

## Influence of kinetin on storage protein mobilization and ultrastructure of plastids in excised lupine cotyledons grown in darkness

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### Abstract

Storage protein disappeared first from the peripheral and later from central parts of the lupine cotyledon. Kinetin (500  $\mu$ M) stimulated this process; its effect was most prominent after longer times of incubation.

In plastids large, crystalline prolamellar bodies were observed. They sometimes split into smaller parts during the course of the experiment, especially in the kinetin-treated material. Kinetin in darkness did not stimulate formation of thylakoid membranes (neither grana nor primary lamellae).

### INTRODUCTION

Detached cotyledons are an excellent material for studying the influence of various growth regulators: they are isolated from hormonal factors of the embryonic axis and contain so much storage material that they can develop independently, even in darkness, for rather a long time. Lupine cotyledon cells contain large amounts of storage protein, lipids, hemicelluloses and starch. The study of detached lupine cotyledons grown in light revealed that kinetin stimulated their growth and mobilization of the reserves (Wesołowska, 1972), what is in good agreement with the works of the other authors (Banerji and Laloraya, 1965; Kursanov et al., 1969; Rijven, 1972; Kulaeva, 1973; Woźny and Szwejkowska, 1975).

Plastids are also influenced by kinetin and other cytokinins. Benzylaminopurine accelerated development of chloroplasts in excised pumpkin cotyledons (Khokhlova et al., 1971); kinetin stimulated chlorophyll

synthesis and chloroplast development in detached lupine cotyledons (Młodzianowski and Gezela, 1974).

It seemed interesting to compare the influence of kinetin on detached lupine cotyledons grown in light (Wesołowska, 1972; Młodzianowski and Gezela, 1974) and in darkness. Thus, the aim of the present study was to investigate the effect of kinetin on the mobilization of the protein reserves and the development of plastids in excised cotyledons grown in darkness.

#### MATERIALS AND METHODS

Seeds of *Lupinus luteus* cultivar Express were soaked in water for 12 hours and germinated for 6 days in darkness. After this time the cotyledons were excised from the embryonic axis and placed in Petri dishes containing 20 ml water (control) or kinetin solution (500  $\mu$ M). One cotyledon from every seed was incubated in water, the second — in kinetin, and these pairs were later analysed. Samples for microscopy were taken after 1, 6 and 12 days of incubation (Fig. 1). These samples always included the inner epidermis, palissade mesophyll, spongy mesophyll and outer epidermis for light microscopy; and inner epidermis, palissade mesophyll and a few layers of spongy mesophyll for electron microscopy.

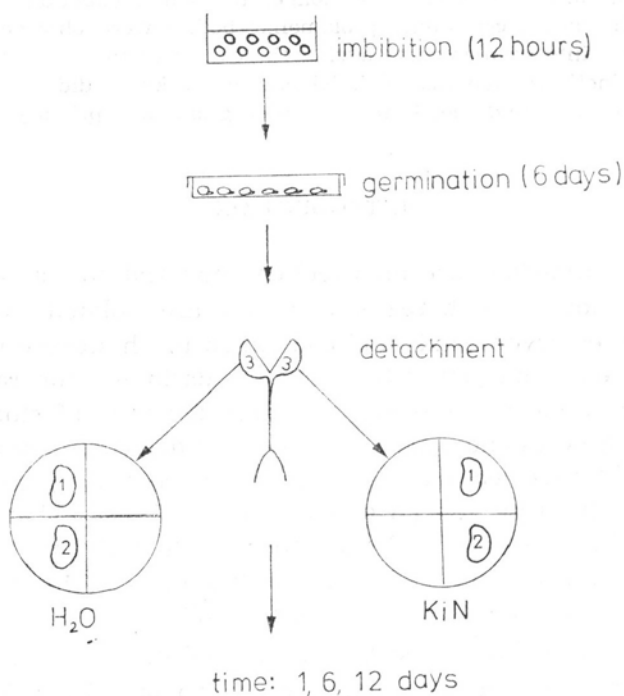


Fig. 1. Scheme of the experiment

Material for light microscopy was fixed in FAA (90 ml of 70% ethanol + 5 ml of acetic acid + 5 ml of 40% formalin), dehydrated in ethanol and embedded in paraffin. The sections were 15  $\mu$ m thick. Protein was stained with bromophenol blue according to Mazia et al. (1953).

Material for electron microscopy was fixed in 5% glutaraldehyde in 0,1 M cacodylate buffer pH 7,2 for 24 hours at +4°C, postfixed for 2 hours in 2% OsO<sub>4</sub> in the same buffer, dehydrated through ethanol, acetone and propylene oxide series and embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a JEM 7A microscope. Epon 1  $\mu$ m sections were viewed in light microscopy after protein staining with toluidine blue according to Kay (1965).

## RESULTS AND DISCUSSION

### 1. Mobilization of storage protein

During incubation of isolated cotyledons either in water or in kinetin solution the amount of storage protein gradually diminished. Kinetin distinctly stimulated this process; this could be observed even after 24 hours of incubation (Figs. 2—3). Cross-sections of cotyledon from water (Fig. 2) showed many dark stained protein bodies. The cotyledon from kinetin was thicker and contained much less protein bodies (Fig. 3). After 6 days of incubation the effect of kinetin on storage protein mobilization was more pronounced, but the most visible difference between kinetin-treated and control material was observed after 12 days of incubation. The control cotyledon still contained storage protein, mainly in the central part of the spongy mesophyll (Fig. 4). Cotyledon cells from kinetin were without visible protein reserves, they were much larger than the former (Fig. 5).

Further details are shown on photographs of Epon sections (Figs. 6—11). After 24 hours of incubation in water in the cells of palissade mesophyll there were large, homogeneous protein bodies (Fig. 6a); in the spongy mesophyll (Fig. 6b) vacuoles were filled with large, partly digested, protein masses. In the mesophyll of the cotyledon incubated in kinetin (Fig. 7a-b) only small protein bodies could be observed in the central vacuoles. After 6 days of the experiment reserve protein in the palissade mesophyll disappeared (Figs. 8a, 9a). In the spongy mesophyll of the control cotyledon there were small protein bodies which sometimes formed clumps (Fig. 8b); the cotyledon incubated in kinetin contained only trace amounts of storage protein (Fig. 9b). After 12 days in the control cotyledon only small protein fragments could be seen in vacuoles of the spongy mesophyll (Fig. 10b); cells from kinetin culture were very large, with central "empty" vacuole (Fig. 11a-b).

Mobilization of storage protein started in the peripheral parts of the cotyledon and advanced into its central part. The same was observed in other Legumes (Smith and Flinn, 1967; Briarty et al., 1970; Harris et al., 1975) and detached lupine cotyledons grown in light (Wesołowska and Młodzianowski, 1976), where storage protein first disappeared from the epidermis and palissade mesophyll.

Degradation of a single protein body may be accomplished in various ways in various species; these processes were studied by many authors. In excised lupine cotyledons protein bodies were gradually digested (one could observe holes in them and erosion of their edges) or they fused together. Large protein bodies and masses diminished during the course of our experiment and in the end there were "empty", large vacuoles. These results agreed with earlier observations (Wesołowska and Młodzianowski, 1976; Młodzianowski, 1978).

Incubation of the excised lupine cotyledons in kinetin solution in darkness stimulated mobilization of storage protein. This was most distinct after longer times of the experiment (see Figs. 4—5). The same effect was obtained in detached lupine (Wesołowska and Młodzianowski, 1976), pumpkin Kulaeva, 1973) and cucumber (Woźny and Szweykowska, 1975) cotyledons grown in light. Kinetin also stimulated in darkness growth and vacuolation of the cells, as it did in light (Wesołowska, 1972).

## 2. Ultrastructure of plastids

In the cells of the cotyledon germinated in water numerous plastids with various profiles could be seen (Fig. 12a-c). They included large starch grains and prolamellar bodies which had a regular, crystalline structure (Fig. 12b); long, single lamellae could be seen in their peripheral part. The cell walls in this stage were thick, especially in the corners of the cells; in vacuoles there were large protein bodies (Fig. 12a).

In the course of our experiment no development of plastids was observed — the occurring changes should rather be called degradation. In the peripheral parts of prolamellar bodies long, single thylakoids could be observed (Fig. 13a). After longer times of incubation in kinetin some prolamellar bodies were dispersed (Fig. 13b) — only small fragments of crystalline structure remained, connected with long thylakoids. Small plastoglobuli also occurred. But even after the longest time of incubation large, crystalline prolamellar bodies could also be seen.

In all the cotyledons grown in kinetin medium (Fig. 13a-b) and only in the 12-day-old water culture plastids with darkstaining matrix

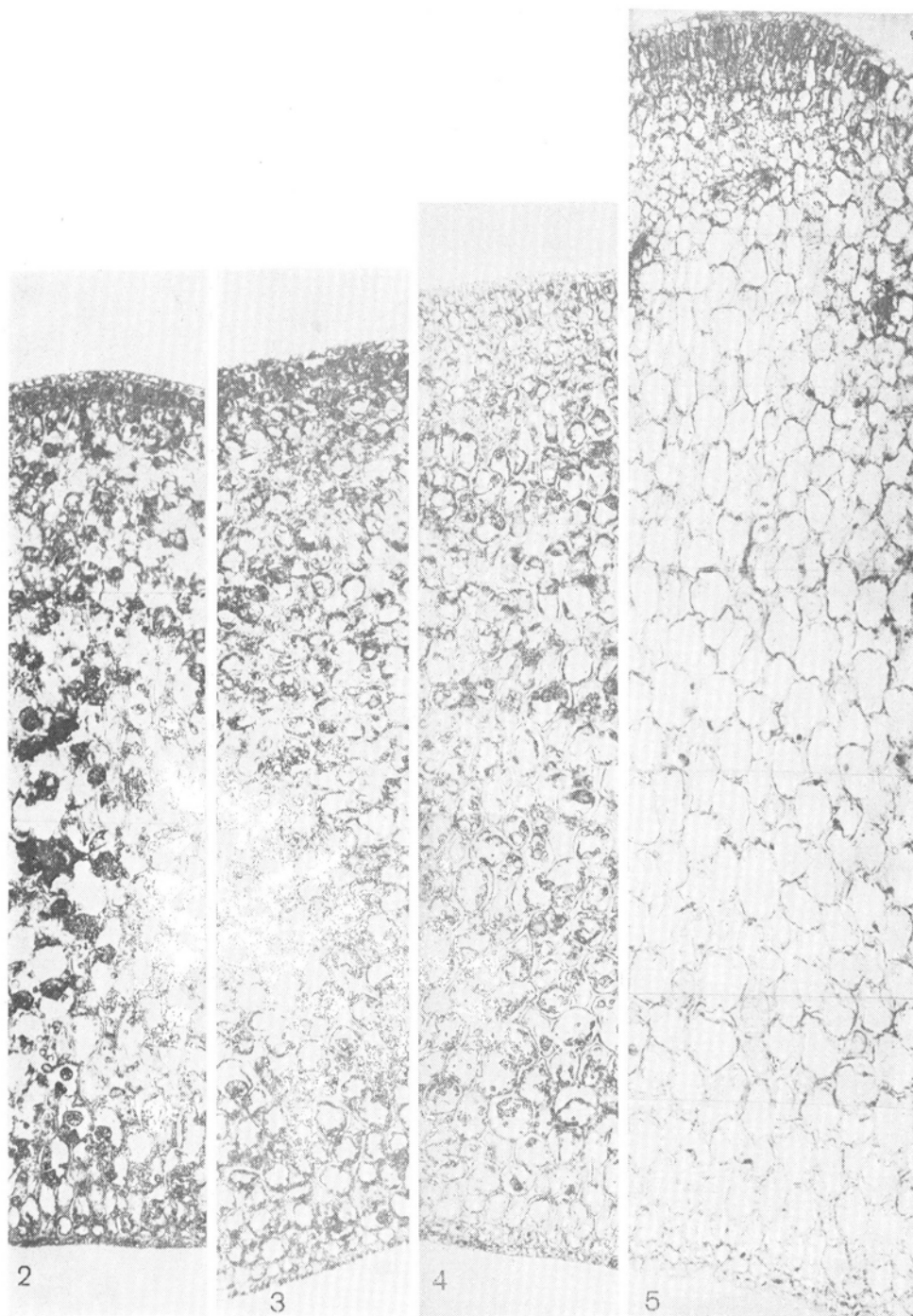


Fig. 2—3. Cross sections of lupine cotyledons grown 24 hours in water (Fig. 2) and in kinetin solution (Fig. 3). Paraffin sections stained with bromophenol blue.  $\times 500$   
 Fig. 4—5. Cross sections of lupine cotyledons grown 12 days in water (Fig. 4) and in kinetin solution (Fig. 5). Paraffin sections stained with bromophenol blue.  $\times 500$

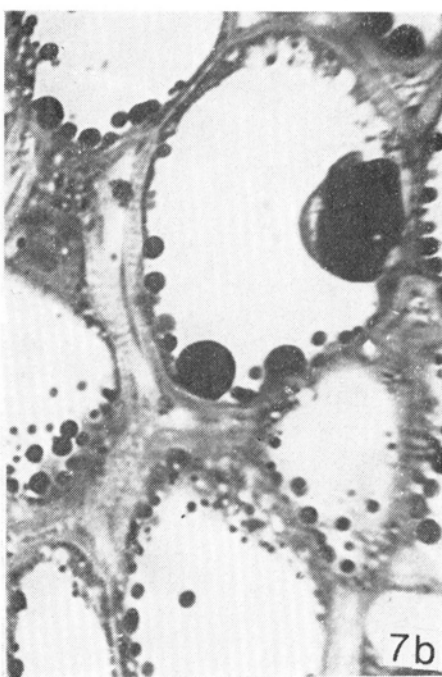
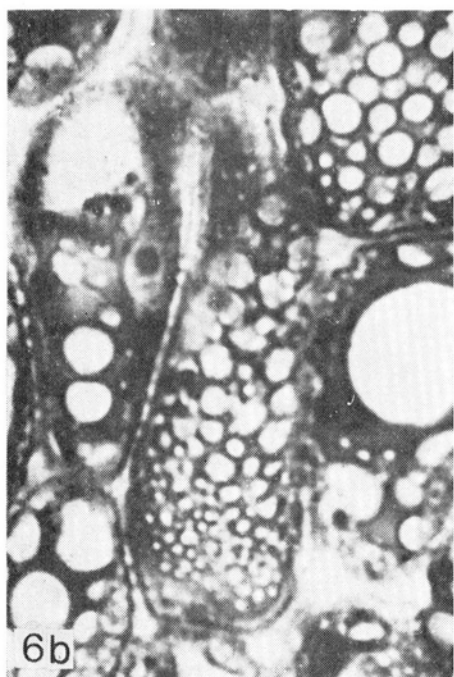
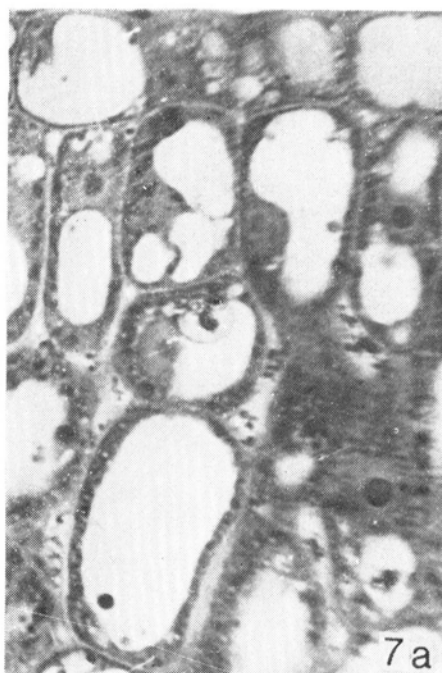
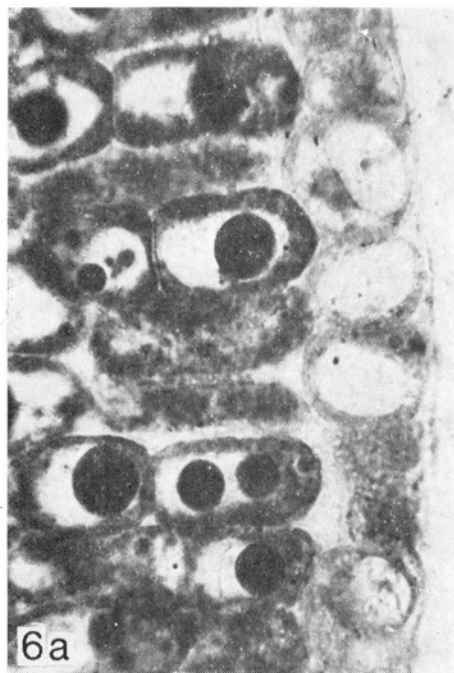


Fig. 6—7. Epon cross sections of lupine cotyledons stained with toluidine blue.  
 $\times 1200$

Fig. 6. Cotyledon grown 24 hours in water. Fig. 7. Cotyledon grown 24 hours in kinetin solution

*a* — epidermis and palisade mesophyll; *b* — spongy mesophyll

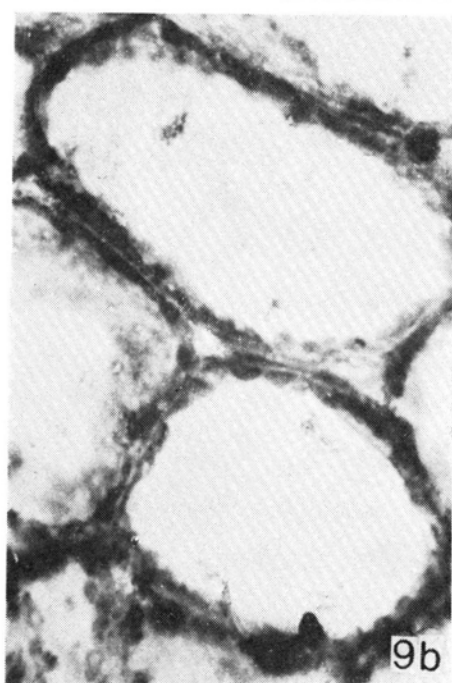
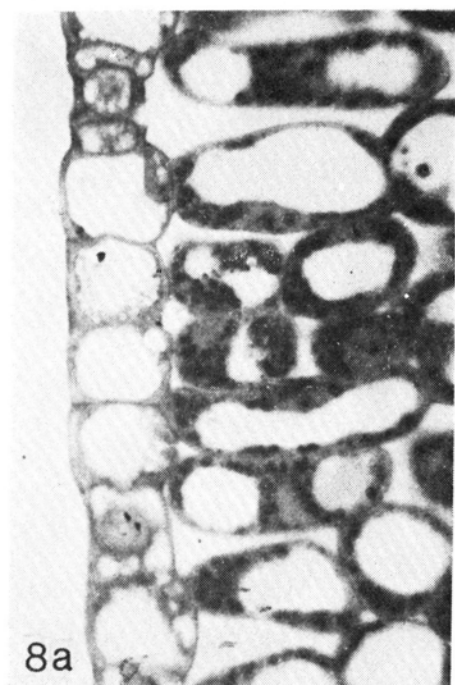


Fig. 8—9. Epon cross sections of lupine cotyledons stained with toluidine blue.  
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Fig. 8. Cotyledon grown 6 days in water. Fig. 9. Cotyledon grown 6 days in kinetin solution  
 a — epidermis and palissade mesophyll; b — spongy mesophyll

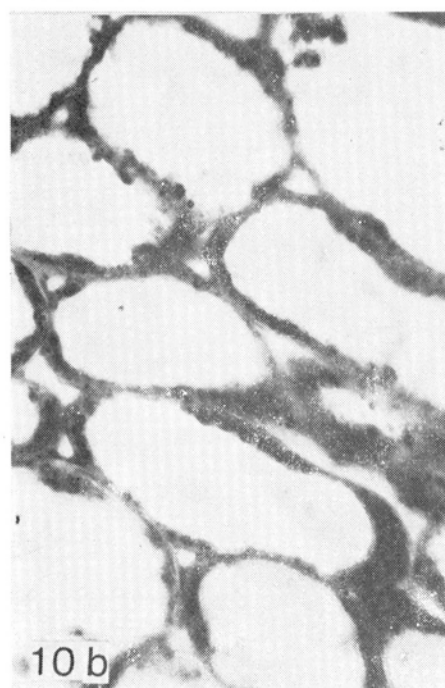
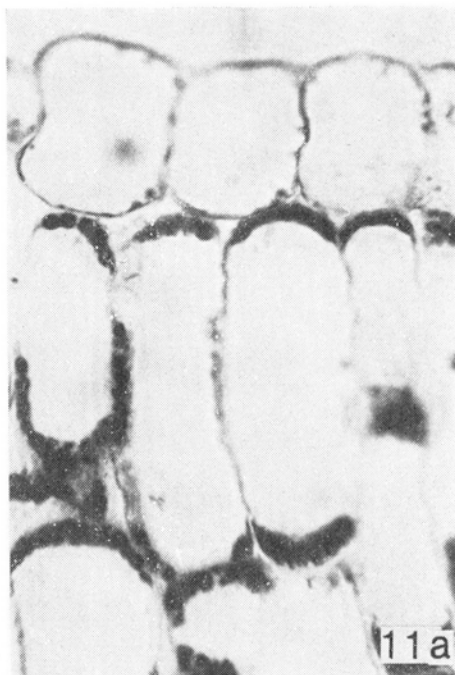
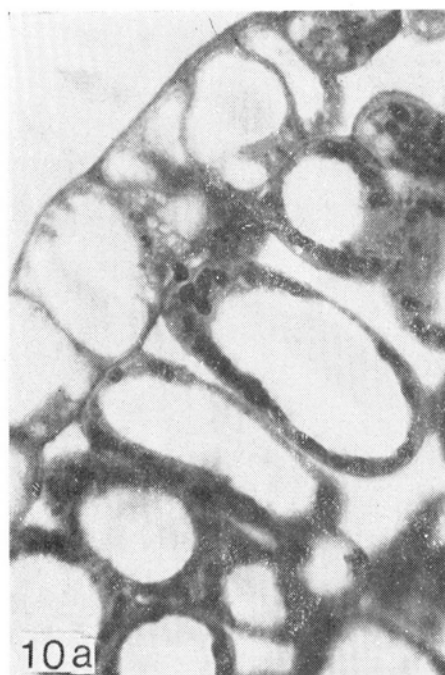


Fig. 10—11. Epon cross sections of lupine cotyledons stained with toluidine blue.  
 $\times 1200$

Fig. 10. Cotyledon grown 12 days in water. Fig. 11. Cotyledon grown 12 days in kinetin solution

*a* — epidermis and palissade mesophyll; *b* — spongy mesophyll

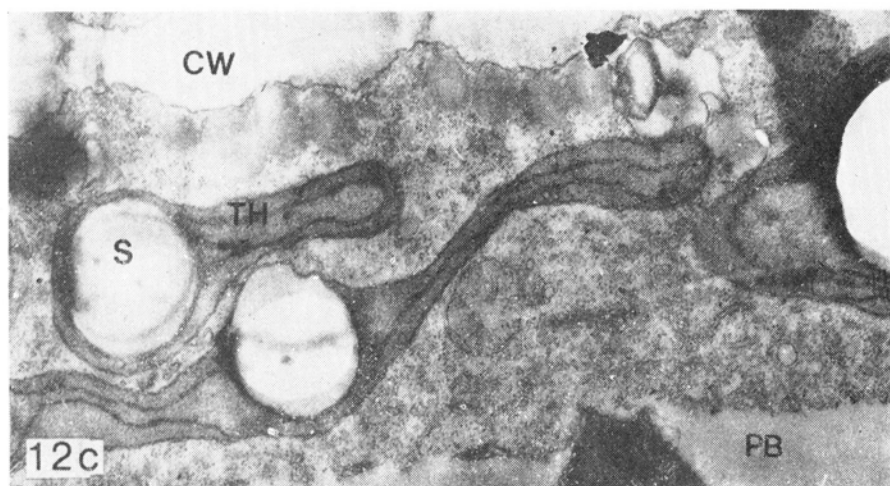
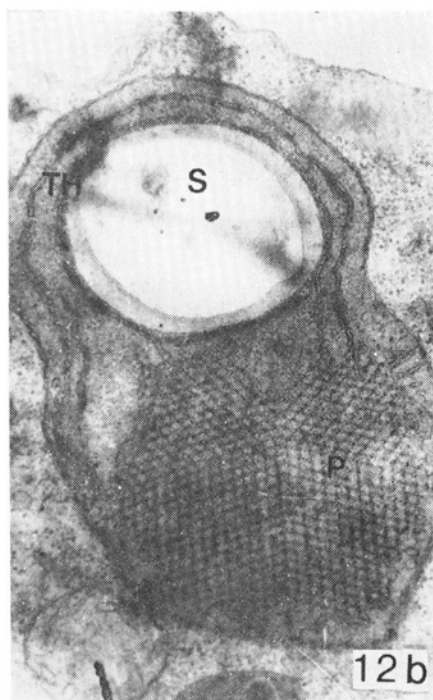
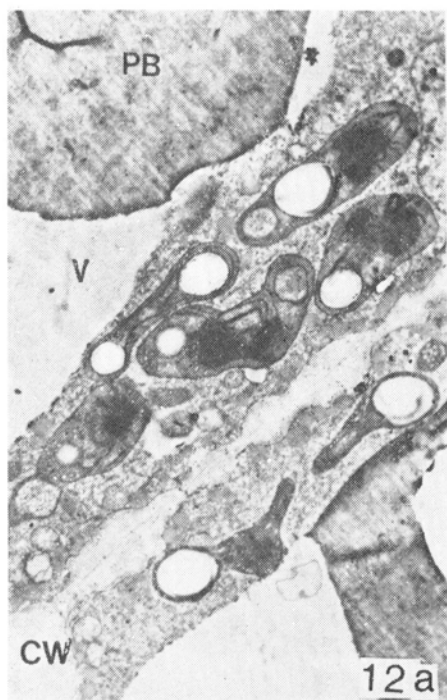


Fig. 12. Electron micrographs of lupine cotyledons germinated in water:  
 a) Fragment of a cell  $\times 3500$ , b) Plastid with large prolamellar body  $\times 20\,000$ , c) Plastid with  
 primary thylakoids  $\times 10\,000$

CW — cell wall, P — prolamellar body, PB — protein body, PL — plastoglobuli, S — starch,  
 TH — thylakoid, V — vacuole

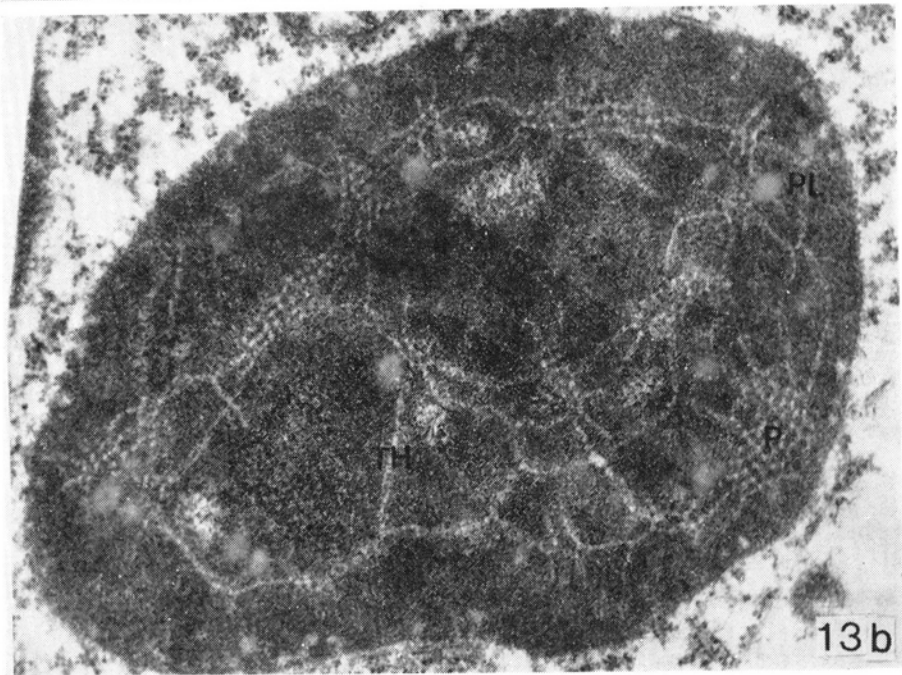
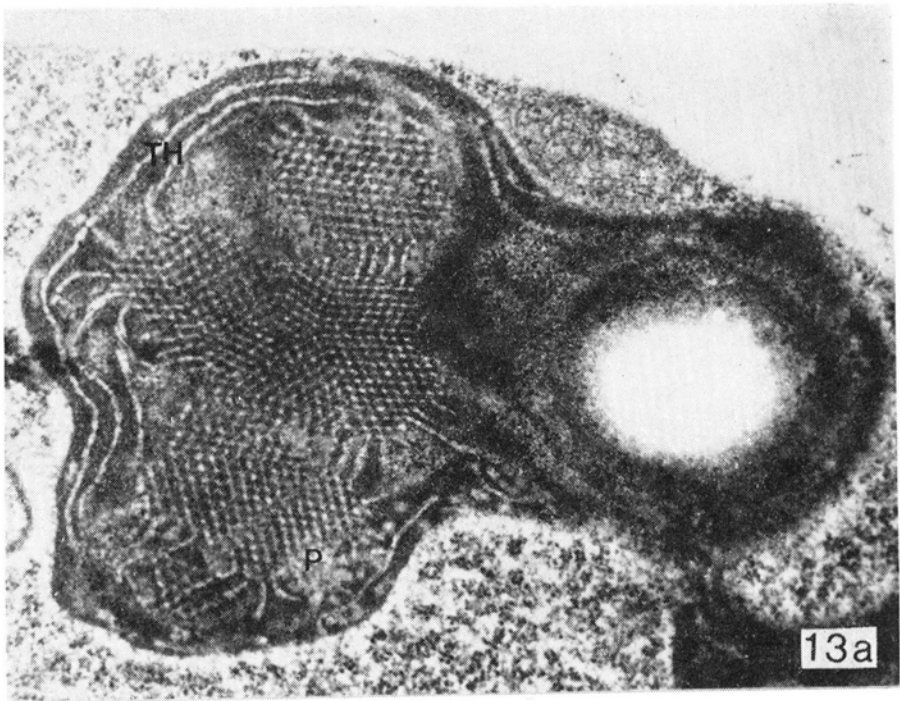


Fig. 13. Plastids in cotyledons grown 24 hours (a) and 6 days (b) in kinetin solution.  $\times 25\,000$ . Notations as in Fig. 12.

occured. It is difficult to explain such staining. A similar phenomenon was observed in leaves of *Rubus* (Ljubešić, 1970) and *Cichorium* (Młodzianowski and Młodzianowska, 1973). This dark-staining matrix could be connected with some stage of plastid differentiation in darkness.

It is also interesting that plastids from detached lupine cotyledons had such diverse profiles. Probably it was because they were grown in unnatural conditions (darkness, deficiency of the axis) for a rather long time.

Plastids in dry and imbibed lupine cotyledons contained only plastoglobuli and grains of phytoferritin (Młodzianowski and Gezeła, 1974). In the present investigation phytoferritin was not observed; probably it had been used up to build prolamellar bodies. Kinetin and other cytokinins are known to stimulate chloroplast development in detached lupine (Młodzianowski and Gezeła, 1974) and pumpkin (Khokhlova et al., 1971) cotyledons. This stimulation was not observed in darkness; only some kind of degradation occurred — starch disappeared, prolamellar bodies split into a few small parts connected with single thylakoids (see Fig. 13b). Data from the literature (Piatelli et al., 1971; Koehler, 1972; Hall, 1973) indicate a close correlation between kinetin and light-dependent processes. Plastid development is surely one of them, but our results showed that kinetin was not able to substitute light in the process of chloroplast differentiation.

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*Wpływ kinetyny na mobilizację białka zapasowego i ultrastrukturę plastydów w izolowanych liścieniach łubinu hodowanych w ciemności*

**Streszczenie**

Białko zapasowe zanikało najpierw z obwodowych części liścienia łubinu, a później — z centralnych. Kinetyna (500  $\mu\text{M}$ ) stymulowała ten proces; jej efekt był zwłaszcza wyraźny po dłuższych czasach inkubacji.

W plastydach obserwowano duże, krystaliczne ciała prolamellarne. Mogły one rozpadać się na mniejsze w czasie trwania doświadczenia, szczególnie w materiale traktowanym kinetyną, ale nie zanikały całkowicie. Kinetyna w ciemności nie stymulowała ani tworzenia gran, ani prostych tylakoidów.