitotic activity in the germinating seeds has been described in various way. Some authors show that DNA synthesis precedes cell division in the activated embryos. The spreading pattern of these processes may be fully convergent as in the Vicia faba primary root where the wave of DNA synthesis is followed by the wave of synchronic mitoses (Jacob and Bovey 1969). In lettuce roots DNA synthesis precedes the mitoses (Fainbrun and Klein 1962) and in *Zea* roots DNA synthesis outstrips labelling of mitoses by 3 hrs. However, it was also detected in *Zea* roots that the first mitoses preceded nuclear labelling (Stein and Quastler 1963). A similar process was observed in the roots of *Xantus* (Arnason et al. 1966), *Allium cepa* (Bryant 1969a and b, Jensen et al. 1960) and *Glycine max.* (Miksche 1966). The divergence of these results can be due to the fact that in some species a great number of embryonic cells enter quiescence before DNA replication, whereas in others they do so afterwards. Moreover the result may be also affected by the different permeability of the seed coat to the precursor or by other reasons, e.g. even a well permeating precursor may not be included into replicating DNA (Bewley and Black 1983).

In germinating rape seeds the beginning of mitotic activity is strictly

dependent on DNA synthesis. First labelled are the nuclei in the basal part of the dermatogen sector I, i.e. exactly at the same site where 12 hours later the first mitoses will appear. This labelling is, then, an indication of mitotic DNA synthesis. At this time no other cells of the root proper body have labelled nuclei. Therefore, it would seem that they should be in phase G_1. As can be concluded from our unpublished studies, in the radicle germinating in ^{3}H-thymidine solution, only 3.5 per cent of the first mitoses were unlabelled, that is such number of cells attained phase G_2 before quiescence. All other cells were in phase G_1. Similarly, the radicles of some other species contained both G_1 and G_2 cells as for instance the embryos of *Vicia faba* (Jakob and Bovey 1969), soybean and wheat (Avanzi et al. 1963), *Triticum durum*, *Hordeum vulgare*, *Zea mays*, *Pisum sativum* and *Crepis capillaris* (Bewley and Black 1983). Cells solely in G_1 are reported in the quiescent embryo of *Pinus pinea*, *Lactuca sativa*, *Allium cepa* and *Tradescantia paludosa* (Bewley and Black 1983).

The acropetal wave of DNA synthesis leads to reduction of the zone of G_1 nuclei and enlargement of the zone of G_2 nuclei localized above the DNA synthesis zone. Thus, for example, when the zone of labelled nuclei occupies the lower half of sector I and upper half of sector II, the cells situated just above neither reveal labelling nor divide, so it can be presumed that they are in phase G_2. Several hours later mitoses appear in the former DNA synthesis zone. The first mitoses occur in the basal root part where 12 hours earlier the first labelled nuclei had appeared. The long time interval between synthesis and mitosis is striking. Perhaps the first mitotic cycle in the germinating embryos is much longer than in the cells with full metabolic activity in growing roots. Consistent with this presumption is the appearance of the first labelled mitoses in barley 20 hrs after the first labelled nuclei appeared (Arnason et al. 1966). Against this hypothesis are, however, other data (Fainbrun and Klein 1962) according to which in lettuce embryos labelled mitoses appear already 1 hour after the labeled nuclei, that is within a much shorter period than the one usually reported for phase G_2. Bryant (1969a and b) found that the mean duration of the mitotic cycle was very similar in onion roots of different lengths. The shortest roots examined (5 mm long) were probably already fully active. This result might, therefore, be different if the first mitotic cycle were studied in shorter roots.

In rape the zone of mitoses formed in the basal part of root sector I shifts in apical direction, at a certain distance behind the DNA synthesis zone. When the DNA synthesis zone approaches the promeristem, a new cycle of DNA synthesis begins in the basal part of sector I. It is of endoreplicating nature as the cells of this zone started to elongate without undergoing further divisions just after the first cell division cycle. The cells of sector I localized below, underwent after the second DNA synthesis a second

mitotic cycle. This mitotic zone shifts in apical direction as did the first one. The first and the second zone of mitosis separated by a zone of cells in phase G_1, S and G_2. This zone diminishes along with root growth so that the second zone of mitoses approaches the first one. It may be suggested that this results from the difference in the duration of the mitotic cycle in various root parts. There are no doubts (Clowes 1961a and b) that in the apical root meristem the initials and their closest derivatives, constituting the quiescent centre, divide less frequently than do the cells in their vicinity. Cells in the basal meristem part at the elongation zone border also divide more slowly, as demonstrated even on smaller cell families by Lopez-Saez et al. (1966). It may be inferred from this and many other papers (e.g. Holmes et al. 1955, Erickson and Sax 1956, Jensen et al. 1960) that the cells localized in the central meristematic zone divide fastest. Thus, as the result of mitotic cycle extension in apical direction of the root cells in the first mitotic cycle are approached by waves of successive mitotic cycles released in the most active root meristem zone. In the root, 41 hours after the start of imbibition, when the first mitotic cycle zone is in sectors IV and V, whereas that of the second one in sector III, the third mitotic cycle begins in sector II. Along the root of 30 mm long seedlings, 57 hrs after the start of imbitition the four successive mitotic zones can be identified, i.e. of the first, second, third and fourth mitotic cycle. The successive mitotic cycles begin lower and lower, as the cell activation zone shifts in apical direction.

Shifting towards the apex, the first mitotic cycle zone restricts the range of the so far inactive zone of G_1 cells, which according to Clowes (1958), Miksche (1966) and Miksche and Greenwood (1966) may be treated as the quiescent centre (quiet zone). In the oldest examined roots this zone includes only cells of the intermediate layer of the promeristem, thus, it exactly corresponds to the quiescent centre in 5 mm long roots of *Sinapis alba* as described by Clowes (1958). Later this centre considerably enlarges. A similar development of the quiescent centre occurs, according to the same author, in developing adventitious roots of *Pistia* and *Euchoria* and, according to Miksche (1966) and Miksche and Greenwood (1966), in the roots of *Glycine max*. germinating seeds. Most likely the quiescent centre in the growing rape roots will attain the typical and constant form. Its shape and size depend on the activation timing and the degree expansion in apical direction of DNA synthesis activity.

RESUMPTION OF RADICLE GROWTH

In the activated radicle there is close convergence of starch and DNA synthesis and localization. At first in two distant regions (the hypocotyl

cells above the radicle boundary and the columella cells on the root cap tip) starch synthesis and accumulation begin. Then endoreplicating DNA synthesis follows. These biosyntheses and others resumed in activated cells enable growth initiation in these parts of the embryo axis. In the radicle two growth waves from opposite directions translocate, the main one shifting through the root proper body from the earlier activated hypocotyl, directed acropetally, and the second one shifting through the root cap from the earlier activated part of the columella, directed basipetally. The resumed radicle growth is differentiated. The first basal sector of the radicle and the root cap are the first to elongate. Then, growth in the successive lower sectors of the meristematic radicle is resumed.

Acknowledgment

The author wishes to express his warmest thanks to prof. B. Rodkiewicz and to prof. H. Teleżyński for their valuable help in elaboration of the research results and final version of the text. Warm thanks are also due to J. Auguścik, M. Sc. and U. Lechnio, M. Sc. for their excellent technical assistance.

REFERENCES

Arnason T. J., El-Sadek L. M., Minocha J. L., 1966. Effects of some variations of treatment-methods on mutation frequency in barley treated with some monofunctional alkylating agents. Can. J. Genet. Cytol. 8: 746-755.

Avanzi S., Brunori A., D'Amato F., Nuti Rouchi V., Scaracia Muguozza G. T., 1963. Occurance of 2C (G_1) and 4C (G_2) nuclei in the radicle meristems of dry seeds in *Triticum durum* DESF. It is implication in studies on chromosome breakage and on developmental processes. Caryologia 16: 553-558.

Beevers H., 1961. Metabolic production of sucrose from fat. Nature 191: 434-436.

Beneš K., 1968. On the stainability of plant cells walls with alcian blue. Biol. Plant. (Praha) 10: 334-346.

Berlyn G. P., 1972. Seed germination and morphogenesis. In: Seed biology. Kozlowski T. T. (ed.), Academic Press, New York-London. vol. I, pp. 223-312.

Bewley J. D., Black M., 1983. Physiology and biochemistry of seeds in relation to germination. Vol. 1. Development, germination and growth. Springer, Berlin-Heidelberg-New York.

Brumfield R. T., 1942. Cell growth and division in living meristems. Amer. J. Bot. 29: 533-543.

Bryant T. R., 1969a. DNA synthesis and cell division in germinating onion. I. Onset of DNA synthesis and mitosis. Caryologia 22: 127-138.

Bryant T. R., 1969b. DNA synthesis and cell division in germinating onion. II. Mitotic cycle and DNA content. Cyryologia 22: 139-148.

Capesius I., Bopp M., 1970. Über die Synthese von DNS in den Keimpflanzen von *Sinapis alba* L. Planta (Berl.) 94: 220-228.

Capesius I., Bopp M., 1974. Evidence of DNA synthesis in hypocotyl and cotyledon of inhibited and non inhibited seedlings of *Sinapis alba*. Planta (Berl.) 118: 171-181.

Clowes F. A. L., 1958. Development of quiescent centres in root meristems. New Phytol. 67: 85-88.

Clowes F. A. L., 1961a. Duration of the mitotic cycle in a meristem. J. Exp. Bot. 12: 283-293.

Clowes F. A. L., 1961b. Rates of mitosis in a partially synchronuous meristem. New Phytol. 61: 111-118.

Clowes F. A. L., 1968. The DNA content of the cells of the quiescent centre and root cap of *Zea mays*. New Phytol. 67: 631-639.

Dawidowicz-Grzegorzewska A., Lewak S., 1978. Anatomy, histochemistry and cytology of dormant and stratified apple embryos. I. General observations and changes in the starch content during after-ripening of seeds. New Phytol. 81: 99-103.

Evenari M., Klein S., Anchori H., Fainbrun N., 1957. The beginning of cell division and cell elongation in germinating lettuce seeds. Bull. Res. Counc. Israel 6D: 33-37.

Ericson R. O., Sax K. B., 1956. Rates of cell division and cell elongation in the growth of the primary root of *Zea mays*. Proc. Amer. Phil. Soc. 100: 499-512.

Feinbrun Naomi, Klein S., 1962. ^{3}H-thymidyne incorporation into cell nuclei in germinating lettuce seed. Plant Cell Physiol. 3: 407-413.

Frisch B., Nagl W., 1979. Patterns of endopolyploidy and 2C nuclear DNA content (Feulgen) in *Scilla* (*Liliaceae*). Plant Syst. Evol. 131: 261-276.

Goodwin R. H., Stopka W., 1945. Growth and differentiation in the root tip of *Phleum pratense*. Amer. J. Bot. 32: 36-46.

Haber A. H., Luippold H. J., 1960. Separation of mechanisms initiating cells division and cell expansions in lettuce seed germination. Plant Physiol. 35: 168-173.

Hejnowicz Z., 1956. Wzrost i różnicowanie w korzeniu *Phleum pratense* . I. Rozmieszczenie wzrostu podłużnego w korzeniu. Growth and differentiation in the root of *Phleum pratense*. I. Growth distribution in the root. Acta Soc. Bot. Pol. 25: 459-478.

Hejnowicz Z., 1959. Growth and cell division in the apical meristem of wheat roots. Physiol. Plant. 12: 124-138.

Hejnowicz Z., Brodzki P., 1961. The growth of root cells as the function of time and their position in the root. Acta Soc. Bot. Pol. 29: 625-644.

Holmes B. E., Mec L. K., Hornsy S., Gray L. H., 1955. The nucleic acid content of cells in the meristematic, elongating and fully elongated segments of roots of *Vicia faba*. Exp. Cell. Res. 8: 101-113.

Jakob K. M., Bovey F., 1969. Early nucleic acid and protein synthesis and mitoses in the primary root tips of germinating *Vicia faba*. Exp. Cell Res. 54: 118-126.

Jensen W. A., Kavaljian L. G., Martinot S., 1960. The incorporation of ^{3}H-thymidine by developing root tip cells. Exp. Cell Res. 20: 361-367.

Kuraś M., 1978. Activation of embryo during rape (*Brassica napus* L.) seed germination. I. Structure of embryo and organisation of root apical meristem. Acta Soc. Bot. Pol. 47: 65-82.

Kuraś M., 1980. Activation of embryo during rape (*Brassica napus* L.) seed germination. II. Transversal organization of radicle apical meristem. Acta Soc. Bot. Pol. 49: 387-395.

Kuraś M., 1984. Activation of embryo during rape (*Brassica napus* L.) seed germination. III. Ultrastructure of dry embryo axis. Acta Soc. Bot. Pol. 53: 171-186.

Kuraś M., Teleżyński H., 1978. Influence of hydroxurea on the course of germination and growth of rape (*Brassica napus* L.) seedlings. Acta Soc. Bot. Pol. 47: 131-141.

Lopez-Saez J. F., Gimenez-Martin G., Gonzalez-Fernandez A., 1966. Relative velocity of the division cycle in the sister cells. Experientia 22: 501-502.

Mc Manus J. F. A., 1948. Histological and histochemical uses of periodic acid. Stain Technol. 23: 99-108.

Miksche J. P., 1966. DNA synthesis in primary root of *Glycine max*. during germination. Can. J. Bot. 44: 789-794.

Miksche J. P., Greenwood M., 1966. Quiescent center in the primary root of *Glycine max*. New Phytol. 65: 1-4.
Nagl W., 1978. Endopolyploidy and polyteny in differentiation and evolution. North Holland, Amsterdam.
Nikolaeva M. G., 1967. Physiology and deep dormancy of seeds. Nauka, Leningrad.
Nitsau J., Lang A., 1966. DNA synthesis in the elongating non dividing cells of the lentil epicotyl and its promotion by gibberelin. Plant Physiol. 41: 965-970.
Olszewska M. J., Osiecka R., 1983. The relationship between 2C DNA content, life cycle type, systematic position and the dynamics of DNA endoreplication in parenchyma nuclei during growth and differentiation of roots in some dicotyledonous herbaceus species. Biochem. Physiol. Pflanzen 178: 581-599.
Pelc S. R., La Cour L. F., 1959. The incorporation of ^{3}H-thymidine in newly differentiated nuclei of roots of *Vicia faba*. Experientia 15: 131-133.
Pollock B. M., Olney H. D., 1959. Studies of the rest period. I. Growth, translocation, and respiratory changes in the embryonic organs of the after-ripening cherry seed. Plant Physiol. 34: 131-142.
Sachs J., 1873. Über das Waschstum der Haupt und Neben-Wurzeln. Arb. Bot. Inst. Wurzburg, 1.
Sass J. E., 1940. Elements of botanical microtechnique. Ames, New York-London, pp. 69-71.
Stein O. L., Quastler H., 1963. The use of tritiated thymidine in the study of tissue activation during germination in *Zea mays*. Amer. J. Bot. 50: 1006-1011.
Tykarska T., 1976. Rape embryogenesis. I. The proembryo development. Acta Soc. Bot. Pol. 45: 3-16.
Tykarska T., 1979. Rape embryogenesis. II. Development of embryo proper. Acta Soc. Bot. Pol. 48: 391-422.
Tykarska T., 1980. Rape embryogenesis. III. Course embryo development in time. Acta Soc. Bot. Pol. 49: 369-385.
Tykarska T., 1982. Rape embryogenesis. IV. Appearance and disappearance of starch during embryo development. Acta Soc. Bot. Pol. 51: 381-387.
Villiers T. A., 1971. Cytological studies in dormancy. I. Embryo maturation during dormancy in *Fraxinus excelsior*. New Phytol. 70: 751-760.
Wolf S., 1954. Some aspects of the chemical protection against radiation damage of *Vicia faba* chromosomes. Genetics 39: 356-364.

Aktywacja zarodka w czasie kiełkowania nasion rzepaku (Brassica napus). IV. Kiełkujący zarodek. Pierwsze strefy mitoz, syntezy skrobi i DNA oraz wzór ich rozprzestrzeniania

Streszczenie

Korzeń zarodkowy w nasieniu rzepaku jest cały pokryty czapeczką. Boczne warstwy czapeczki są wyraźnie oddzielone granicami, które nie zmieniają się zasadniczo w czasie rozwoju. Posłużyły one zatem do wyznaczenia sektorów dermatogenu i peryblemu. Opierając się na tym, zbadano w kolejnych fazach rozwoju korzenia długość odpowiadających sobie sektorów, liczbę ich komórek oraz zlokalizowano w nich syntezę skrobi, DNA oraz podziały komórkowe. Wszystkie te procesy zaczynają się w stałej kolejności, w określonych strefach zarodka. Pierwszym obserwowanym przejawem aktywności zarodka jest synteza skrobi. Rozpoczyna się ona w dwu ośrodkach: w apikalnej części kolumelli oraz w derma-

togenie hypokotyla nad granicą z korzeniem. Z aplikalnej części kolumelli aktywacja syntezy skrobi przesuwa się bazypetalnie na całą kolumellę i centrum inicjalne oraz na boczne części czapeczki. Z dermatogenu hypotokyla przemieszcza się w głąb kory oraz w obu kierunkach wzdłuż osi zarodka. W dermatogenie i periblemie korzenia, strefa aktywacji obejmuje najpierw bazalny sektor a później stopniowo coraz niższe sektory. Bezpośrednio przez wykiełkowaniem następuje zetknięcie się bazalnej i apikalnej strefy aktywacji. Skrobia występuje wtedy w całym korzeniu.

W bazalnej części korzenia, podczas syntezy skrobi zaczyna się synteza DNA oraz wzrost komórek. W sektorach korzenia, które zaczęły rosnąć i syntetyzować DNA zaczynają się podziały komórkowe. Granica strefy dzielących się komórek przesuwa się akropetalnie w pewnej odległości za dolną granicą strefy syntezy DNA i strefą wydłużania komórek. Można to porównać z rozprzestrzeniającymi się kolejnymi falami aktywacji. W ślad za pierwszą falą aktywacji rozprzestrzeniają się przynajmniej trzy następne fale, po czym następuje asynchronizacja tych procesów w wierzchołku korzenia. Aktywacja czapeczki jest częściowo niezależna od akropetalnej fali aktywacji, a jest wynikiem rozprzestrzeniającej się słabszej fali bazypetalnej.