

Protein bodies formation in yellow lupin seeds

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Abstract

The ultrastructure of cotyledon cells is described at five stages of lupin seed development, distinguished on the basis of their morphological features. It was found that the endoplasmic reticulum and dictyosomes participate in the synthesis and transport of storage protein, and that protein is deposited in the central vacuole or in newly forming ones. In the investigated zone of lupin cotyledon cells two forms of protein bodies were observed differing in the contrast and compactness. Both were the simple protein bodies. Parallely to storage protein synthesis, thickening of the cell walls was observed which may indicate synthesis and deposition of hemicellulose in them. Storage lipids surrounding the protein bodies form in the end stages of seed development.

Key words: cotyledons, protein bodies, *Lupinus luteus*

INTRODUCTION

Seeds of leguminous plants contain a high amount of storage proteins (mainly globulins — Derbyshire et al. 1976) which in mature seeds may constitute about 80 per cent of all proteins (Millert and Thomson 1975). These proteins are present in the form of distinct protein bodies and serve as the main source of nitrogen and carbon skeletons for the developing seedlings (Ashton 1978, Derbyshire et al. 1976).

The literature on protein mobilisation in the process of germination is abundant, however, the formation of protein bodies is as yet not definitively elucidated. Recent reports confirmed the earlier hypotheses that in *Fabales* reserve proteins are synthesised on polysomes connected with the endoplasmic reticulum (ER) (Bollini and Chrispeels 1979, Bollini et al. 1982, Higgins and Spencer 1981, Püchmel

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et al. 1979). These newly synthesised proteins accumulate in the vacuoles (e. g. Weber and Neumann 1980 and references quoted by them, Yoo and Chrispeels 1980). Mature protein bodies should, therefore, be considered as structures homologous to vacuoles. The problem of protein transport from the granular ER to the vacuole still remains controversial in the literature. It results from electron-microscopic studies of leguminous plants that dictyosomes may take part in this transport like in animal cells (Bergfeld et al. 1980, Davey and Van Staden 1978, Harris and Boulter 1976). Numerous authors, however (among them Bollini et al. 1982, Neumann and Weber 1978, Weber and Neumann 1980), believe that at the present state of knowledge proof thereof is not sufficient.

The object of the present investigations was yellow lupin seed. The ultrastructure of protein bodies in dry lupin seeds and the ultrastructural changes accompanying protein hydrolysis (Młodzianowski 1978) are already known. The present study concerns the formation of protein bodies in the cotyledons of lupin during seed development.

MATERIAL AND METHODS

As material for investigations served yellow lupin (*Lupinus luteus* L. cv. Jantar). Small fragments of cotyledons taken from the middle part of the adaxial side were used for microscopic examination. They were fixed in 5 per cent glutaraldehyde in 0.1 M cacodylate buffer and afterwards in 2 per cent osmium tetroxide in the same buffer, then dehydrated in an increasing gradient of aqueous acetone and in propylene oxide and finally embedded in Epon 812. Sections were cut on Tesla BS 490 and LKB "Ultratome III" microtomes with glass knives. Semi-thin sections (3 μ m) were stained with toluidin blue (after Kay 1965) and ultrathin ones were contrasted with uranyl acetate and lead citrate. Photographs were taken with the use of Amplival (Zeiss) and JEM 7A (JEOL Co) microscopes.

RESULTS AND DISCUSSION

The development stages of seeds were established according to the seed diameter, their colour and that of the cotyledons and the morphology of the pods (Fig. 1). These successive stages were the object of the present studies. As first was considered the stage when the cotyledons could be mechanically isolated from the seeds. Stage I consists of uniformly coloured light green seeds of 4-5 mm size. The cotyledons in them are about 3 mm in diameter, dark green and soft. The cells of the epidermis and parenchyma of such cotyledons have the thin cell walls (Fig. 2)

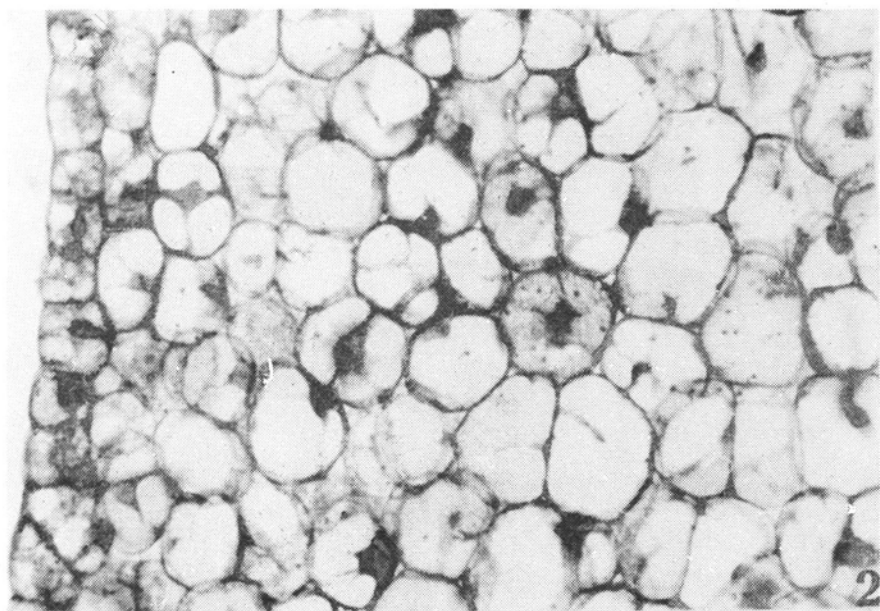
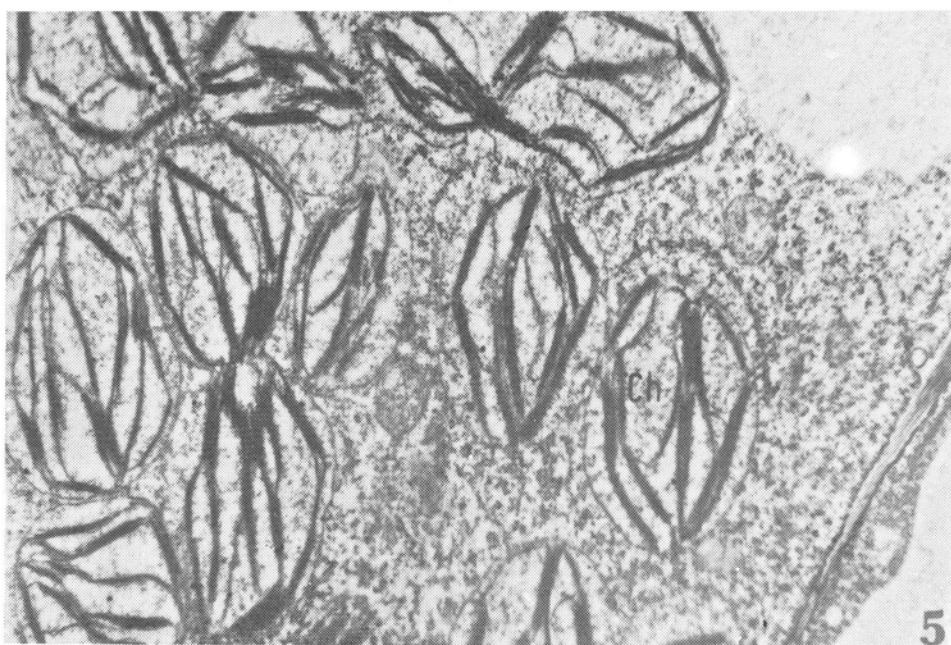
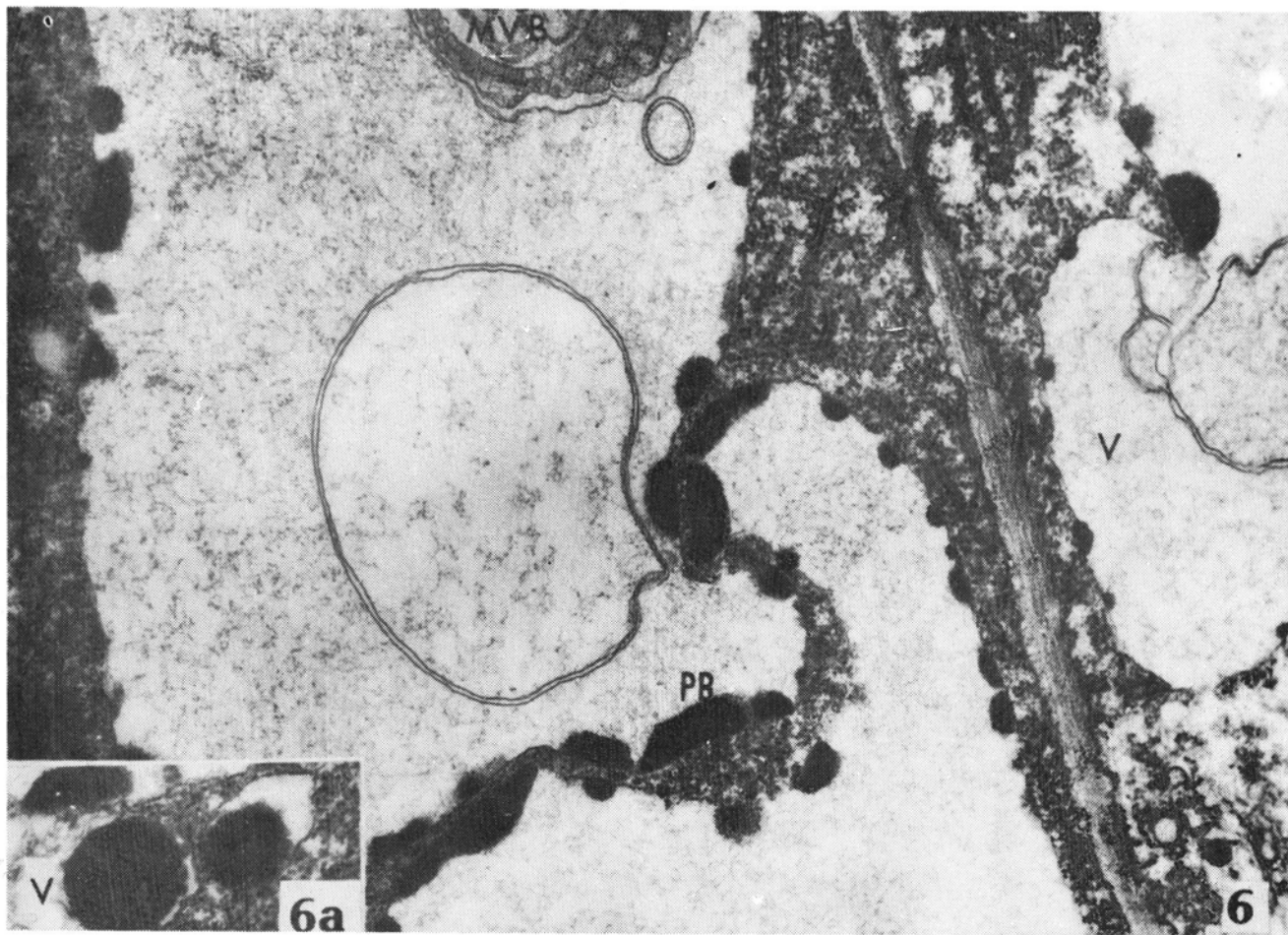


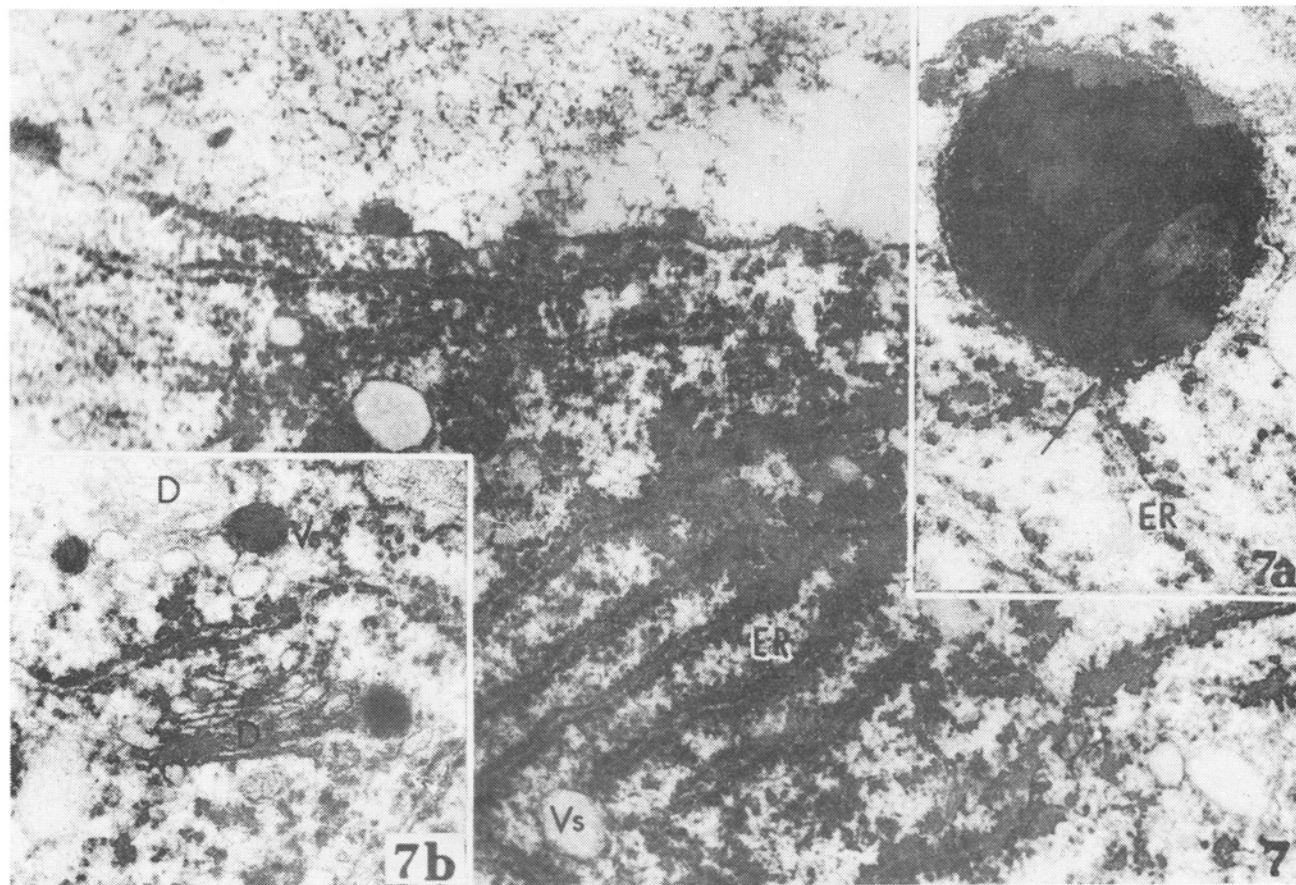
Fig. 2. Cross section through lupin cotyledon. Meristematic cells of palisade parenchyma and parts of spongy parenchyma are visible. Stage I of seed development. X 500
 Fig. 3. Cross section through cell endosperm. Stage I of seed development. X 600



Fragments of palisade parenchyma cells from cotyledon. Stage I of seed development
 Fig. 4. Picture of nucleus (N) characteristic for cells of this stage with ring-shaped nucleolus.
 Vacuoles (V) do not yet contain reserve protein. Cell walls (CW) thin. X 9000
 Fig. 5. Numerous still dividing plastids (Ch). X 10 000



Figs. 6 and 6a. Fragments of palisade parenchyma cells from cotyledon. Stage II of seed development. Both within the central vacuole (Fig. 6) and in the small ones in the cytoplasm (V)—Fig. 6a reserve protein (PB) has appeared (dark spheres adhering to tonoplast). The vacuole shown in Fig. 6 is undergoing fragmentation, within it a multivesicular body (MVB) is visible. X 23 000



Fragments of palisade parenchyma cells from cotyledon. Stage II of seed development

Fig. 7. Rich endoplasmic reticulum (ER) of granular type. X 45 000

Fig. 7a. Ending of endoplasmic reticulum profile (arrow) in the from of a vesicle containing material similar in electron density to reserve protein. X 66 000

Fig. 7b. Dictyosomes (D) with vesicles (Vs). In them electron-dense contents similar in this respect to reserve protein. X 40 000

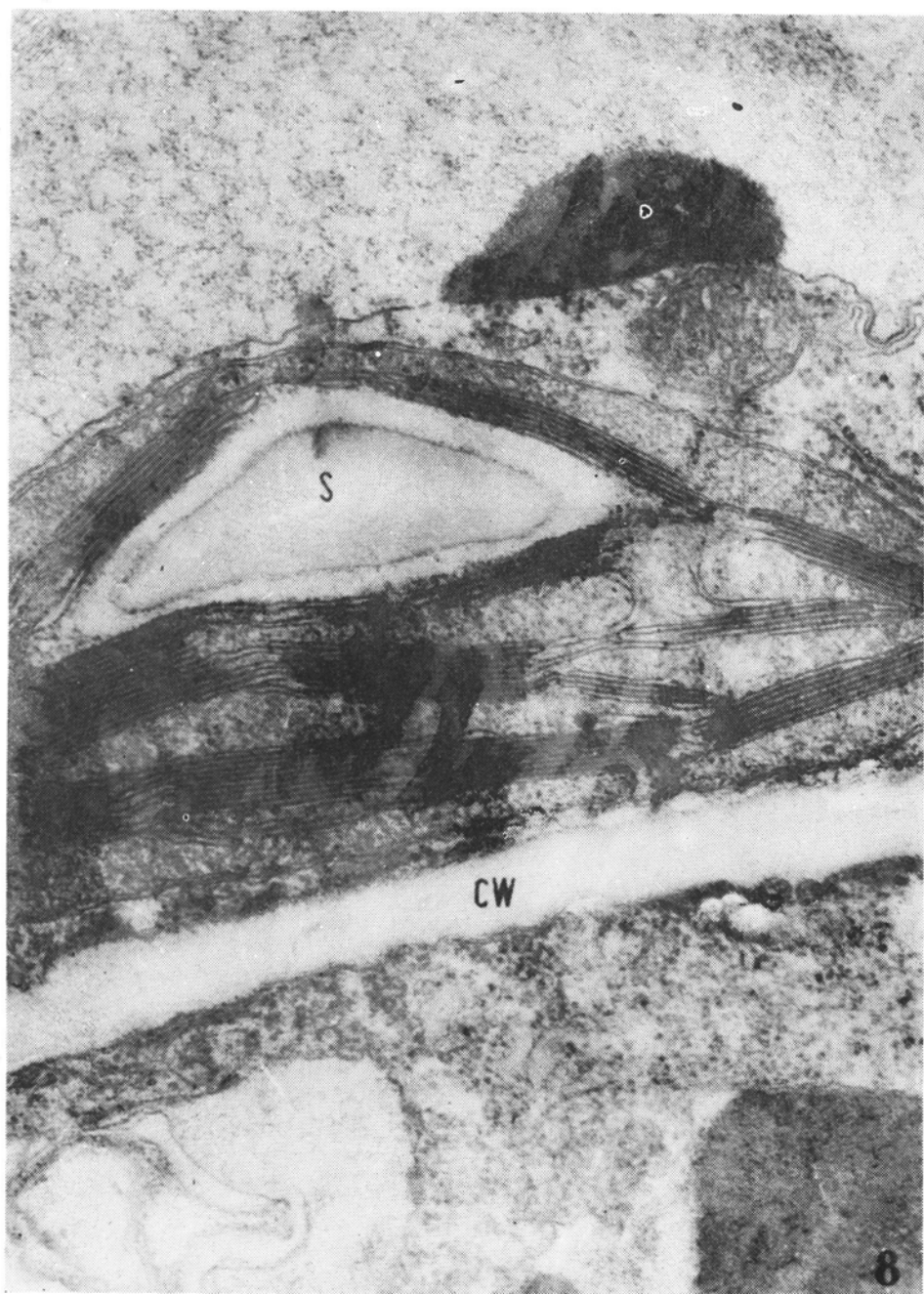


Fig. 8. Fragment of palisade parenchyma cells from cotyledon. Stage II of seed development. The structure of chloroplast containing starch (S) does not differ from that of chloroplasts in green leaves. CW — cell wall, PB — protein body. X 30 000

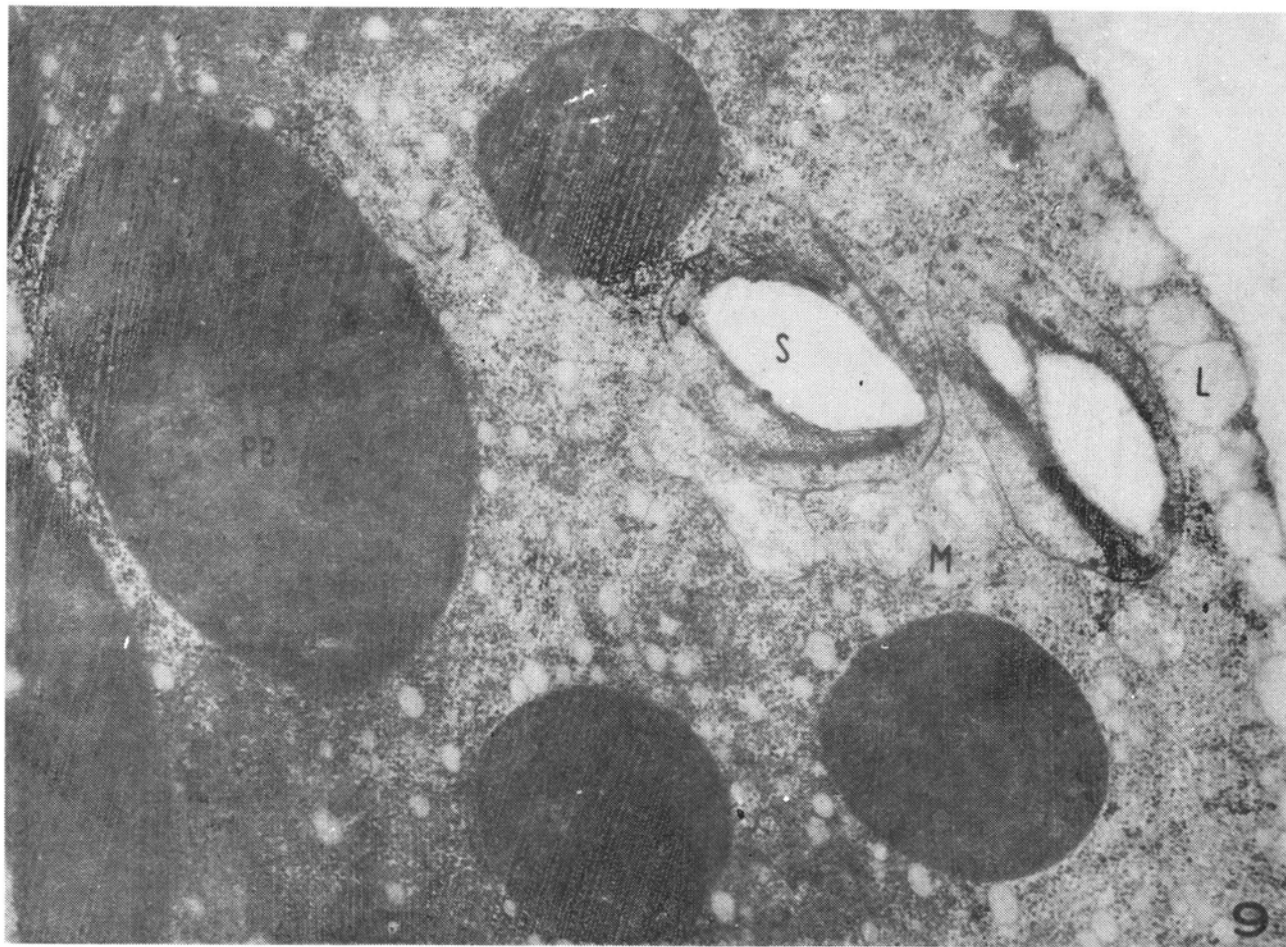


Fig. 9. Fragment of palisade parenchyma cells from cotyledon. Stage III of seed development. Large protein bodies (PB) are visible and irregularly dispersed spherosomes in the cytoplasm. Only few of them adhere to the protein bodies. Plastids with large starch grains (S) have a distinctly reduced thylakoid system (as compared with previous stage). L — lipid body, M — mitochondrion. X 18 000

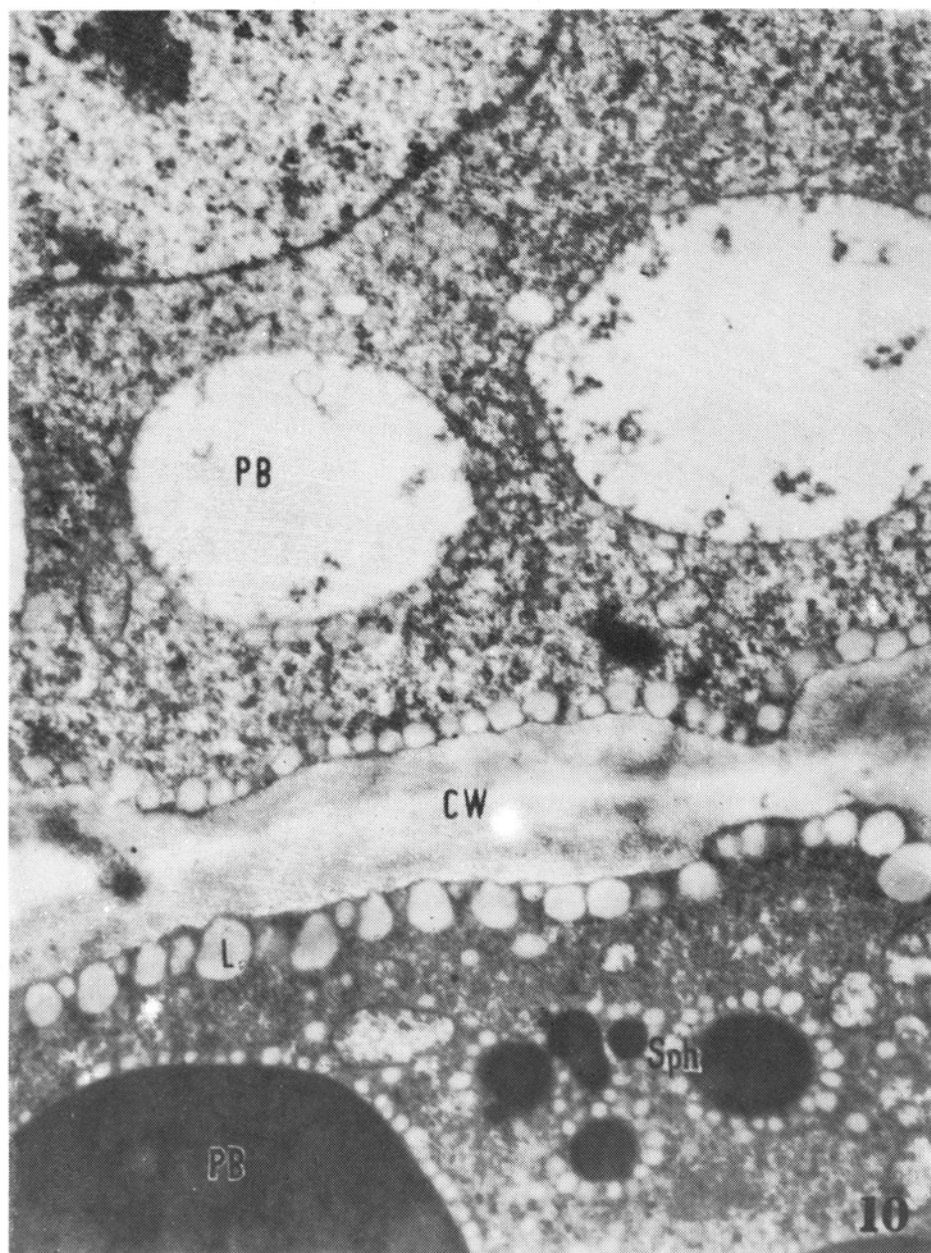
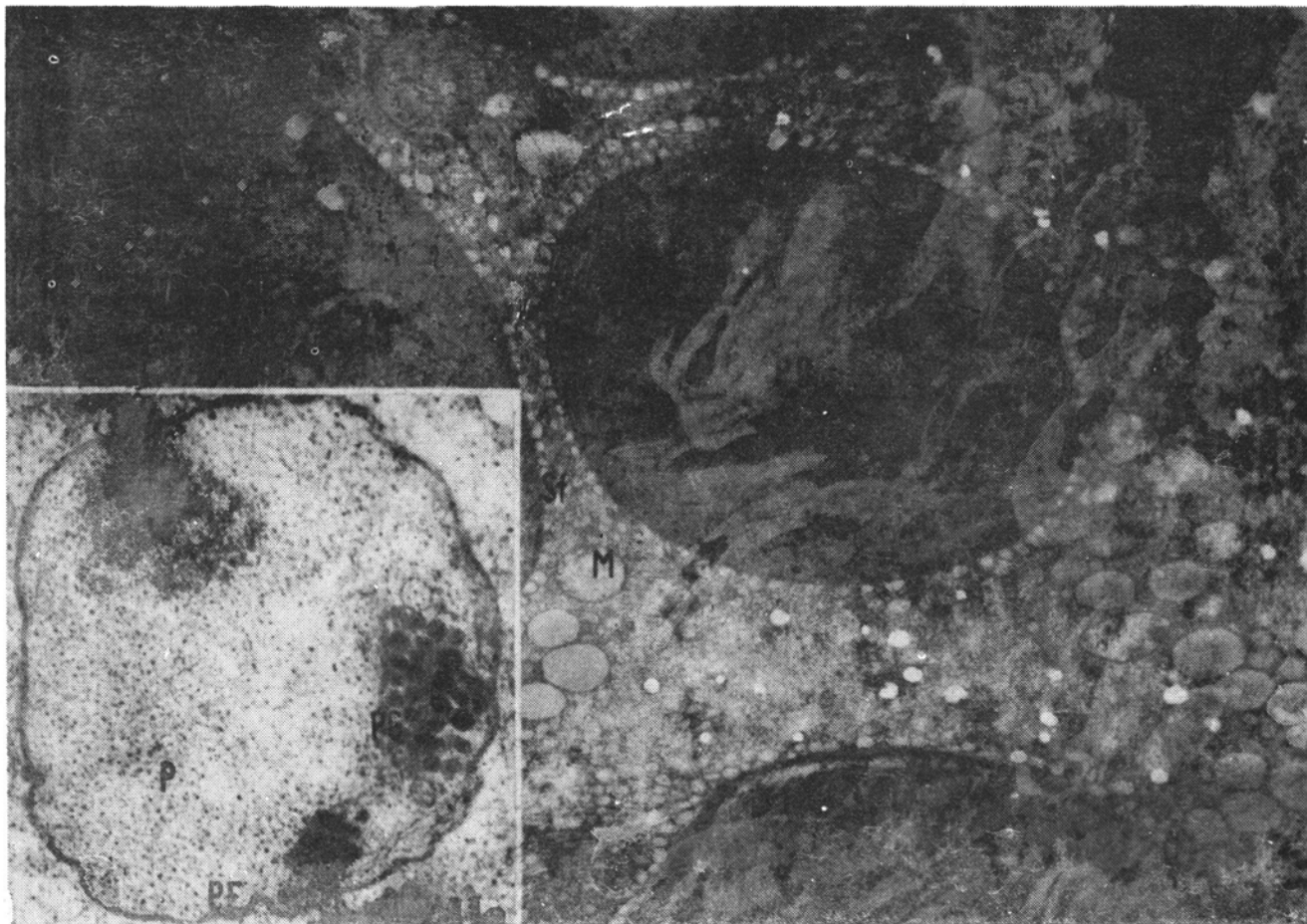


Fig. 10. Fragment of palisade parenchyma cells from cotyledon. Stage IV of seed development. Two forms of protein bodies (PB) are visible. The cell walls (CW) are markedly thickened (as compared with previous stage). Lipid bodies (L) adhere to them on protoplast side. Electron-dense protein bodies are surrounded by spherosomes (Sph). X 15 000



Figs. 11 and 11a. Fragments of palisade parenchyma cells from cotyledon. Stage V of seed development. Protein bodies (PB) surrounded by spherosomes (Sf). Fig. 11 — X 18 000.

Fig. 11a. Plastid (P) characteristic for this stage with residual thylakoids. Plastoglobules (PG) and phytoferritin (F) present in stroma. PE — plastid envelope. X 34 000

and joined by numerous plasmodesmata. Most of the cell volume is occupied by a large central vacuole (Fig. 2) with delicate fibrillar deposits (Fig. 4). The cytoplasm includes a large number of dictyosomes and free ribosomes and a small amount of endoplasmic reticulum. Mitochondria are scarce, and chloroplasts numerous (Fig. 5) with a well developed thylakoid system. Chloroplasts containing assimilation starch were only exceptionally noted. Many profiles shows division of plastids by constriction (Fig. 5). The nuclear envelope has numerous pores and the nucleoli are of ring-like type with a granular component on the periphery and a "nucleolar vacuole" in the centre (Fig. 4). Seeds of stage I contain dense endosperm of cellular type (Fig. 3) easy to isolate in the form of a glassy lump.

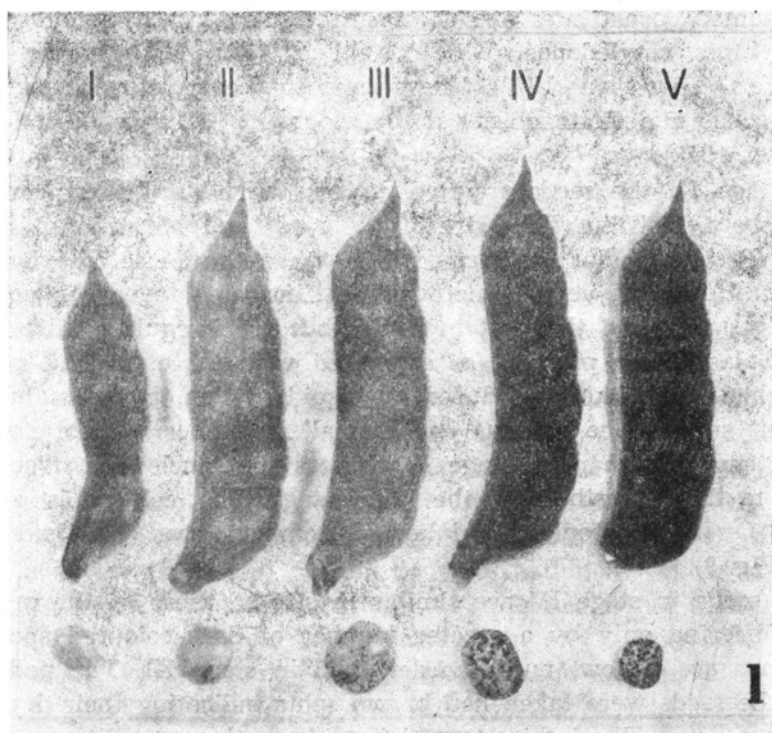


Fig. 1. Distinguished stages of yellow lupin seed and pod development

Stage II is characterised by light green 6-9-mm seeds. The endosperm in these seeds is completely digested and the size of the cotyledons does not as a rule differ from that of the seeds. In the vacuoles of the cotyledon cells beginning of storage protein deposition can be seen, most frequently spherical or of flattened sphere shape always adhering to the tonoplast (Fig. 6). Beside large vacuoles there appeared in the cytoplasm small ones with reserve protein (Fig. 6a). Pictures were sometimes ob-

served indicative of fragmentation of the vacuole (Fig. 6). Multivesicular bodies were also seen in the cytoplasm, most frequently in the vacuoles (Fig. 6). A characteristic difference as compared with stage I is the appearance of numerous endoplasmic reticulum profiles of granular type (Fig. 7). In the ER cisterns electron-dense delicate structures are visible and at the endings of the ER profiles a slight distension and vesicles containing material similar in electron-density to reserve protein are noted (Fig. 7a). Dictyosomes are as numerous as in the preceding stage, with contents in their vesicles which in electron-density are similar to reserve protein deposited in the vacuole (Fig. 7b). The structure of chloroplasts in the cotyledon cells is similar to that in green leaves (Fig. 8). The cell walls are thicker than in stage I, this being possibly evidence of the beginning of deposition in them of reserve hemicelluloses. The latter, namely, constitutes one of the three main kinds of storage material in lupin cotyledones (Krietowicz 1955, Młodzianowski and Wesołowska 1975). They begin to accumulate only when the seed reaches a definite stage of development (Davey and Van Staden 1978).

In stage III the seeds are light green with but little pronounced brown spots (mottling). The size of the seeds and cotyledons is about 10 mm. Protein bodies are formed (Fig. 9). In the dense cytoplasm and, beside granular ES, very numerous free ribosomes appear. Spherosomes do not yet surround the protein bodies but are irregularly dispersed in the cytoplasm. The plastids (as compared with those of the previous stages) have a markedly reduced thylakoid system, but contain rather abundant starch. The nucleus has a smaller number of pores in the nuclear envelope than in stage I, whereas the nucleolus structure is similar to that described for the latter stage. The cell walls are thickened. On the protoplast periphery a compact layer of lipid bodies forms (Fig. 9).

The seeds in stage IV are similar in size to those of the preceding stage, differing only by a greater number of dark-coloured spots. The cotyledons are yellow, much harder than in stage III. The pods from which the seeds were taken had brown spots indicating their drying up. At ultrastructure level this stage differs from the preceding one by an almost complete lack of endoplasmic reticulum and plastids deprived of starch. Figure 10 shows fragments of two neighbouring cells. Their comparison indicates that there occur in the cells of the zone examined of lupin cotyledons at least two forms of protein bodies. One containing protein which after fixation is compact and electron-dense. It should be stressed that the observed protein exhibited such a form from the earliest stage of formation of protein bodies, that is stage I. The second form, consists of protein bodies with a loose structure. In the cells of the examined lupin cotyledons the first type dominates.

In stage V the white seeds marked distinctly with black spots are somewhat smaller than in the preceding stage. The cotyledons are yellow and hard. Such seeds were derived from completely brown and dry pods. The protein bodies in this stage are surrounded by spherosomes (Fig. 11). The size of these bodies in the particular cells differs, but in general in the epidermis they are smaller than in the remaining cells. The cytoplasm ultrastructure is similar to that in stage IV. The mitochondrial cristae are reduced and their matrix is electron-translucent. Thylakoids disappear almost completely in the plastids, whereas the number of plastoglobules increases and phytoferritin appears. The outline of the plastid envelopes is still discernible but their profiles are undulated (Fig. 11a). In structure the cells of this stage resemble those of cotyledons of dry seeds stored for several months after the harvest (Młodzianowski 1978). The only noticeable difference concerns the membrane which in dry seeds can hardly be identified.

The present authors failed to confirm the observations of Davey and Vant Staden (1978) carried out on cotyledons of white lupin. The latter authors described, namely a periodical formation of reserve protein not associated with the membranes. Observations up to date did not allow to demonstrate the presence in yellow lupin of reserve protein formed directly in the ground cytoplasm. The suggestions in the literature for or against the involvement of the Golgi apparatus in reserve protein transport have been entirely on morphological evidence (e. g. Briarty 1980, Craig et al. 1979, Harris 1979). The latest results of Chrispeels et al. (1982) do not rule out the possibility that the Golgi apparatus is involved in transport of reserve protein, but neither do they lend support to that suggestion. The finding in dictyosomal vesicles of stage II of contents resembling in electron density storage protein deposited in the vacuole suggests the cooperation of the Golgi apparatus in the process of protein body formation in yellow lupin. The results of Bechtel and Gaines (1982) indicate that this apparatus plays an important role in the concentration and transport of storage protein to the vacuole during development of the endosperm in cereals. Boulter (1981) suggests the possibility of this transport by way of vesicles of dictyosomal origin as an alternative mechanism to transport by vesicles from the ER.

The problem of translocation of reserve protein from the site of its synthesis in granular ER to the place of its accumulation in vacuoles requires, however, further detailed and extensive investigation.

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REFERENCES

- Ashton F. M., 1976. Mobilization of storage proteins of seeds. *Ann. Rev. Plant Physiol.* 27: 95-117.
- Bechtel D. B., Gaines R. L., 1982. The presence of protease-digestible material in Golgi vesicles during endosperm development of selected cereals. *Amer. J. Bot.* 69: 880-884.
- Bergfeld R., Kühnl T., Schopfer P., 1980. Formation of protein storage bodies during embryogenesis in cotyledons of *Sinapis alba* L. *Planta* 148: 146-156.
- Bollini R., Chrispeels M. J., 1979. The rough endoplasmic reticulum is the site of reserve-protein synthesis in developing *Phaseolus vulgaris* cotyledons. *Planta* 146: 487-501.
- Bollini R., Van der Wilden W., Chrispeels M. J., 1982. A precursor of the reserve-protein, phaseolin, is transiently associated with the endoplasmic reticulum of developing *Phaseolus vulgaris* cotyledons. *Physiol. Plant.* 55: 82-92.
- Boulter D., 1981. Biochemistry of storage protein synthesis and deposition in the developing legume seed. *Adv. Bot. Res.* 8: 1-31.
- Briarty L., 1980. Stereological analysis of cotyledon cell development in *Phaseolus*. II. The developing cotyledon. *J. Exp. Bot.* 31: 1387-1398.
- Chrispeels M. J., Higgins T. J. V., Craig S., Spencer D., 1982. Role of the endoplasmic reticulum in the synthesis of reserve proteins and the kinetics of their transport to protein bodies in developing pea cotyledons. *J. Cell Biol.* 93: 5-14.
- Craig S., Goodchild D. J., Hardham A. R., 1979. Structural aspects of protein accumulation in developing pea cotyledons. I. Qualitative and quantitative changes in parenchyma cell vacuoles. *Aust. J. Plant Physiol.* 6: 81-98.
- Davey J. E., Van Staden J., 1978. Ultrastructural aspects of reserve protein deposition during cotyledonary cell development in *Lupinus albus*. *Z. Pflanzenphysiol.* 89: 259-271.
- Derbyshire E., Wright D. J., Boulter D., 1976. Legumin and vicilin, storage proteins of legume seeds. *Phytochem.* 15: 3-24.
- Harris N., 1979. Endoplasmic reticulum in developing seeds of *Vicia faba*. A high voltage electron microscope study. *Planta* 146: 63-69.
- Harris N., Boulter D., 1976. Protein body formation in cotyledons of developing cowpea (*Vigna unguiculata*) seeds. *Ann. Bot.* 40: 739-744.
- Higgins T. J. V., Spencer D., 1981. Precursor forms of pea vicilin subunits. Modification by microsomal membranes during cell-free translation. *Plant Physiol.* 67: 205-211.
- Kay D. H., 1965. Techniques for electron microscopy. Blackwell Sci. Publ., Oxford.
- Krietowicz W., 1955. *Biogchemia roślin*. PWRiL, Warszawa.
- Millerd A., Thomson J., 1975. Storage proteins of legume seeds: Potential for change? CSIRO Div. Plant Indust. Genet. Rep. 3: 58-68.
- Młodzianowski F., 1978. The fine structure of protein bodies in lupine cotyledons during the course of seed germination. *Z. Pflanzenphysiol.* 86: 1-13.
- Młodzianowski F., Wesołowska M., 1975. Morphological aspects of starch and cell wall material mobilization in developing lupine cotyledons and the effect of kinetin on these processes. *Acta Soc. Bot. Pol.* 44: 529-536.
- Neumann D., Weber E., 1978. Formation of protein bodies in ripening seeds of *Vicia faba* L. *Biochem. Physiol. Pflanzen* 173: 167-180.

- Püchel M., Müntz K., Parthier B., Aurich O., Bassüner R., Manteufel R., Schmidt P., 1979. RNA metabolism and membrane-bound polysomes in relation to globulin biosynthesis in cotyledons of developing field beans (*Vicia faba* L.). Eur. J. Biochem. 96: 321-328.
- Weber E., Neumann D., 1980. Protein bodies, storage organelles in plant seeds. Biochem. Physiol. Pflanzen 175: 279-306.
- Yoo B. Y., Chrispeels M. J., 1980. The origin of protein bodies in developing soybean cotyledons: a proposal. Protoplasma 103: 201-204.

Tworzenie się ciał białkowych w nasionach łubinu żółtego

Streszczenie

Opisano ultrastrukturę komórek liścieni w wyróżnionych, na podstawie cech morfologicznych, pięciu stadiach rozwoju nasion łubinu. Stwierdzono, że w syntezie i transporcie białka zapasowego uczestniczyły siateczka śródplazmatyczna i diktiosomy, a odkładanie białka następowało do centralnej wakuoli lub do tworzących się nowych wakuol. W badanej strefie komórek liścieni łubinu obserwowano dwie formy ciał białkowych, różniące się stopniem zwartości i kontrastu. Obie stanowiły proste ciała białkowe. Równoległe z syntezą zapasowego białka obserwowano grubienie ścian, co może świadczyć o syntezie i odkładaniu się w nich hemiceluloz. Lipidy zapasowe otaczające ciała białkowe tworzyły się w końcowych stadiach rozwoju nasion.