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# Activation of rape (*Brassica napus* L.) embryo during seed germination. III. Ultrastructure of dry embryo axis

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Abstract

Mature dry winter rape (*Brassica napus* L.), var. *oleifera*, cv. Górczański embryos were studied in the light and the electron microscope. Considerable modifications and regression of the cell ultrastructure were noted in the resting embryo as compared with the metabolically active cells. The degree of regression of the ultrastructure differed in the particular organs and tissues of the embryo. Of most regressed character are the cells of the storage organs — the hypocotyl and cotyledones. They are almost completely filled with protein and lipid bodies. The small spaces between them are filled with dense cytoplasm with a lobular nucleus and not numerous, difficult to identify, plastids and mitochondria. The cells of the shoot primordium and radicle, particularly of the protoderm at the boundary of the hypocotyl and root and columella of root cap have a less regressed ultrastructure. They contain less storage material, a less dense cytoplasm and nearly all cell organelles with a normal appearance. The mitochondria are quite numerous with rather large cristae. Plastids are large with characteristic infolds filled with cytoplasm and some lamellae and a few agglomerations of plastoglobules. The nucleus is lobular with distinctly double and porous nuclear envelope and uniformly dense nucleolus. These cells do not contain dictyosomes and the ER is reduced to short, mostly rough cisternae and vesicles. Cells within the columella itself are also differentiated. The least regression of ultrastructure is seen in the cells of external layers containing the most numerous and most active looking mitochondria and more ER structures. The promeristem cells are similar to those of the deeper columella layers but their mitochondria are more regressed. The cells of the lateral parts of the cap and radicle cells, distant from the promeristem are more similar to the hypocotyl cells.

*Key words: Brassica napus L., rape, ultrastructure, dormant embryo.*

## INTRODUCTION

Seeds are a very convenient object in the investigations of structure and function in plant ontogenesis. Changes in metabolic activity in the course of their development, dormancy and germination are, mainly, connected with structural modifications which mostly can be visualised in the electron microscope. Therefore, in the last two decades numerous papers have appeared dealing with cell ultrastructure at various phases of embryo development. Most of these publications concern the phases exhibiting a high metabolic activity, that is the final period of embryogenesis (Engleman 1966) or the initial stage of germination (Bagley et al. 1963, Nieuwdorp and Buys 1964, Srivastava and Paulson 1968, Durzan et al. 1971, Swift and O'Brien 1972, Mia and Durzan 1977, Webster and Leopold 1977). Quite a lot of information has been accumulated on these stages of embryonal development. There is much less of it concerning, the cell ultrastructure of the fully ripe dry dormant embryo. Although some studies as those of Nieuwdorp (1963), Yatsu (1965) and Paulson and Srivastava (1968) are wholly devoted to this problem and in many others the embryo structure is described at least fragmentarily, it is still commonly considered that the knowledge on this subject is insufficient. The main cause of this are the technical difficulties connected with investigation of the ultrastructure of mature embryos. Their dry cells almost completely filled with storage substances and covered with thick cell walls are just a little permeable to fixatives and epoxy resins (Setterfield et al. 1959, Bagley et al. 1963, Nieuwdorp 1963, Öpik 1965, Yatsu 1965, Paulson and Srivastava 1968, Mollenhauer and Totten 1971a, Swift and O'Brien 1972, Webster and Leopold 1977 and others).

In the present investigations, after the application of various methods of fixation to the dry embryo, best proved the fixation in glutaraldehyde with a short postfixation in $KMnO_4$. In these investigations the ultrastructure of all the embryo organs is compared, but mainly that of the columella and root cap cells, since the extremely regular spacial arrangement of the cells in this part of the embryo makes identification of the same layers possible in various embryos. This greatly facilitates the obtention of precise information on the structure of cells in the successive steps of swelling and germination of seeds. The results of these studies together with the earlier published ones (Kuraś 1978, 1980) concerning the anatomical structure of the axis of the mature embryo will serve as a reference point in anatomic-developmental, cytochemical and ultrastructural investigations on the factors determining the germination process.

## MATERIAL AND METHODS

Seeds of winter rape (*Brassica napus* L.), var. *oleifera*, cv. Górczański were derived form plants cultivated on our experimental field which in the end phase of ripening were covered with a foil roof. The seeds were collected successively as they ripened. For the experiment we only chose seeds of the same size and with a completely black husk. They were highly uniform, germinated almost simultaneously and in 100 per cent. From the isolated embryos the tips of roots and shoots, the middle part of the hypocotyl and 1-mm fragments of cotyledones were cut off and fixed for 12 h in 3 per cent glutaraldehyde, pH 6.8 (0.1 M cacodylate buffer). They were postfixed in 2 per cent aqueous $KMnO_4$ for 20 min or in 2 per cent $OsO_4$ for 2 h. The material was dehydrated in ethanol and embedded in Epon 812. Longitudinal sections about 1 $\mu$m thick were stained in 0.1 per cent toluidine blue. Ultrathin sections were stained for 30 min in uranyl acetate solution and for the next 30 min in Reynolds reagent and then inspected in a BS-500 Tesla electron microscope.

## RESULTS

In a mature dry embryo prepared out from a dry seed the cell structure is greatly differentiated. Even in the light microscope differences can be seen in the structure of the particular organs (Figs. 1-11). They are mainly the result of differentiation in the size and structure of storage materials — protein and lipid bodies. These bodies are not numerous in the cells of the shoot and root apical meristems and particularly in the columella and protederm cells at the boundary of the rootlet and hypocotyl. The cells of the remaining parts of the embryo — the hypocotyl and cotyledones — are almost completely filled with storage material. The main component of cells of these organs are protein bodies. Their structure is similar to that in other plant species: they are surrounded by a single membrane especially well visible after fixation in $KMnO_4$. They are usually completely filled with homogeneous ground substance with numerous globoids. There are, however, no crystalloids there (Figs. 12-16). The protein bodies usually occupy the central part of the cell, but in various organs and tissues of the same organs they differ in size, shape and structure.

In the mesophyll of cotyledone cells, where the main storage material are lipids, the protein bodies are less numerous than in the hypocotyl. They are mostly small, spherical or oval and freely disseminated over the whole cell (Figs. 2 and 3). In the basal part of the cotyledones the protein bodies attain very large sizes, their shapes are irregular and ameboidally folded (Fig. 5). They most frequently are tightly packed

## Legend to plates and photographs

Abbreviations used: Ci — cytoplasm islet, co — columella, cor — cortex, Cw — cell wall, dc — dermatocalyptrogen, en — endoderm, ER — endoplasmic reticulum, Gl — globoid, Gv — Golgi vesicles, icc — plerome initial, ico — columella initial, iec — layer of central connecting cells, L — lamellae, Lb — lipid body, M — mitochondrion, mv — metaxylen vessel mother cells, N — nucleus, Nu — nucleolus, Pb — protein body, pd — protoderm, pe — pericycle, Pg — plastoglobules, Pl — plastid, pr — procambium, prm — promeristem, rc — root cap, V — vacuole.

Plate I. Cytological differentiation of successive zones of embryo, upper part. Longitudinal semithin section. Glut./$OsO_4$. Toluidine blue. × 700

Fig. 1. Diagram of embryo structure with marked zones of structure and ultrastructure investigations. 1 — protoderm and parenchyma of central part of cotyledones, 2 — parenchyma of basal part of cotyledones, 3 — cortex of upper hypocotyl part, 4 — plumule, 5 — cortex of central hypocotyl part, 6 — hypocotyl-radicle boundary, 7 — radicle promeristem and columella

Fig. 2. Cotyledon. Protoderm and palisade parenchyma. Cells completely filled with lipid and protein bodies (black)

Fig. 3. Cotyledon. Spongy parenchyma. Storage material as in palisade parenchyma

Fig. 4. Plumule. Protein bodies (grey and black), between them wide areas of cytoplasm (clear)

Fig. 5. Cotyledon base cells. Protein bodies (black and grey), scare lipids and small areas of cytoplasm

Fig. 6. Cortex cells of upper part of hypocotyl. Storage material as in Fig. 5

Fig. 7. Cortex cells of central part of hypocotyl. Protein bodies smaller but more numerous fill almost the whole cell

Plate II

Fig. 8. Differentiation of protein bodies in successive tissues of hypocotyl-radicle boundary. Few protein bodies in the protoderm and an increase of their number in cortex cells. Numerous small spherical protein bodies in central cylinder with exception of central metaxylem vessel where these bodies are absent

Fig. 9. Promeristem. Cells with homogeneous clear cytoplasm without storage substances

Fig. 10. Protein bodies in cells of lateral root cap parts and their absence in columella cells

Fig. 11. Protein bodies in the two lowest layers of the lateral root cap parts and first columella layer

Plate III. Ultrastructure of cotyledone and hypocotyl cortex cells.

Fig. 12. Cotyledone mesophyll. Numerous lipid bodies and single protein bodies. Glut./$OsO_4$. × 9000

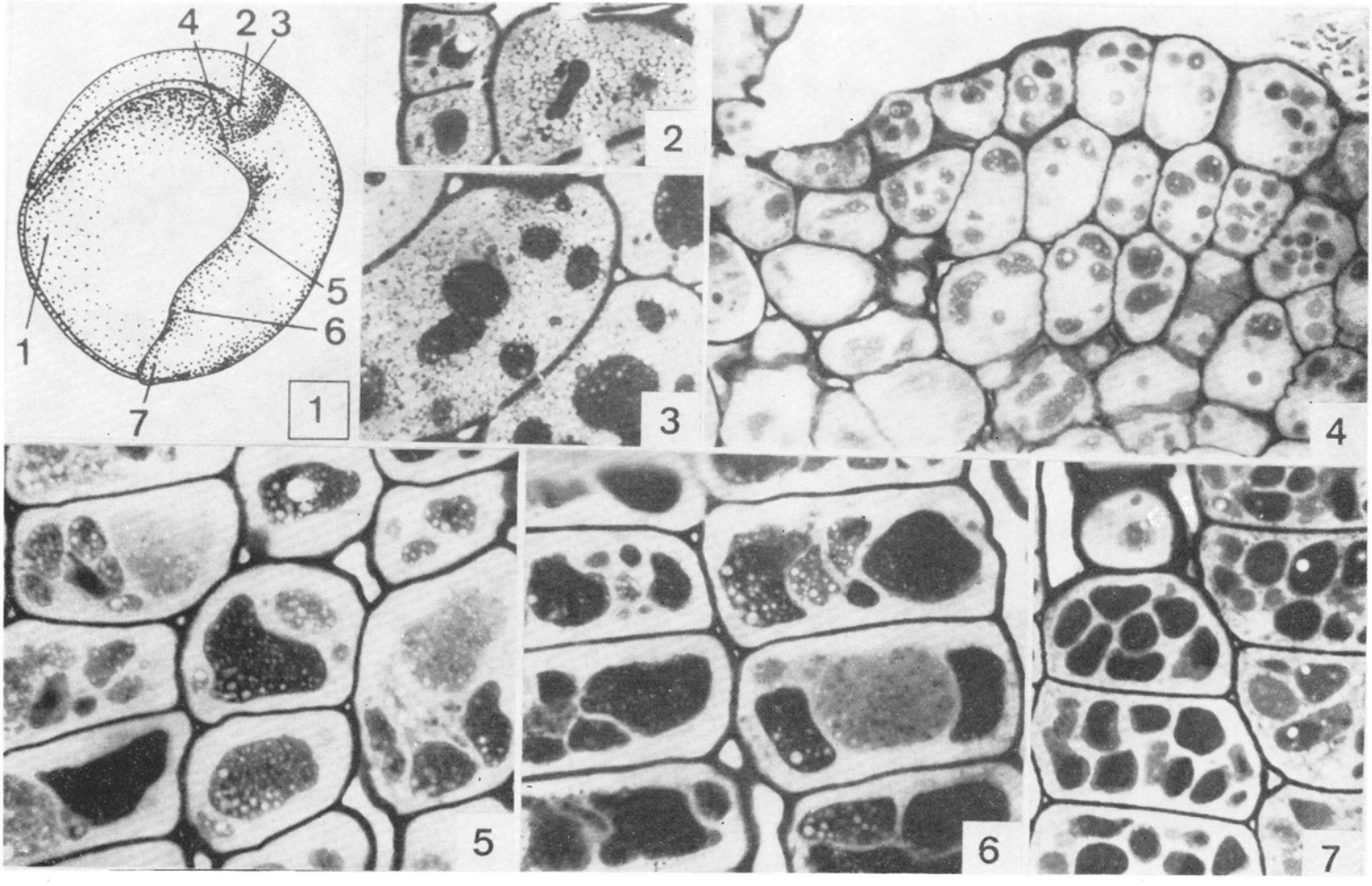
Fig. 13. The same as in Fig. 12 only after postfixation in $KMnO_4$. Lipid bodies are deformed. Plastids and mitochondria without distinct internal structure. Glut./ /$KMnO_4$. × 23 000

Fig. 14. Hypocotyl cortex. Protein bodies, lipids and amorphous plastids with clear ground substance. Glut./$OsO_4$. × 10 000

Fig. 15. Lobulate nucleus situated between protein bodies in hypocotyl cortex cell. Glut./$KMnO_4$. × 10 000

Plate IV. Ultrastructure of hypocotyl cortex, plumule and protoderm cells at hypocotyl-radicle boundary

Fig. 16. Hypocotyl. Protein body with globoids, lipid bodies and space filled with cytoplasm with tightly packed ribosomes Glut./$OsO_4$. × 30 000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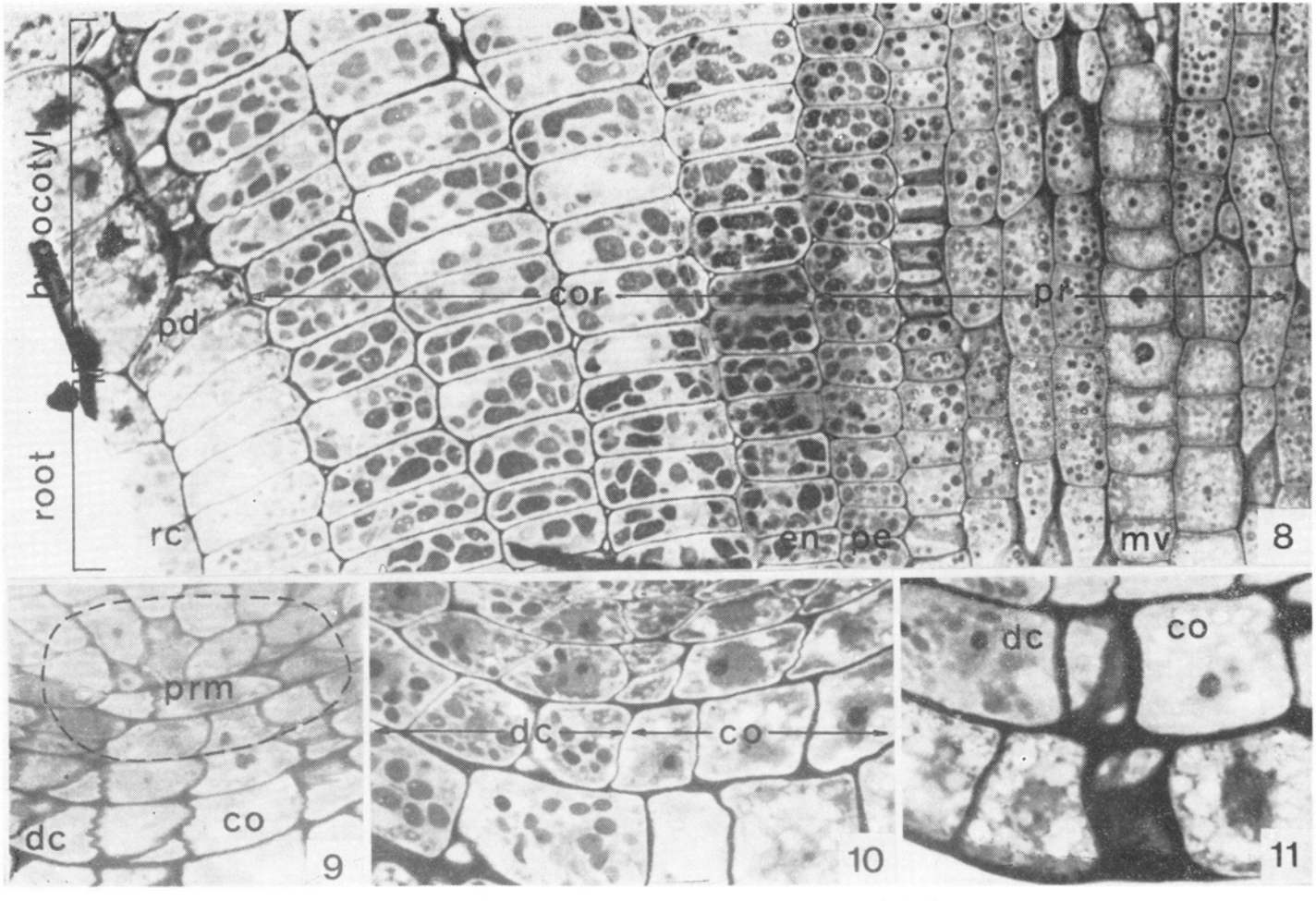
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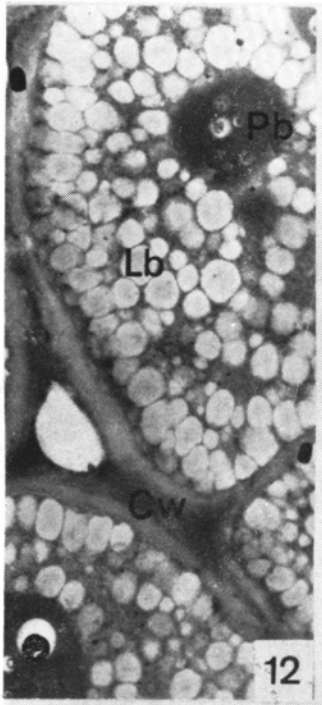
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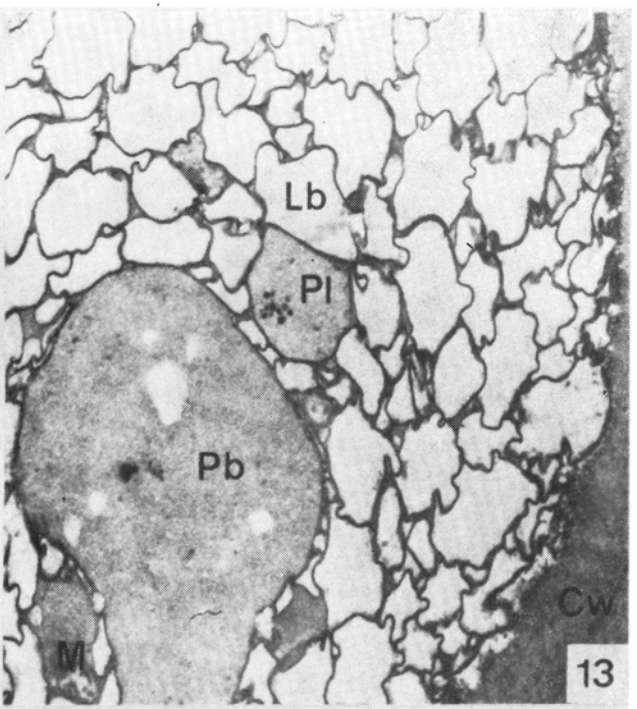
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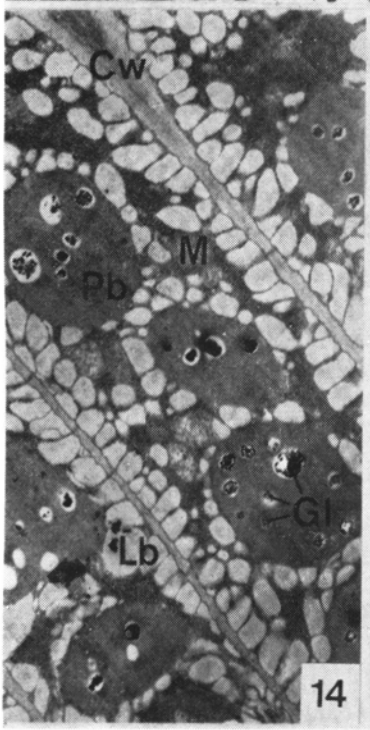
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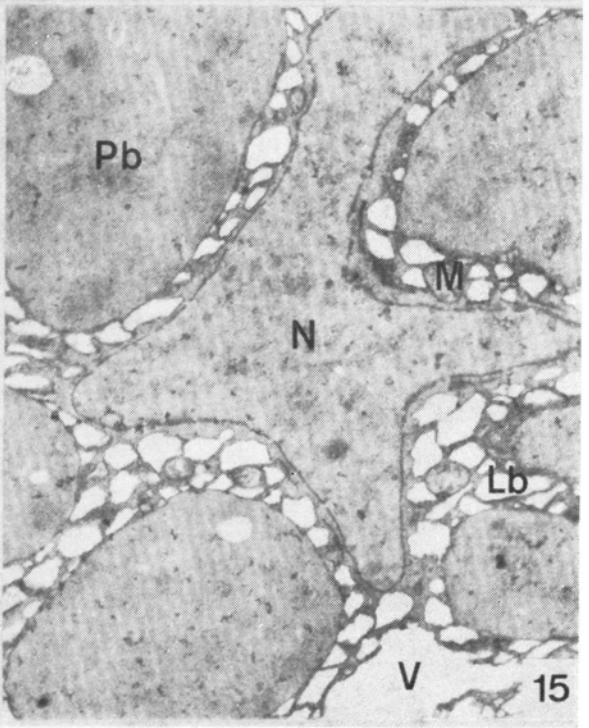
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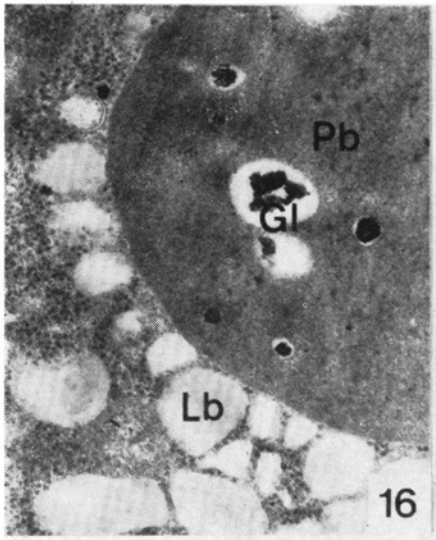
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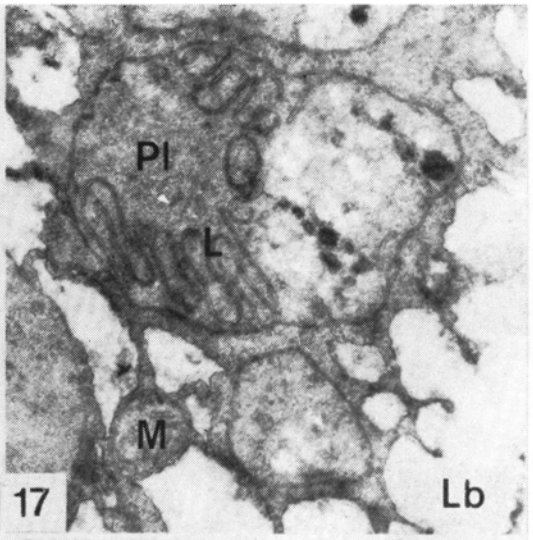
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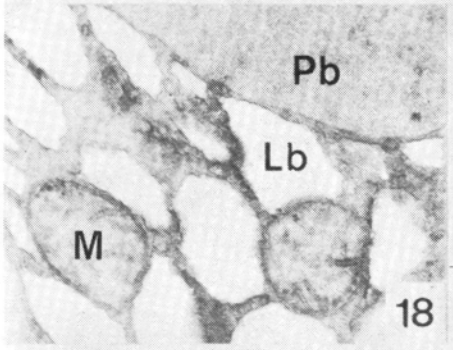
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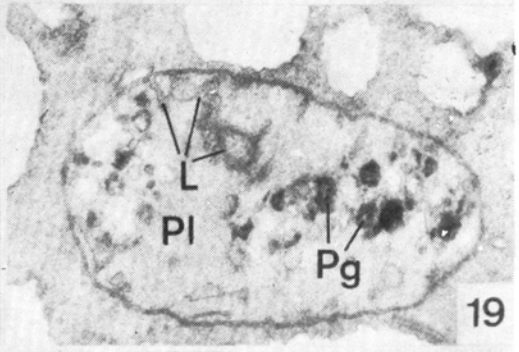
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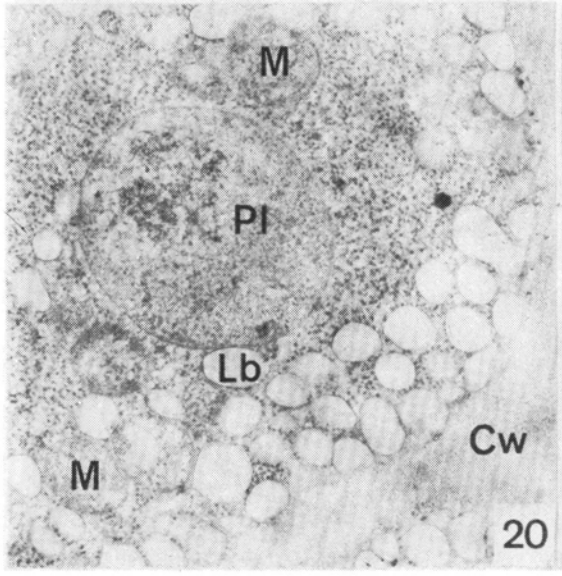
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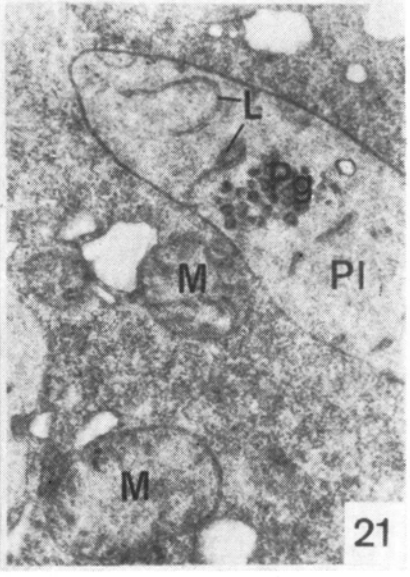
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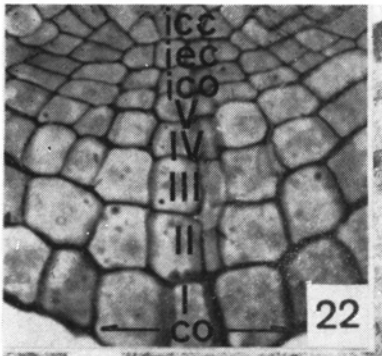
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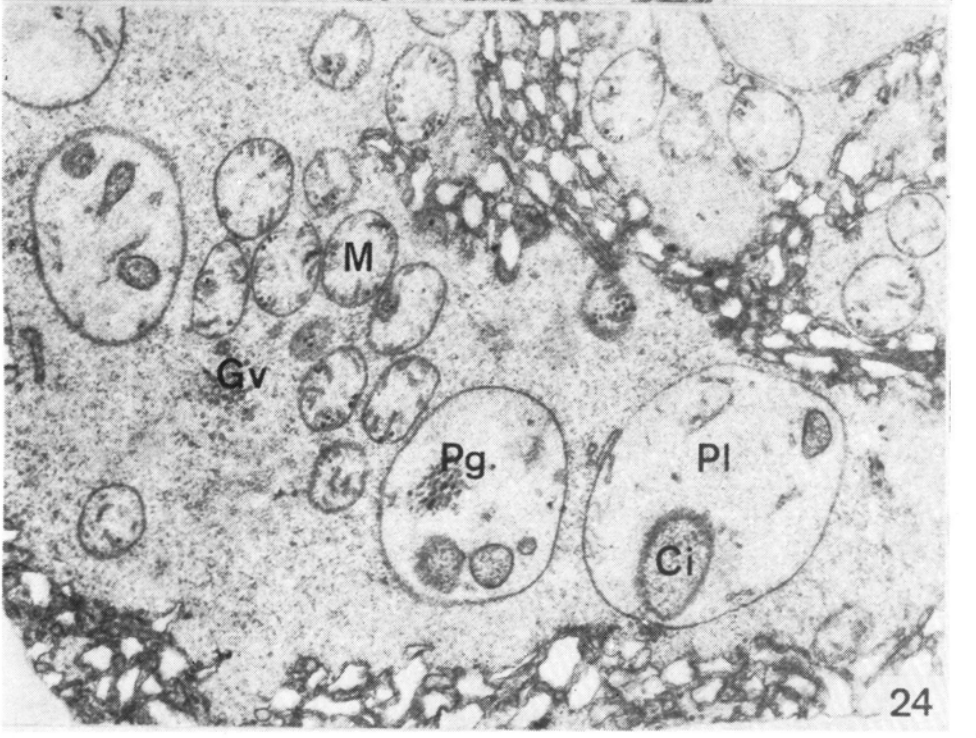
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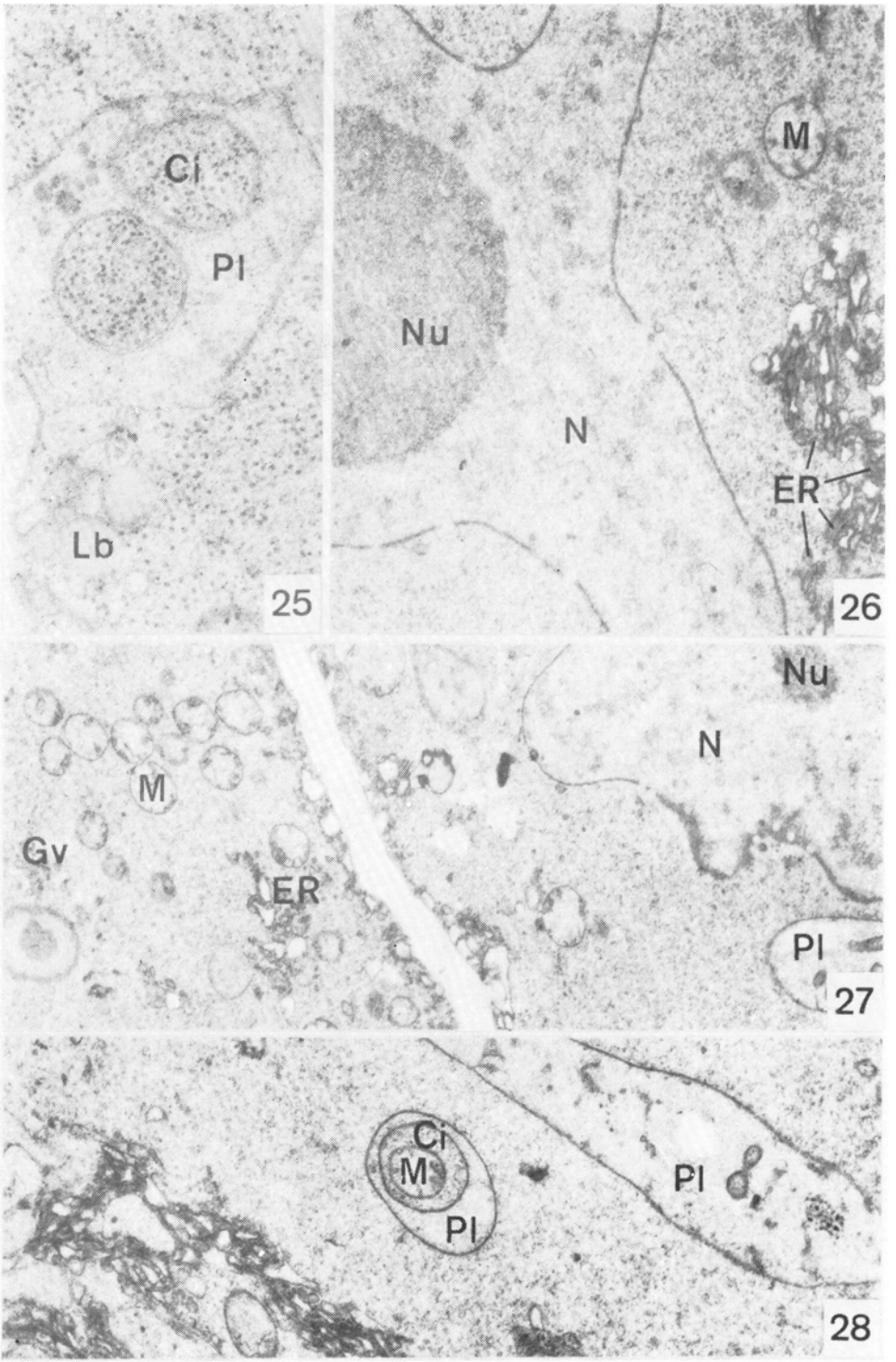
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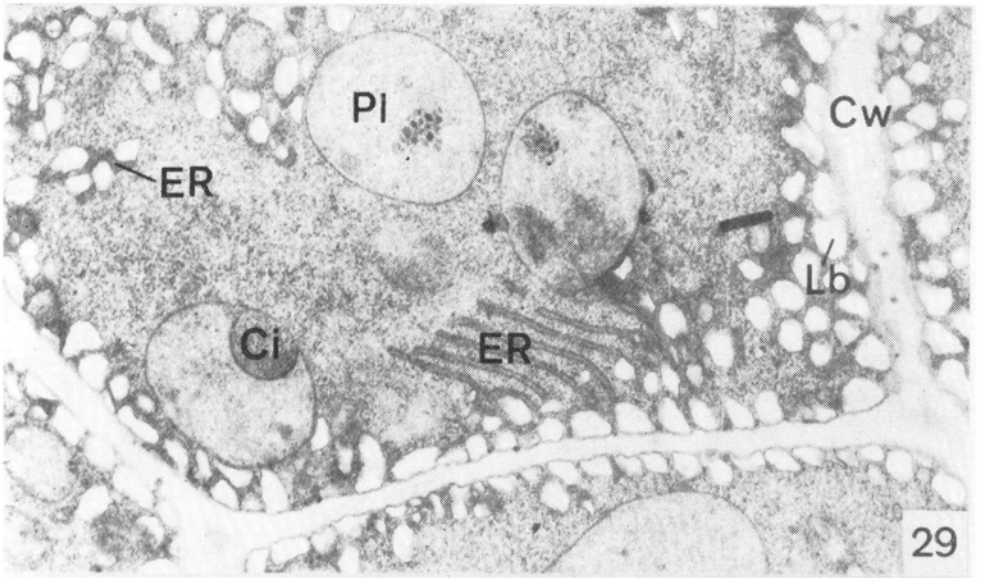
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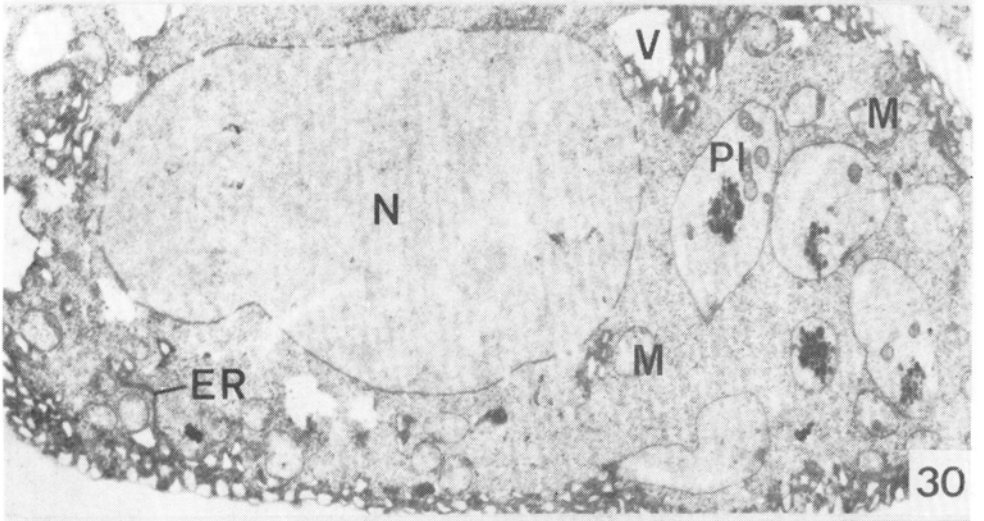
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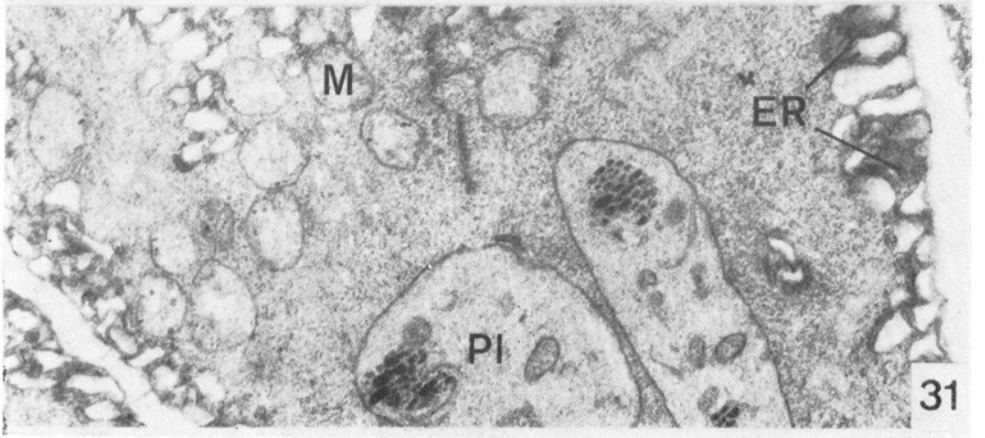
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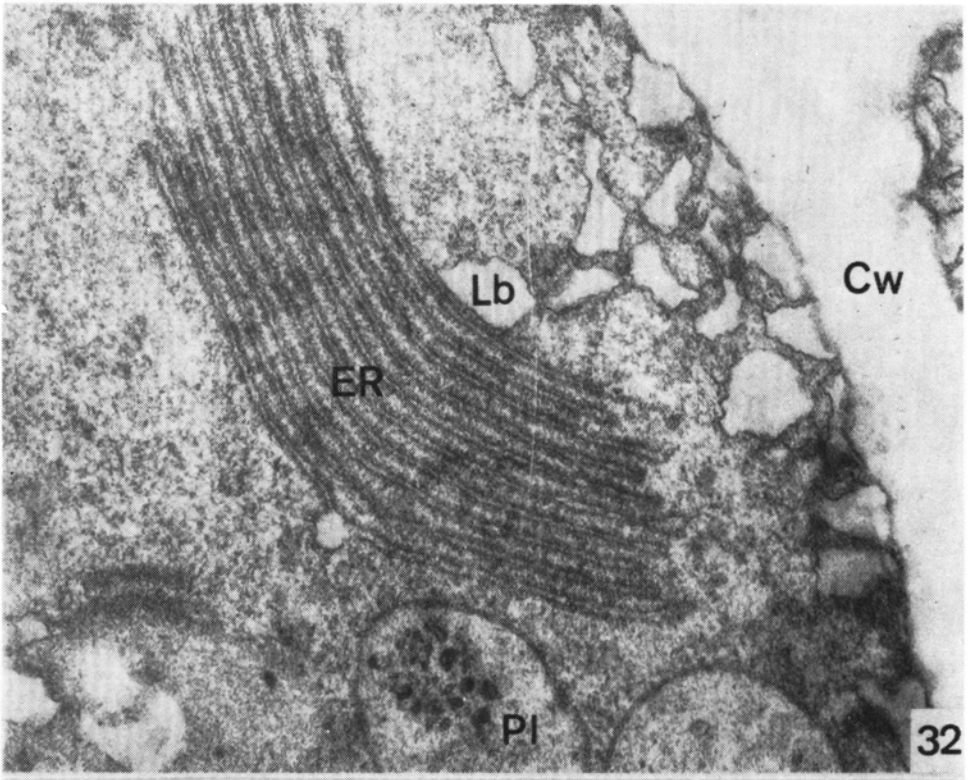
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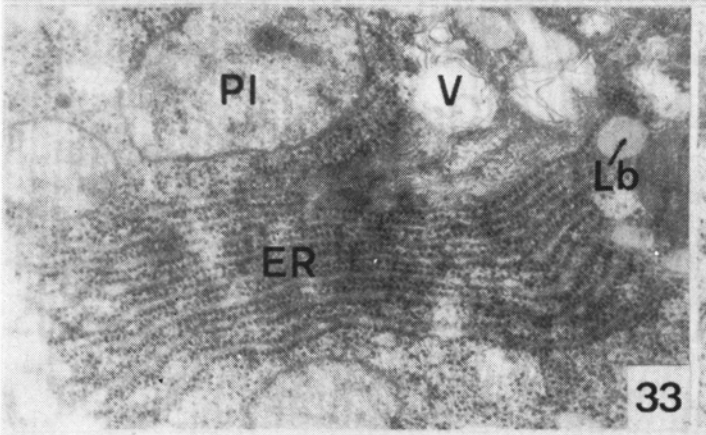
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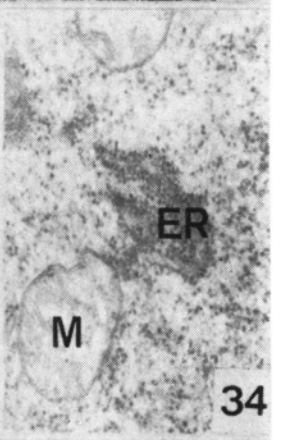
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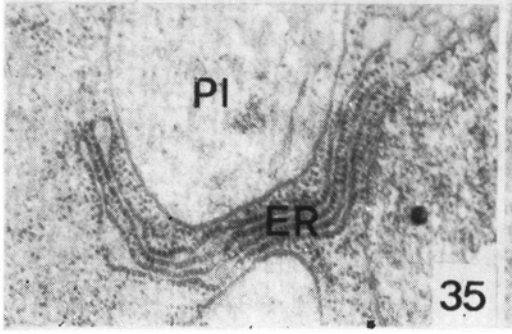
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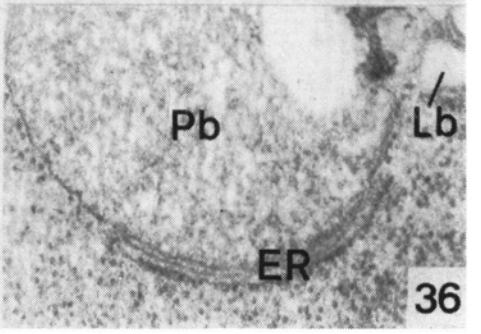
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Figs. 17-19. Hypocotyl. Plastids with plastoglobules and scarce lamellae and single mitochondria with rather distinct cristae. Glut./$KMnO_4$. × 23 000

Fig. 20. Plumule. Rarefied cytoplasm and plastids and mitochondria with electron-transparent reticular ground substance. Glut./$KMnO_4$. × 23 000

Fig. 21. Protoderm of hypocotyl-radicle boundary. Cells of this zone like those of the columella (Figs. 22-27) contain little storage substance, rarefied cytoplasm and active-looking mitochondria and plastids. Glut./$KMnO_4$. × 26 000

Plate V. Ultrastructure of the two lowest columella layers

Fig. 22. Longitudinal section through promeristem and columella with leyers marked in successive order as they are discussed in the text. Glut./toluidine blue. × 450

Fig. 23. Columella layer I. Protein bodies without globoids. Lipid bodies form strands in cytoplasm and surround protein bodies. Numerous mitochondria with cristae. Agglomerations of dictyosomal vesicles. Glut./$KMnO_4$. ×23 000

Fig. 24. Columella layer II. Numerous active-looking mitochondria and plastids with closed areas (infolds) in cytoplasm. Protein bodies absent. Lipid bodies arranged as in first columella layer. Glut./$KMnO_4$. ×23 000

Plate VI. Ultrastructure of columella layers II and III

Fig. 25. Layer II. Rarefied cytoplasm with single ribosomes. Plastids with two cross sections of depressions filled with cytoplasm. Glut./$OsO_4$. ×32 000

Fig. 26. Layer II. Creased nucleus with porous envelope and homogeneous nucleolus. Glut./$KMnO_4$. ×23 000

Figs. 27 and 28. Columella layer III. Ultrastructure similar to that of layer II cells. Glut./$KMnO_4$. Fig. 27 — ×14, Fig. 28 — ×23 000

Plate VII. Ultrastructure of initial centre cells. Glut./$KMnO_4$

Fig. 29. Central cylinder initial cell. Oval small plastids with plastoglobules but no lamellar structures. Small number of clear mitochondria and rare aglomerations of ER cisternae. ×23 000

Fig. 30. Layer of central connecting cells. Almost oval nucleus, numerous but small mitochondria and plastids without membraneous structures. ×14 000

Fig. 31. Initial cell of columella. Numerous mitochondria without cristae with electron-transparent ground substance. ×23 000

Plate VIII. ER in columella and metaxylem vessel mother cells

Figs. 32 and 33. Metaxylem vessel mother cells. Parallel arrangement of rough ER cisternae. Fig. 32 — Glut./$KMnO_4$. Fig. 33 — Glut./$OsO_4$. ×30 000

Figs. 34-36. Columella. Sporadically appearing, almost exclusively in external layers, diverse ER forms. Glut./$OsO_4$. ×23 000

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and fill almost completely the cell interior, restricting greatly the zone of presence of lipids. On the cell cross section there are usually several of them, varying in size, but sometimes the lumen of the whole cell is filled with only one large centrally situated protein body. Protein bodies in the subcotyledone cortex of part of the hypocotyl have a similar appearance (Fig. 6). As we proceed, however, towards the root base, the dimensions of the protein bodies gradually diminish, but their number increases to such a degree that, tightly packed, they fill almost completely the cell interior (Fig. 7). More frquently than in the cells of the cotyledone base they are of oval shape, but mostly they are polyhedral. They have such a shape in the cortex of nearly the whole hypocotyl and that of

the basal part of the rootlet. Further in apical direction their number and size decrease down to the complete disappearance in the cells of the inintial centre and columella (Figs. 8-11), but they persist in the cells of the lateral part of the cap.

Very important are also the histological differences in the protein bodies within the particular embryonal organs (at the boundary of the hypocotyl and radicle — Fig. 8). In the protoderm of all organs they are smaller and less numerous than in the cortex, they disappear, moreover, almost completely on the coneave side of the maximal hypocotyl curvature and at the boundary of the hypocotyl and radicle. The protein bodies in the cells of the central cylinder of the hypocotyl and radicle or in the procambium of the cotyledones differ even more from the others (Fig. 8). Here they are very small, exactly spherical and frequently deprived of globoids. They are very numerous in the central cylinder of the whole hypocotyl and the basal part of the radicle, and their numbers decrease and size diminishes in apical direction. The protein bodies disappear completely in the zone of the initial centre. Neither are they to be found in the plate of maternal metaxylem cells (Fig. 8).

Protein bodies also show wide differences even within the particular cells. This is manifested in a varying basophilicity. After staining with toluidine blue, the protein bodies observed in one cell might become blue-green or pink or may have an intermediate colour. The differences also appear in the electron density of the cell ground substance. Beginning with completely amorphous very electron dense substance through various stages of lower density to completely electron transparent with a reticulate substrate. Most numerous protein bodies with a very compact ground substance are found in the cotyledones and in the upper part of the hypocotyl. In the central part of the latter and in the radicle, especially in its apical part, electron transparent bodies are more numerous with a completely loosened ultrastructure and electron empty protein vecuoles.

The lipid bodies show smaller differences. After fixation in $OsO_4$ they are usually of spherical or oval shape and after postfixation in $KMnO_4$ they are irregular with creased surface. The interior of the lipid bodies is completely uniform and electron transparent or grey. Similarily to protein bodies, they are surrounded by a single membrane, especially well visible after fixation in $KMnO_4$. Lipid bodies are most numerous and largest in the cotyledone parenchyma where they occupy greater part of the space than protein bodies (Figs. 2, 3, 12 and 13). Lipid bodies are less numerous in the cells of other organs, where protein is the basic storage material. In such cells lipid bodies are smaller, form a wall lining and build by a single layer around the protein bodies (Figs. 5, 8, 14 and 15). Sometimes they form small agglomerations lying freely in the dense

cytoplasm. Lipid bodies are particulary scarce in the collumella cells and external in the layers of the hypocotyl-radicle boundary (Figs. 5-9).

The described differences in the protein and lipid bodies are connected with the degree of ultrastructural modification in the organisation of cell organelles. The accumulation of protein and lipid bodies in the cells of storage tissues restricts the free spaces in the cytoplasm and leads to the far going reduction of the contained organelles structure. This is most pronounced in the cotyledones, where, beside the cell nucleus, highly degraded plastids and mitochondria could be only sporadically identified (Fig. 13). Plastids of very irregular shapes with electron transparent ground substance contain usually one agglomeration of some, few not very regular electron dense plastoglobules, and they contain no lamellar structures or starch. The scarce and small mitochondria are also deformed and contain residual cristae and very electron transparent ground substance.

The picture of the fine structure of hypocotyl cortex cells is similar (Figs. 14-19). They contain, however, somewhat less storage material, large spaces are occupied by the cytoplasm with more distinct and numerous plastids and mitochondria in them. At the same time the contours of these organelles are less deformed and more regular. Only the cell nucleus preserves its characteristic stellar shape enforced by the neighbouring protein bodies (Fig. 15). The plastids also have larger plastoglobule agglomerations and better preserved countours of lamellary structures. The mitochondria, although not numerous, are of regular oval shape with scarce but long cristae (Figs. 17-19).

The cells of the remaining organs, in spite of considerable dehydration and the state of dormancy, preserve a meristematic appearance. They contain wide areas of rarefied cytoplasm with a small amount of storage material and more distinctly outlined and less modified organelles. The organs of meristematic appearance differ in the degree of reduction of the fine structure of their cells. Less active seem to be cells of the shoot apical meristem (Fig. 20). In spite of the rarefied cytoplasm they still contain rather numerous protein bodies with globoids and large quantities of lipid bodies. From among the cytoplasmic organelles only scarce plastids and mitochondria are present. They are, however, very small, rounded, with a greatly rarefied ground substance and without a distinct membraneous internal structure (Fig. 20), this indicating a very low metabolic activity.

Cells of the columella preserve the highest degree of ultrastructural organisation. They resemble the metabolically active cells (Figs. 22-32). They contain still less storage material than the plumule cells. Lipid bodies form a discontinuous layer along the walls or have the shape of strands running irregularly in the cytoplasm, particularly in the zone of protein bodies agglomeration and organelles (Figs. 22-31).

Protein bodies are almost exclusively present in the first external layer and they are much smaller than in the cells of the remaining zones of the embryo and their ground substance is much less dense and deprived of globoids (Fig. 23). In the external layers of the columella there sometimes also appear minute electron empty vacuoles.

Nearly all organelles with the exception of dictyosomes, microtubules and microfilaments are visible in the columella cells (Figs. 22-31). The cell nucleus is irregular in shape and very strongly folded (Figs. 26 and 27), only in the cells of the initial centre it remains oval (Fig. 30). It is surrounded by distinct nuclear envelope. The chromosome territory and chromocentres are only little pronounced, particularly after fixation with $KMnO_4$. The nucleolus is well outlined and as in the cotyledone cells and hypocotyl it is compact, electron dense, almost homogeneous and fibrillar, without a distinct granular part (Fig. 26).

Plastids are numerous, their shape is spherical, elogated, oval or irregular (Figs. 22-37). A characteristic feature of columella plastids are numerous different-sized infolds filled with cytoplasm. The plastid stroma, less electron dense than the cytoplasm is separated from the latter by a continuous double membrane. Within the stroma there usually are one or more electron dense agglomerations of minute plastoglobules. They in general are more numerous in cells of the outer layers.

The cells of the columella do not have the developed endoplasmic reticulum. Only sometimes, particularly in the external layers, short cisternae were noted, pushed in between, the lipid bodies and forming with them characteristic layers coating the walls or layers surrounding the protein bodies or forming else different-sized agglomerations lying freely in the cytoplasm (Figs. 23, 24 and 26-28). In the outer layers the single vesicles and short cisternae or even larger groups of longer cisternae arranged parallelly to the plastids were seen sometimes free-lying in the cytoplasm or adherent to the protein bodies (Figs. 34-36). Cisternae were very rare in the cells of the initial centre, which have the appearance of elongated single tubules (Figs. 29, 30). In the initial cells of metaxylem vessels and their closest derivatives, several times the characteristic and extensive characteristic parallel systems of rough cisternae ER were detected (Figs. 32, 33).

In the columella cells Golgi structures are not noticeable, but in the cytoplasm there commonly appear the minute vesicular bodies, which are electron transparent and surrounded by a single smooth membrane, forming frequently agglomerations (Figs. 23, 24). On account of the dimensions of these vesicles and the shape of their agglomerations, it may be assumed that they arose as the result of dictyosome fragmentation.

Numerous single ribosomes were observed in the cytoplasm of the columella cells like in the cells of the remaining organs after fixation

in $OsO_4$ (Fig. 25). Their number is, however, much lower than in the storage cells of the hypocotyl and cotyledones. In spite of the deformations, due to fixation in $KMnO_4$ of material fixed in glutaraldehyde, ribosomes could be recognised even in the cells of the deep cap layers and initial centre where the slowly penetrating postfixative had a weak influence (Fig. 32).

All htese data indicate that the fine structure of the cells of the columella, particularly of its external layers is much less reduced than in the remaining cells of the redicle and hypocotyl. The columella cells differ, thus, even from those of the lateral cap parts which contain much more lipid bodies, typical protein bodies with globoids and much more reduced organelles.

The promeristem cells (Figs. 29-31) the ultrastructure of which is rather similar to that of cells of the deeper columella layers differ from them mostly by regression of the structure of mitochondria, that is reduction of their number and size of their cristae.

Cells of the protoderm and external cortex layers lying at the boundary of the hypocotyl and radicle are ultrastructurally similar to those of the columella (Fig. 21), whereas the remaining radicle cells, especially those of the cortex and lateral parts of the cap resemble rather cells of the hypocotyl.

## DISCUSSION

On account of great technical difficulties, the knowledge of the ultrastructural organisation of the cells of a mature dry embryo is not satisfactory. Papers dealing with this problem are rare and the results are fragmentary and even sometimes controversial. The importance of such studies is stressed in them mostly because of the need for establishing a model of the ultrastructural organisation of the cells, conditioning a minimal level of metabolic processes during dormancy and at the same time rapid resumption of these processes during germination. All authors stress the reduced level of ultrastructural organisation in dry embryos, which is the consequence of gradual physiological maturation and, as its consequence, water deficit leading to a depression of metabolic activity. At the same time the presence of nearly all organelles typical for normally active cells is reported. The presence of the latter is a condition for the maintenance of life in dormant seeds and their rapid passage to a state of activity at germination time. An obvious consequence of dehydration is a densification of the cytoplasm, more compact packing of all the organelles and storage substances and a reduction of volume, frequently connected with their deformation. Particularly ribosomes are tightly packed. They appeared in the cells of all the examined dry

embryos (Paulson and Srivastava 1968, Yoo 1970, Hallam 1972, Swift and O'Brien 1972) and even in cells of lethally dried *Zea mays* roots (Nir et al. 1969). As demonstrated in the present investigations, ribosomes occur also in large numbers in the cells of all organs of the dormant rape embryo. In the cells of the storage organs thery are so densely packed in the cytoplasm that it is difficult to ascertain whether they are joined into polysomes. It is certain, however, that in the plumule and columella cells of meristematic appearance only single ribosomes are seen. It is possible that, according to the findings of Klein and Ben Shaul (1966), Paulson and Srivastava (1968) and Nir et al. (1969), ribosomes occur only singly in all dry embryos, since polysomes, very numerous in early embryogenesis, disappear with the cessation of protein synthesis during ripening of seeds (Hallam 1972). In the epithelium of the scutellum, however, of the dry barley embryo rough ER was found (Nieuwdorp 1963). Similar conditions as in the epithelium occur in the columella and the metaxylem vessels mother cells of rape. Ribosomes surround lipid vesicles, protein bodies and mitochondria in the cells of the wheat scutellum (Swift and O'Brien 1972), and Hallam (1972) describes their presence on the surface of ER cisternae in the neighbourhood of aleurone grains of rye radicles.

The essential difference in the ultrastructural picture of cells of the dry embryo as compared with that of active meristematic cells, is the generally observed reduction of membraneous structures, mainly of the ER (Nieuwdorp 1963, Yatsu 1965, Horner and Arnott 1965, Paulson and Srivastava 1968, Yoo 1970, Hallam 1972, Swift and O'Brien 1972, Webster and Leopold 1977). The observations of these authors agree completely with ours. ER in dry embryos may be present in the cytoplasm as scarce single and short tubules or different-shaped vasicles, frequently deformed and crushed between lipid bodies agglomerations occurring mainly at the cell wall. ER cisternae may also be present in the neighbourhood of cell organelles, for instance around plastids (Yoo 1970) and mitochondria (Webster and Leopold 1977), but they have not been found close to the surface of the cell nucleus. Sometimes much larger quantities of ER have been noted. Webster and Leopold (1977) claim that, although very small amounts of ER are present in the cells of dry cotyledones (sometimes only small cisternae around protein bodies and mitochondria), there is so much of it in the root cap cells that it completely fills the cytoplasm with tubules and vesicles. Setterfield et al. (1959) also found smooth and rough ER in the pea radicle. Both these cases of occurrence of large amounts of ER structures do not find confirmation either by the authors of all the other papers or in the present study. They rather seem to indicate that seeds being no more or not yet in a state of dormancy were used. It may be concluded that ER may assume different forms in dor-

mant embryos in dependence on the degree of maturity of the seeds, the conditions of their storage and the kind of cells examined. A particularly characteristic form is exhibited by the parallel systems of rough epithelium cisternae in metaxylem initial cells, similar to those described earlier by Villiers (1971) in cells of the dormant larch embryo. These regularly arranged piles of cisternae, according to this author, may be a manifestation of oxygen deficit in the final phase of seed maturation, whereas all other forms of ER structures appearing in masses (among them those described by Webster and Leopold (1977), are rather not typical for dry embryos and may indicate a beginning of metabolic activation. Evidence of this would be the observations of Klein and Ben-Shaul (1966) who claim that in dry bean embryos there are few ER structures, but that they become abundant as soon as after one hour of soaking. It might be assumed typical for dry embryos, therefore, an extremal reduction of ER structures. The latter disappeared completely in rape seeds dying because of storage at elevated temperature, depressed humidity and oxygen deficit (Kuraś and Auguścik, unpublished). The presence of ER structures may, thus be assumed as indicating metabolic activity and viability of the seeds.

Very characteristic for the dry embryo cells is the complete absence of Golgi structures. Their presence was not noted in any part of the rape embryo, neither was it in embryos of other plant species (Yatsu 1965, Paulson and Srivastava 1968, Swift and O'Brien 1972, Crevecoeur et al. 1976). Sometimes, however, in dormant seeds structures may be identified which probably are modified dictyosomes. For instance we observed in the rape collumella vesicle agglomerations which arose probably owing to fragmentation of Golgi cisternae. Hallam (1972) also describes groups of vesicles and compact torsioned cisternae in cells of the dry embryo radicle of rye. Nir et al. (1969) observed even almost normally looking dictyosomes in maize roots after their lethal dehydration. This means that as the result of dehydration, disorganisation of dictyosomes occurs slowly. Their reconstruction, on the other hand, is relatively rapid, Swift and O'Brien (1972) saw numerous dictyosomes of normal appearance as early as 3 h after germination in the wheat scutellum cells. They drew the conclusion from this fact that in stabilisation of the membrane structure of dictyosomes, hydrophobic bonds play a decisive role. Since, according to these authors 3 h is too short a time for dictyosome formation *de novo*, they must probably be self-assembled during hydration from the dispersed units which existed in the resting embryo. This concept is very probable and the more interesting that it may also concern the mechanism of transformation of the ER structures which most probably play a dominant role in the regulation of the course of metabolic processes in germinating seeds.

All the remaining organelles do not undergo such serious transformation during maturation, and, although the degree of their ultrastructural modification depends laterly on their situation in the embryo, even in the case of their extreme reduction, as it happens in the storage organs, they preserve their distinctiveness and structural continuity over the entire period of embryogenesis and dormancy. This concerns above all the cell nucleus, plastids and mitochondria.

The cell nucleus, although in dry embryo cells it is greatly creased and of irregular shape, has in all parts of the embryo a typical structure resembling that of active cell nuclei, with a distinct double and porous membrane as reported among others by Yoo (1970), Hallam (1972), Swift and O'Brien (1972), Crevecoeur et al. (1976), Webster and Leopold (1977). This nucleus differs, however, from that of metabolically active cells by a lower electron density of the nucleoplasma (Paulson and Srivastava 1968, Yoo 1970, Hallam 1972, Webster and Leopold 1977) attested by all authors, a high chromatin condensation and a close adherence to the nuclear envelope (Swift and O'Brien 1972). The nucleolus is also compact and its electron density is increased, making impossible identification of the granular and fibrillar parts characteristic for active nuclei.

Plastids are present in all organs of the dry rape embryo. Their structure is different in its various parts, but they can be identified even in case of far advanced degradation. The least modified plastids as compared with those in active tissue were observed in the columella of the root cap. They were, however, of varying shapes with numerous infolds filled with cytoplasm. Other features of plastids were typical for the dry embryo and have been described in many other plant species (Nieuwdorp 1963, Yatsu 1965, Yoo 1970, Webster and Leopold 1977). They are surrounded by a double membrane, the ground substance is of low electron density, with scarce lamellae, they do not contain starch, similarly as in the case of soybean cotyledones (Webster and Leopold 1977), *Gossypium hirsutum* (Yatsu 1965) and barley scutellum (Nieuwdorp 1963). They contain, however, electron dense plastoglobules, particularly numerous in the meristematic cells of the columella and at the boundary between the hypocotyl and radicle.

Last modified of all organelles are the mitochondria, although their appearance varies in various parts of the embryo. In the cotyledones and hypocotyl they were very scarce with indistinct contours and a highly degenerated internal structure. Mitochondria are numerous in the plumule and in radical promeristem, they are distinctly outlined though small, spherical with electron transparent ground substance and incompletely formed cristae. Mitochondria with a fully active appearance are present in the external layers of the columella. It results from the available descriptions of the dry embryo fine structure which say that the ap-

pearance of mitochondria is generally noted in other plant species. Doubtless is also the presence of a distinct double membrane in mitochondria (Setterfield et al. 1959, Nieuwdorp 1963, Yatsu 1965, Paulson and Srivastava 1968, Webster and Leopold 1977 and others), their internal structure may in various species, however, differ widely. They may have long cristae which frequently remain in direct contact with the internal membrane and seem normal as in active tissue cells (Yatsu 1965, Nir et al. 1969, Yoo 1970 and others). According to other authors, however, the cristae are poorly developed, scarce, small and thin, with blurred contours (Swift and O'Brien 1972, Crevecoeur et al. 1976, Webster and Leopold 1977) or they may be completely absent. If we compare these data with the present ones and assume that in the embryo of any plant the degree of cytologic-histological differentiation is similar to that in rape embryos, one may suppose that the ultrastructural differences between the mitochondria are not so much the result of specific differences but might be caused by the investigation on various parts of the embryo.

Beside the modification of cell organelle structure, most characteristic for the resting embryo is the mass accumulation of storage material. The rape embryo contains protein bodies and lipid droplets, but there is no starch. The protein bodies are surrounded by a single membrane consisting of ground substance with globoids. Lipids appear in the form of single spherical vesicles with homogeneous grey contents (after fixation in $OsO_4$). This, according to Yatsu (1965) is due to the high content of unsaturated fatty acids. The presence of a single membrane, which used to raise controversies, causes at present no doubts. It is very well visualised after postficsation in $KMnO_4$ applied at present; moreover, Mollenhauer and Totten (1971b) demonstrated the presence of membraneous vesicles after lipid extraction.

The distribution of storage materials in the dry embryo cell is very characteristic. Protein bodies as a rule are present in the central part of the cell, whereas lipid bodies accumulate at the plasmalemma and around the protein bodies. Such a distribution of lipid bodies is, according to Yoo (1970), a reflection of the metabolic inactivation of the cell. A confirmation of this is the fact that in rye embryo cells both in the final phase of embryogenesis and the arly stage of germination the lipid bodies are distributed over the whole area of the cell (Hallam 1972). As established in the quoted paper, the protein and lipid bodies are also irregularly distributed in the organs of the dry embryo. This is closely connected with different degrees of modification of the cell ultrastructural organisation. Such wide ultrastructural differences within the mature dry embryo must in turn determine a definite order of its activation during seed germination. This order must be determined before the

ripening of the seeds or even during early embryogenesis, as found for the rape embryo by Tykarska (1976, 1979).

The results obtained in the present study unequivocally suggest that the complete disapearance or far going changes in the dynamic membraneous structures and the considerable reduction of the fine structure of cell organelles with simultaneous mass accumulation of storage material constitute the base for the entrance of the embryo into relative dormancy. In some of its parts, however, the structure of mitochondria remains almost unchanged, ribosomes and ER are abundant and, owing to the irregular shape of plastids and nuclei, the exchange surface increases, making possible in dry embryo cells basic metabolic processes and survival through the period of dormancy and at the same time rapid activation immediately after swelling of the seeds.

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## *Aktywacja zarodka podczas kiełkowania nasion rzepaku. III. Ultrastruktura osi suchego zarodka*

Streszczenie

Badano dojrzałe, suche zarodki rzepaku ozimego (*Brassica napus* L.) odm. Górczański za pomocą mikroskopu świetlnego i elektronowego. Stwierdzono znaczne modyfikacje i uwstecznienia ultrastruktury komórek spoczynkowego zarodka w porównaniu z komórkami metabolicznie aktywnymi. Stopień uwstecznienia ultrastruktury jest różny w poszczególnych organach i tkankach zarodka. Najbardziej uwstecznione są komórki organów spichrzowych — hypokotyla i liścieni. Są one prawie całkowicie wypełnione ciałami białkowymi i lipidowymi. Niewielkie przestrzenie między nimi wypełnia gęsta cytoplazma z płatowatym jądrem i trudnymi do zidentyfikowania, nielicznymi plastydami i mitochondriami. Komórki zawiązka pędu i korzenia zarodkowego a zwłaszcza protodermy na granicy hypokotyla i ko-

rzenia oraz kolumelli czapeczki mają mniej uwstecznioną ultrastrukturę. Mają mniej substancji zapasowych, rzadszą cytoplazmę i prawie wszystkie, normalnie wyglądające organelle komórkowe. Mitochondria są liczne z dość dużymi kristami. Plastydy duże z charakterystycznymi wgłębieniami wypełnionymi cytoplazmą oraz nielicznymi lamellami i kilkoma skupieniami plastoglobul. Jądro jest płatowate z wyraźnie podwójną i porowatą błoną jądrową i jednorodnie gęstym jąderkiem. W komórkach tych nie ma diktiosomów a ER jest zredukowane do krótkich, najczęściej szorstkich cystern i pęcherzyków. Zróżnicowane są również komórki w obrębie samej kolumelli. Najmniej uwstecznioną ultrastrukturę mają komórki warstw zewnętrznych, zawierają one najwięcej i najbardziej aktywnie wyglądające mitochondria oraz więcej struktur ER. Komórki promerystemu są podobne do komórek głębszych warstw kolumelli ale mają bardziej uwstecznione mitochondria. Komórki bocznych części czapeczki i oddalone od promerystemu komórki radikuli są coraz bardziej podobne do komórek hypokotyla.