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## STRUCTURAL AND FUNCTIONAL DIFFERENTIATION OF WHEAT CHLOROPLASTS\*

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## Introduction

The leaves of monocotyledonous plants are especially suitable for the study of chloroplast differentiation. They grow from the basal meristem so that, according to their age, the cells are arranged linearly, the youngest lying at the base. At the same time proplastids develop into chloroplasts in differentiated cells lying a few millimetres above the leaf base. Fine structural changes, occurring in plastids during these processes, are already partly known. The small oval or amoeboidal proplastids in meristematic cells contain only a few tubules or single thylakoids, which are formed by invagination of the inner membrane of the proplastid envelope. Later on, by multiplication, elaborate systems of grana and stroma thylakoids of young chloroplasts are formed (Kirk and Tilney-Bassett 1978, Possingham 1980). Much less is known about the functional differentiation of these plastids. In recently published investigations on biochemical and functional changes, occurring during differentiation of chloroplasts in leaves of maize and wheat, for technical reasons the youngest regions of the leaves were either omitted or not separately studied (Baker and Leech 1977, Boffey et al. 1979, 1980). Preliminary electron microscopic investigations have shown, that it is just in these lowest parts of the leaves that proplastids turn into young chloroplasts. It is unknown when and how the photosynthetic activity begins in these young plastids.

In this paper the photooxidation of diaminobenzidine (DAB) has been used to localize the photosynthetic activity (photosystem I activity) in the thylakoids at ultrastructural level (Wrischer 1978). It is generally

\* Dedicated to Professor Zvonimir Devidé on the occasion of his 60<sup>th</sup> birthday.

accepted that the photooxidation of DAB is mediated by some component(s) of the photosystem I (Porat et al. 1978, Nishimura and Hiraoaka 1979). The result of this photooxidation is an osmiophilic polymer of DAB which accumulates in the membranes and loculi of photosynthetically active thylakoids, and due to its contrast it can be observed in electron microscope.

### Material and Methods

Wheat seedlings (*Triticum aestivum* cv. »super zlatna«) were grown for 12 days at a photoperiod of 15 h light and 9 h darkness daily (2 fluorescent tubes 20 W, 4500 K, 4000 lx at ground level). The coleoptile and the first outermost leaf were removed from the seedling and the second leaf thoroughly detached from the base. The lowest 2 centimetres of the leaf were immediately cut into segments 0.5 mm wide and fixed for 30 minutes in a freshly prepared solution of 2% formaldehyde in 0.05 mol phosphate buffer (pH 7.5). After that the leaf pieces were rinsed in the same buffer (with addition of 5% sucrose) for 30 minutes, and then incubated in a medium containing 1 mg/ml diaminobenzidine (DAB) in phosphate buffer (pH 7.5), first, for 30 minutes in darkness and then for 90 minutes in light (at 4000 lx). The tissue was then rinsed in phosphate buffer for 30 minutes and postfixed in 1% OsO<sub>4</sub> in the same buffer for 60 minutes. After dehydration the leaf pieces were embedded in Araldite. Ultrathin sections were examined without further staining in a Siemens Elmiskop I. For control the leaf pieces were incubated in DAB medium for 120 minutes, in darkness only.

The leaf segments were also fixed in 1% glutaraldehyde in cacodylate buffer (pH 7.2), postfixed in 1% OsO<sub>4</sub> and dehydrated and embedded as usual. The sections were stained with uranyl acetate and lead citrate.

### Results

In the cells of the basal meristem, lying up to 0.5 mm from the leaf base, there were only oval or amoeboidal proplastids (1 — 2 μm in diameter), which contained very few short tubules or single thylakoids (Fig. 1). These internal membranes never showed a positive DAB reaction (Fig. 2). At the same time the inner membranes of mitochondria were darkly stained due to the presence of DAB polymers (Figs. 2, 3, 7). It is known that some components of the mitochondrial electron transport chain also interact with DAB, this reaction being independent of light (Seligman et al. 1968).

The photooxidation of DAB appeared first in the plastids lying in the second leaf segment examined, i. e. 0.5 to 1 mm above the leaf base. In some partly differentiated cells the sparse single thylakoids of the plastids became DAB positive. The photooxidation of DAB was at first very faint and discontinuous, and later more intense (Fig. 3). Exceptionally only some thylakoids in a plastid were DAB positive, while others were still without any positive DAB staining (Fig. 4). The plastid envelopes always remained without reaction (Figs. 3, 4, 7, 8).

When the cells developed further and were pushed by the growing tissue away from the leaf base, the plastids became larger, the

thylakoids in them increased in number and became aggregated into stacks of 2, 3 or more (Figs. 5—8). By further multiplication an elaborate system of grana and stroma thylakoids was formed in young chloroplasts. Both the grana and the stroma thylakoids were always DAB positive (Figs. 7, 8). In cells lying 2 to 3 mm above the leaf base, there were already young chloroplasts with grana. In such chloroplasts starch grains were also present (Fig. 8). In older cells the chloroplasts became still larger and the number of grana thylakoids considerably increased.

Young chloroplasts, lying in segments up to 1 cm from the leaf base, contained one or several small prolamellar bodies, which were always in connection with the grana (Figs. 5, 6). The tubules of the prolamellar bodies did not contain osmiophilic DAB deposits (Fig. 8). In older cells — more than 1 cm above the leaf base — the chloroplasts did not contain prolamellar bodies at all.

As the leaves grew, the number of plastids constantly increased by division. Forms of proplastids and chloroplasts, which could be interpreted as division stages, were observed very frequently (Figs. 3, 8).

Light microscopic examinations showed that in the zone of the first few mm above the leaf base mitoses still occurred (see also Boffey et al. 1979). Therefore, among already differentiated cells, which contained young chloroplasts, there were cells which had just passed mitoses and which contained proplastids (Fig. 5). Their internal membranes did not show a positive DAB reaction. Plastids in epidermal cells, as well as plastids in young sieve tubes were also without positive DAB reaction.

## Discussion

The first sign of the photosystem I activity in thylakoids — as seen by photooxidation of DAB — can be observed in wheat plastids lying 0.5 to 1 mm above the leaf base. Light microscopic control investigations have shown that in this zone also a slight greening of the tissue begins. At first, single thylakoids of the proplastids become DAB positive, and later on — when the proplastids turn into chloroplasts — all thylakoids become darkly stained. These data show that the activity of the photosystem I appears already before the grana are formed. This is also in good accordance with the results of physiological investigations performed on maize, namely that photosystem I develops and is completed earlier than photosystem II and that an efficient  $O_2$  evolution is connected with the fusion of thylakoids into grana (Baker and Leech 1977).

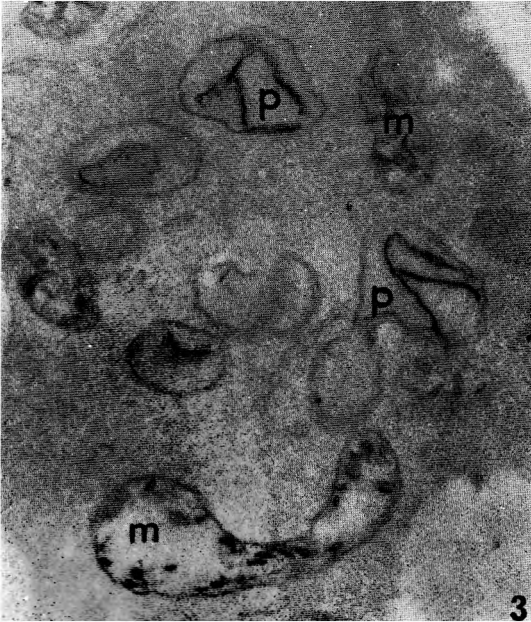
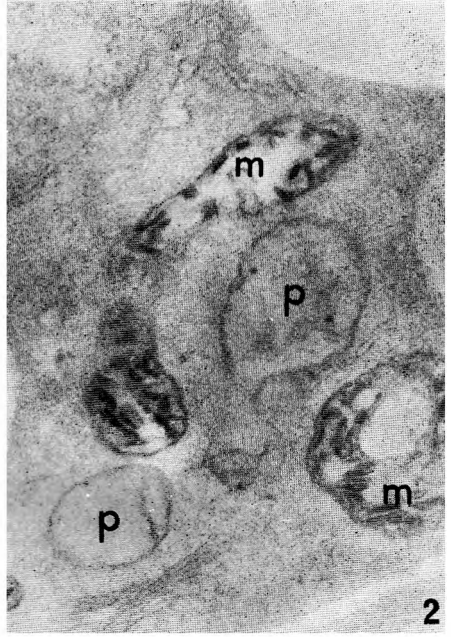
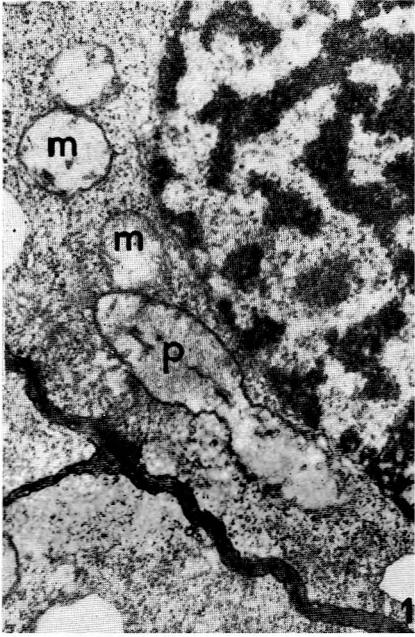
The plastid envelope and the tubules of prolamellar bodies always remain without photooxidized DAB. These facts are in good agreement with the results obtained earlier in studies of chloroplasts and greening etioplasts (Wrischer 1977, 1978). Recently published biochemical and physiological investigations also indicate that the tubules of the prolamellar bodies have neither photosynthetic activity, nor do they contain chlorophyll precursors (Hamp and Wellburn 1978, Lütz and Klein 1979). According to their ultrastructure, the plastids lying in cells at the base of monocotyledonous leaves should not be named etioplasts (Kirk and Tilney-Bassett 1978, Possingham 1980), as prolamellar bodies do not develop in them until they have formed grana. It is believed that prolamellar bodies develop owing to weak illumination at the base of these leaves, which are shaded by others (Brangeon

1973, Baker and Leech 1977). On the other hand, pointing to the fact that prolamellar bodies are formed only by very young chloroplasts in maize leaves, Rascio et al. (1980) claim that the presence of prolamellar bodies is common to all young chloroplasts and represents "a transitional stage in membrane assemblage". Further experiments, using among others, additional shielding or additional illumination of leaves of different age, will be necessary to solve this problem.

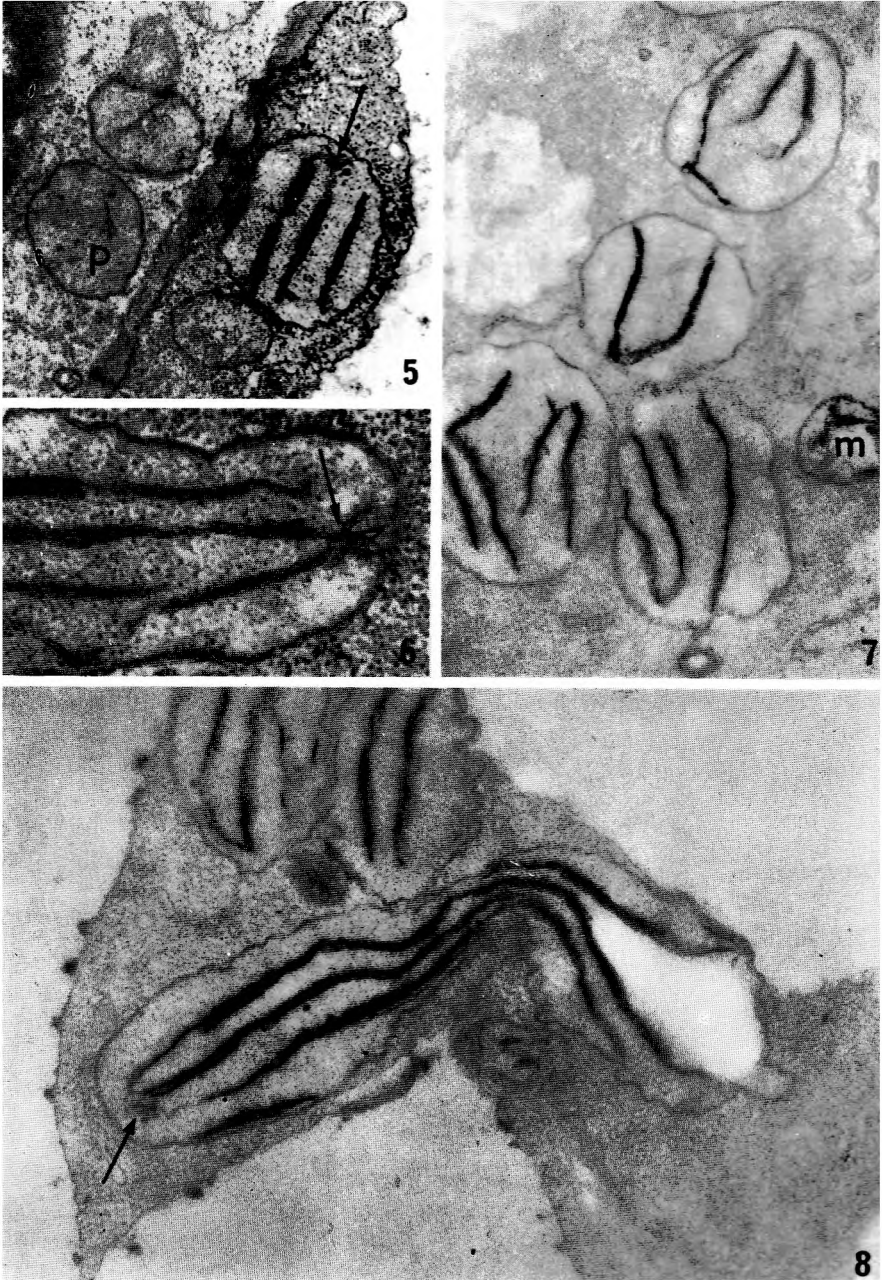
### Summary

Photooxidation of diaminobenzidine (DAB) has been used to detect ultrastructurally the photosynthetic activity (photosystem I activity) in the thylakoids of differentiating chloroplasts of wheat leaves. It has been shown that the accumulation of photooxidized DAB deposits first in single thylakoids of plastids of cells lying 0.5 to 1 mm above the leaf base. Later the thylakoids multiply and aggregate into grana; in leaf segments being 2 — 3 mm above the leaf base, there are already chloroplasts with an elaborate system of grana and stroma thylakoids, which all show a positive DAB reaction. Plastid envelopes and small prolamellar bodies, which develop in young chloroplasts up to 1 cm from the leaf base, remain without photooxidized DAB deposits. The changes described are discussed in connection with the present knowledge of the functional differentiation of chloroplasts.

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- Fig. 1. Part of a meristematic cell from the bottom of the leaf with a proplastid (p) and mitochondria (m). Fixation: glutaraldehyd/OsO<sub>4</sub>; staining with uranyl acetate and lead citrate. 24,000 : 1.
- Fig. 2. Part of a meristematic cell from the bottom of the leaf. Proplastids (p) without DAB reaction. Intratubular DAB staining in mitochondria (m). 20,000 : 1.
- Fig. 3. Part of a cell lying 0.5 — 1 mm above the leaf base. Photooxidized DAB deposits in single thylakoids of the plastids (p). Plastid envelopes without reaction. Positive DAB reaction in mitochondria (m.) 17,000 : 1.
- Fig. 4. The same material as in Fig. 3. Plastid with positive DAB reaction in one thylakoid. The plastid envelope and the other thylakoid are without DAB reaction. 36,000 : 1.
- Fig. 5. Part of two cells lying 1—2 mm above the leaf base. A young chloroplast with grana and a prolamellar body (arrow) in the right cell, two proplastids (p) in the left cell. Fixation: glutaraldehyde/OsO<sub>4</sub>; staining with uranyl acetate and lead citrate. 20,000 : 1.
- Fig. 6. The same material as in Fig. 5. Young chloroplast with grana and a prolamellar body (arrow). 42,000 : 1.
- Fig. 7. Part of a cell lying 1—2 mm above the leaf base. Young chloroplasts with small grana, which are darkly stained owing to the presence of photooxidized DAB. Plastid envelopes without reaction. Positive DAB reaction in the mitochondrion (m). 16,000 : 1.
- Fig. 8. Part of a cell lying 2—3 mm above the leaf base. All grana and stroma thylakoids contain darkly stained DAB. Prolamellar body (arrow) and plastid envelopes without reaction. In the lower chloroplast a starch grain. 32,000 : 1.



Figs. 1—4.



Figs. 5—8.

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## SA Ž E T A K

## STRUKTURNA I FUNKCIONALNA DIFERENCIJACIJA KOROPLASTA PŠENICE

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Koristeći se fotooksidacijom diaminobenzidina (DAB) elektronskim je mikroskopom praćeno pojavljivanje fotosintetske aktivnosti (aktivnosti fotosistema I) u tilakoidima plastida listova pšenice. Nagomilavanje osmofilnih DAB-polimera pojavljuje se najprije u pojedinaćnim tilakoidima plastida iz stanica koje leže 0,5—1 mm iznad baze lista. U nešto starijim stanicama umnažanjem tilakoida izgrađuju se grana, tako da u odsječ-

cima listova koji leže 2—3 mm iznad baze lista postoje već mladi kloroplasti s grana-tilakoidima i stroma-tilakoidima. Svi tilakoidi tog membranskog sistema pokazuju pozitivnu reakciju DAB. Ovojnice plastida i tubuli prolamelarnih tjelešaca u mladim kloroplastima pri bazi lista nikad ne sadržavaju osmiofilne DAB-polimere. Opisane promjene razmatrane su u vezi s današnjim našim znanjem o funkcionalnoj diferencijaciji kloroplasta.

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