ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 3: 381—389
1981

Structural and functional polymorphism of plastids in leaves of *Clivia miniata* Rgl. I. Ontogenesis of plastids in epidermis and guard cells

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(Received: July 10, 1980)

Abstract

During leaf ontogenesis in *Clivia miniata* epidermal and guard cell plastids differing in structure and function differentiate from morphologically equal proplastids. Plastid differentiation proceeds in parallel to cellular differentiation and stops in cells reaching maturity. It is suggested that the developmental sequence of plastids is fixed within cells and controlled by the inner mechanisms.

INTRODUCTION

Mature leaves of both mono- and dicotyledonous plants contain various types of plastids which differ in structure and function. The differences are largely related to the stages of thylakoid development and to the ability of plastids to accumulate starch and/or other inclusions. The heterogeneity of plastids has been found directly linked to their distribution within the leaf blade. Freeman (1973) described three morphological types of plastids in epidermis, guard cells, and mesophyll in leaves of *Opuntia basiliaris* Engelm. Bigel. On comparing the capability of plastids to accumulate starch as well as basing upon the degree of thylakoid development in leaf blade cells of *Bryophyllum*, Sarda et al. (1975) have recognized the following three types: 1) plastids deprived of starch, with well developed thylakoid system of both grana and intergrana; 2) plastids containing starch, with recognizable stroma and grana thylakoids; and 3) plastids abundant in starch, with markedly reduced number of thylakoids. The latter type of plastids has been generally found within the region adjacent to the vascular bundle. These authors did not distinguish, however, the bundle sheath

Figs. 1-6. Plastids of the lower epidermis in leaves of *Clivia miniata*
1. Proplastid from the meristematic zone of the young leaf (0.5 cm in length). 25 500 ×
2. Proplastid with starch grains from the colourless zone of older leaf (10 cm in length). 25 500 ×
3, 4, 5. Successive stages of plastid differentiation; sacks filled with osmiophilic contents near starch grains, within the stroma. 3 — 25 500 ×; 4 — 31 300 ×; 5 — 27 400 ×
6. Amyloplast from the celadon leaf zone. 18 500 ×

layer which is typical for the C_4 plants. Again, Khudairi (1977) described three different types of chloroplasts in leaves of *Xanthium*, as follows: 1) chloroplasts in mesophyll and transfer cells, with normally developed grana; 2) chloroplasts in epidermis and bundle sheath cells, with less numerous thylakoids; and 3) chloroplasts capable of starch-storage in guard cells. Also Miyake and Maeda (1976) have found various ultrastructural types of plastids within different tissues of the leaf blade in *Oryza sativa* L. cv. Norin-8. According to Whatley (1979), plastids occurring in particular cells of primary leaves of *Phaseolus vulgaris* differ in volume and in the amount of accumulated starch already at the initial stages, while at later periods the alterations may be attributed to the degree of thylakoid development. These differences are partially based on cell position within the leaf blade and in part upon cell type as well as the stage of cell differentiation. It is well known that mature chloroplasts may vary in structure and function even in adjacent mesophyll cells. In fact, examples of such heterogeneity can be demonstrated in chloroplasts of the bundle sheath and mesophyll cells of C_4 plants (Laetsch and Price 1969, Kanai and Kashiwagi 1975, Miyake and Maeda 1978).

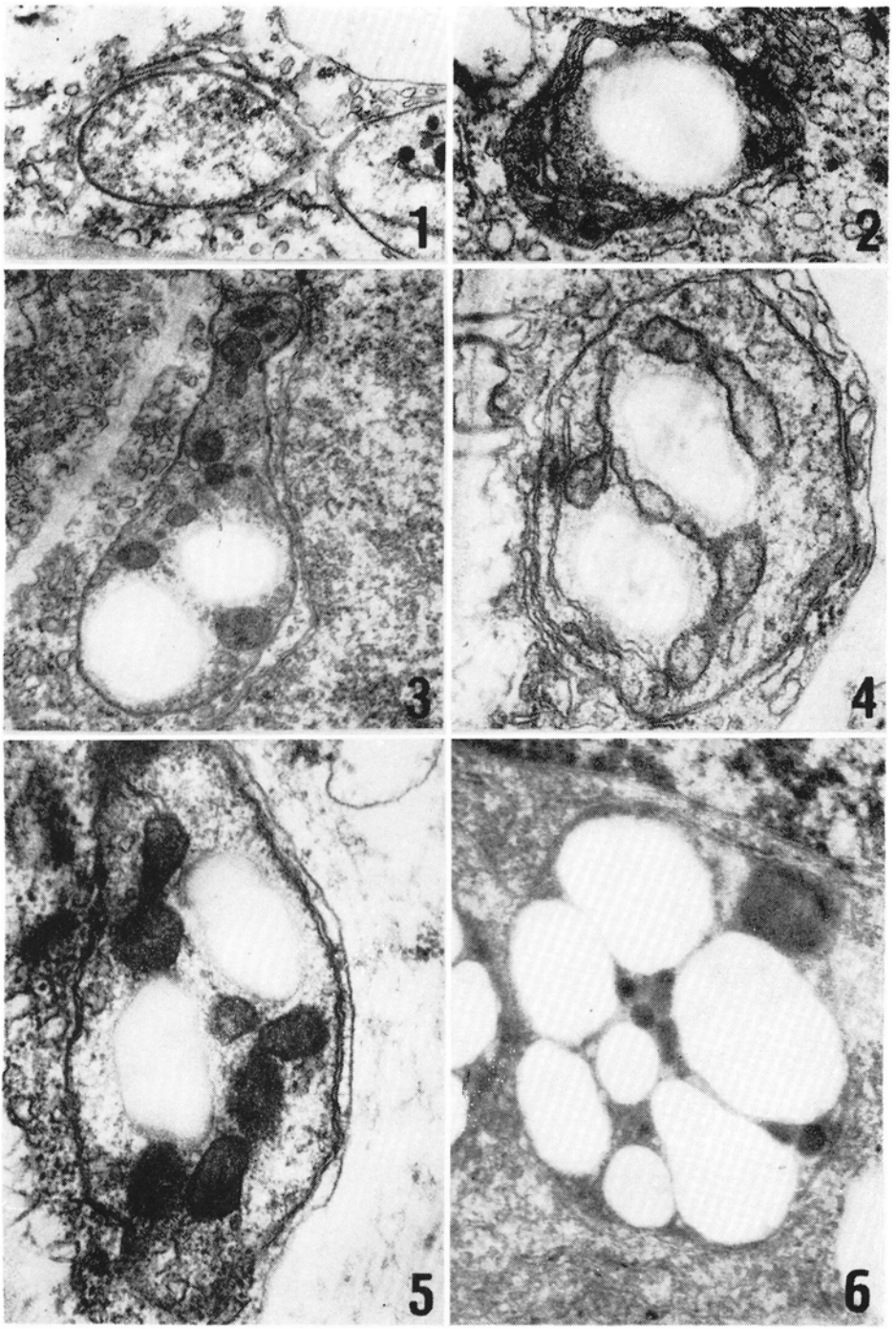
Granick (1961) suggests that the structural variety of plastids may result either from their retention in specific phase of development or from degenerative changes. It seems also possible that the high degree of cellular specialization may be the main factor determining the structure of plastids.

The present paper deals with the sequence of structural and functional changes in plastids during cell differentiation of the lower and upper epidermis and of the guard cells in leaves of *Clivia miniata*. The second part will discuss the structural and functional polymorphism of mesophyll chloroplasts and plastids in vascular tissue of leaves.

MATERIAL AND METHODS

Clivia miniata Rgl. was grown under natural day length conditions. Leaves of various ages as determined by the degree of expansion, were

Plate I



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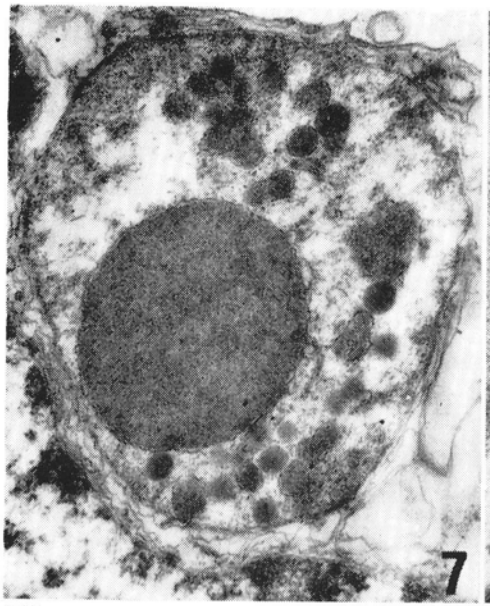
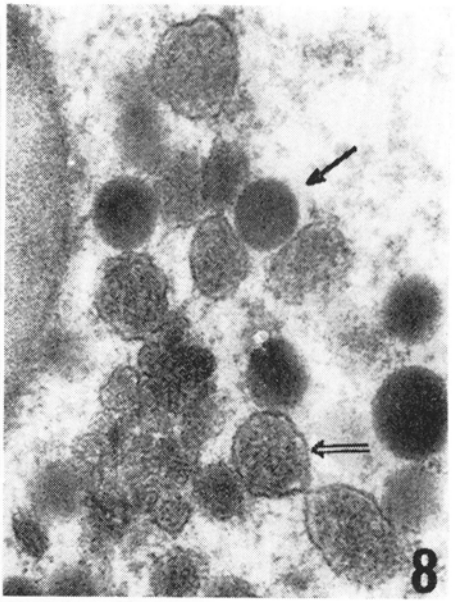
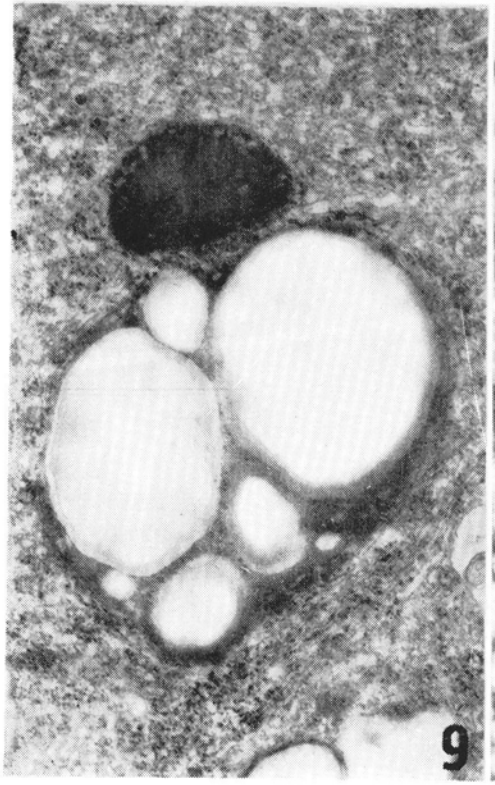
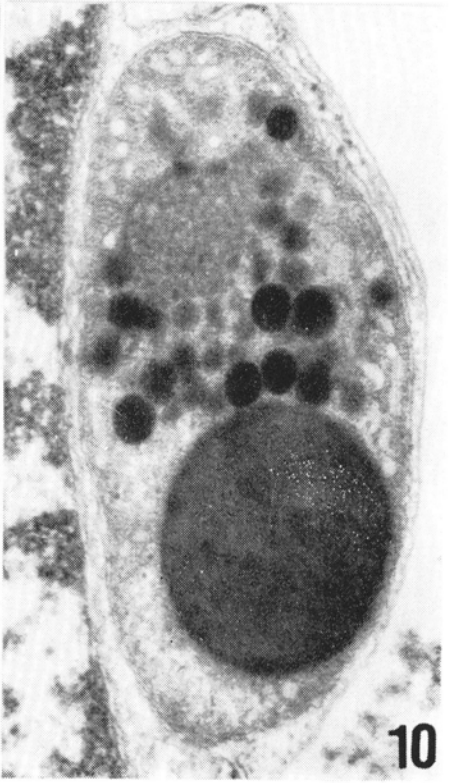








Figs. 7-8. Proteoplasts of the lower epidermis in the apical zone of leaf
7. Proteoplast with large electron dense inclusion enclosed by a single membrane. 17 400 ×
8. Part of the proteoplast with plastoglobuli (single arrow) and sacks with granular contents (double arrow). 40 200 ×
Figs. 9-10. Plastids of the upper epidermis
9. Amyloplast from the middle leaf zone. 25 200 ×
10. Proteoplast from the apical leaf zone; visible: large inclusion, protein sacks, plastoglobuli and prolamellar body. 38 400 ×

prepared for electron microscopy. The length of leaves varied from 10 to 50 cm. The smallest leaves (1 mm-2 cm in length) were also studied. Both leaf pieces of 0.5-1 mm² and the small pieces of the upper and lower epidermis were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer of pH 7.2 for 3 h at 4°C, and postfixed in 1% osmium tetroxide in the same buffer for 1 h at 4°C. This was followed by washing in buffer, dehydration in a graded ethanol series and acetone. The material was embedded in Epon 812.

Some specimens were fixed in 2% osmium tetroxide in the same buffer. Ultrathin sections were made with LKB ultramicrotome III using glass knives, stained with saturated uranyl acetate aqueous solution for 1 h and with lead citrate for about 20 min (Reynolds 1963), and examined under Tesla BS 500 electron microscope.

RESULTS

The initial forms of plastids in both epidermal and guard cells in leaves of *Clivia miniata* are proplastids characterized by a very simple structure (Fig. 1). The first stage of their differentiation is connected with the appearance of starch grains (Fig. 2). Such proplastids occur in epidermal cells of the basal zone in very young leaves as well as in guard cell mother cells and young guard cells. From this stage the process of plastid differentiation in epidermal and guard cells takes place in a different manner.

EPIDERMAL PLASTIDS

In the epidermal cells of the basal colourless zone of older leaves, proplastids increase in size and become elongated, comma-shaped or ameboid in profile. Within the finely granular stroma sacks enclosed by a single membrane occur (Figs. 3, 4, 5), their contents being more and more osmiophilic with age. In cells of the middle celadon zone of the leaf blade plastids assume a regular, oval or circular contours, and

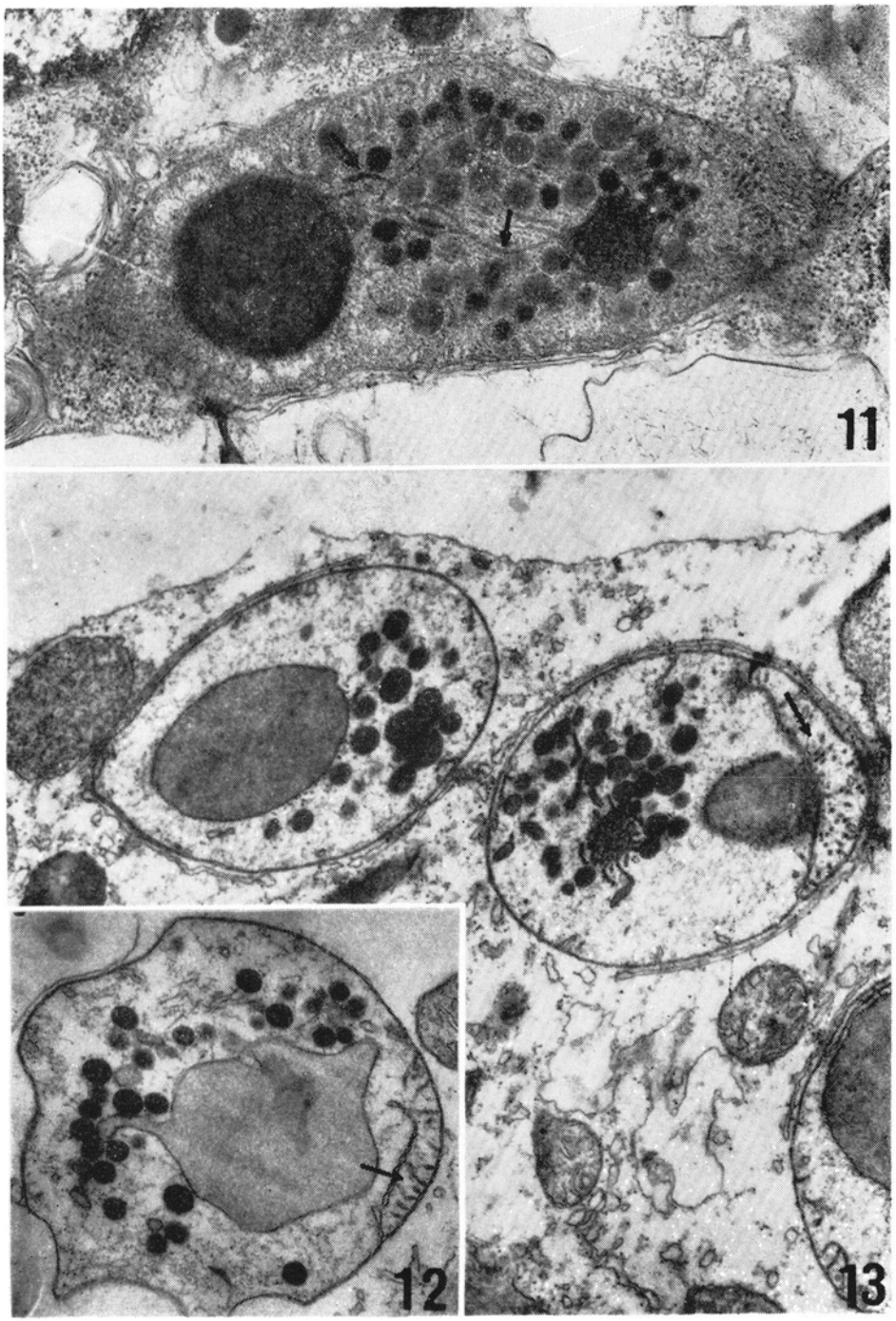
accumulate starch intensely, which leads to the transition of proplastids into amyloplasts (Fig. 6). Sacks filled with dense contents undergo swelling and tend to coalesce. At later developmental stages the amount of starch decreases and a large inclusion surrounded by a single membrane appears in the stroma. The proteinaceous nature of these inclusions has been ascertained by means of the positive mercuric bromophenol blue test (Mazia et al. 1953). Apart from the large inclusion small sacks and plastoglobuli are still present within the stroma (Fig. 7). In mature epidermal cells of the apical leaf region a definite conversion of amyloplasts into proteoplasts takes place, the latter being more complicated in structure.

The same developmental sequences, i.e. from proplastids via amyloplasts into proteoplasts, can be observed in cells of the upper epidermis (compare Figs. 9 and 10). After glutaraldehyde treatment and OsO_4 postfixation the stroma of proteoplasts is dense and finely granular. The proteinaceous inclusion surrounded by a single and faintly visible membrane is homogenous and electron opaque (Figs. 7, 10, 11). Within the group of small osmiophilic inclusions two populations can be distinguished. The first is composed of plastoglobuli (Fig. 8; single arrow), which are typical for plastids; the other consists of sacks enclosed by single membrane and filled with finely granular contents which aggregate into large inclusions (Fig. 8; double arrow). The membraneous and microtubule-like structures are also seen in the stroma at longitudinal and cross sections within some proteoplasts (Fig. 11; arrows). Moreover, aggregates of both vesicles and tubules composing a primitive prolamellar body appear at this stage of plastid development (Figs. 10, 11).

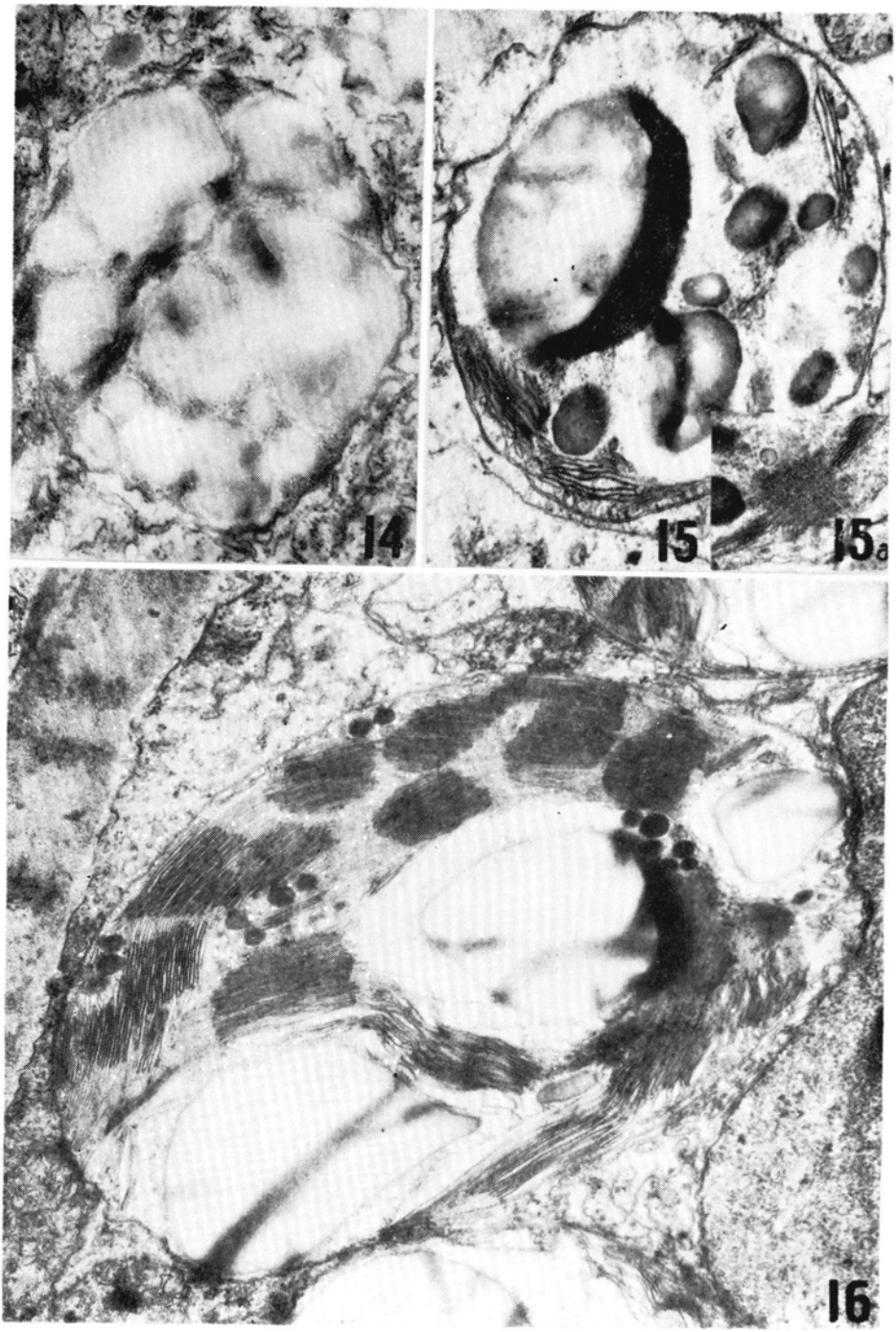
The appearance of proteoplasts in the OsO_4-phosphate buffer-fixed material is unlike that described above, although they contain all the structures reported formerly. The stroma is electron transparent and finely granular; the protein inclusion is more opaque and surrounded by a conspicious single membrane. The proteoplasts contain vesicular prolamellar bodies and microtubule-like structures, the latter being localized at poles. These parts are separated from the remaining fragments of plastids by coalescing vesicles (Figs. 12, 13; arrows), and they resemble images described by Marinos (1967) in plastids of potato tuber buds which were interpreted as "proliferations in the form of pseudopods".

GUARD CELL PLASTIDS

An alternative course of plastid differentiation proceeds within the guard cells of *Clivia miniata*. In the colourless basal zone of older leaves proplastids undergo transformation into amyloplasts with large amounts of starch (Fig. 14). At the middle celadon leaf zone amyloplasts dif-



Figs. 11-13. Proteoplasts from the lower epidermis in the apical zone of leaf 11. Membraneous structures within the dense stroma of proteoplast (single arrow), prolamellar body and microtubule-like structures (double arrow). 25 500 × 12-13. Proteoplasts after OsO_4 fixation; microtubule resembling structures localized at poles (arrow). Membrane surrounding the inclusion is clearly visible. 12 — 16 800 ×; 13 — 23 200 ×



Figs. 14-16. Successive sequences of plastid differentiation in guard cells of leaves in *Clivia miniata*

14. Amyloplast from the colourless zone. 18 500 ×
15. Chloroamyloplast from the celadon zone. 28 200 ×
15a. Prolamellar body in chloroamyloplast. 11 200 ×
16. Chloroplast from the green zone. 22 000 ×

ferentiate into chloroamyloplasts with small numbers of thylakoids and reduced amounts of starch (Fig. 15). Sometimes, the prolamellar body can still be visible (Fig. 15a). In the mature guard cells of the green apical zone there are chloroplasts with well-developed grana and intergrana thylakoids; the starch grains are also present (Fig. 16).

DISCUSSION

The structural and functional heterogeneity observed during ontogenesis of plastids in epidermal and guard cells of leaves in *Clivia miniata* was found to depend on both the developmental stage and the exact function of cells. The youngest cells contain proplastids, older ones — amyloplasts, mature cells — proteoplasts. Similar sequences of plastid development have been described by Badenhuizen (1976) in leaf epidermis of *Helleborus corsicus*. Proteoplasts found in leaves of *Clivia miniata* are characterized by the presence of an electron opaque protein inclusion surrounded by a single membrane. Membrane bound inclusions have been reported in the epidermal plastids of *Beta vulgaris* (Hoefert and Esau 1975), in leaves of *Taraxacum* (Martin and Larbalestier 1977), in the epidermal plastids of developing and mature leaves and cotyledons of sesame (Platt-Aloia and Thomson 1979), and in epidermal plastids of primary leaves in *Phaseolus vulgaris* (Whatley 1979). The protein inclusion of proteoplast in leaves of *Clivia miniata* originate from coalescence of proteinaceous sacks which appear already at initial stages of plastid development. Quite similar process of inclusion formation has been described by Hurkman and Kennedy (1976) in proteoplasts of the P layer cells in primary leaves of seedlings of *Phaseolus aureus*. A different system was reported by Marinos (1967) within plastids in potato tuber buds. The protein body surrounded not by a single, but by the double membrane was shown to originate by invagination of the plastid envelope.

The above inclusions have also been described in plastids from other tissues in a number of plants, and a variety of functions were proposed. In most cases they were found to take place in the process of thylakoid formation (Mikulska 1964, Israel and Steward 1967, Stetler and Laetsch 1969, Boasson et al. 1972; Marty 1973, Ames and Pivorun 1974, Damsz and Mikulska 1976, Platt-Aloia and Thomson 1979). According to Marinos (1967) inclusions in plastids of the potato tuber buds contain nucleic acids and proteins, and tend to disappear when sprouting commences. Similarly, Newcomb (1967) supposes that inclusions in plastids of root tip cells of *Phaseolus vulgaris* serve as a protein storage body. The

proteinaceous inclusions in epidermal plastids of *Clivia miniata* leaves are neither structural materials for the arrangement of thylakoids, nor (as evidenced from electron micrographs) are the reserve substances. Instead, they possibly may result from irreversible changes which accompany the process of plastid senescence. At transitory stages epidermal plastids of *Clivia miniata* leaves are capable of accumulating starch grains, protein bodies and plastoglobuli simultaneously. Marinos (1967) described plastids which concurrently accumulate starch grains, phytoferritin, lipid droplets and intraplastid body in potato tuber buds, and termed them as potentially multifunctional organelles. Platt-Aloia and Thomson (1979) have also noticed the multifunctional capacity of epidermal plastids of sesame leaves cotyledons. Similarly, Hoefert and Esau (1975) suppose that epidermal plastids in leaves of *Beta vulgaris* may store two different proteins under some conditions. According to Miyake and Maeda (1976) the plastids should be regarded as a polymorphous and multifunctional components of cells in higher plants.

The prolamellar body which in plastids of *Clivia* appears at the final stage of the development is utilized during formation of the membranous structures to a low degree, only. This could be observed in a few cases at the cross sections of plastids (Fig. 11). The epidermal plastids of *Clivia* leaves are cell organelles characterized by a specialized structure and function, as compared with those occurring in leaf epidermis of *Zea mays* (Williams 1974) or plastids in *Oryza sativa* (Miyake and Maeda 1976), which judging from the electron micrographs have been inhibited in the development at the stage of proplastid.

During ontogenesis of plastids in guard cells of leaves in *Clivia miniata,* proplastids, amyloplasts, chloroamyloplasts and chloroplasts with well developed grana and stroma thylakoids can be distinguished, which is a feature not common for the guard cells of other plant species. At all developmental stages *Clivia* plastids accumulate large amounts of starch. This is characteristic also for plastids in guard cells of other plants. On the other hand, however, the system of inner membranes is developed differently and was shown to be rather reduced. Thus, it seems reasonable to assume that the degree of thylakoid development is a species-specific feature. Plastids of guard cells in 16 species of grasses have few scattered lamellae (Brown and Johnson 1962). Similarly, chloroplasts of guard cells in *Opuntia ficus-indica* (Thomson and de Journet 1970) and in leaves of *Opuntia basiliaris* (Freeman 1973) are characterized by a very limited grana-fret membrane system. Also Srivastava and Singh (1972) and Williams (1974) have observed plastids with large amounts of starch and poor membrane system in guard cells of leaves in *Zea mays.* Miyake and Maeda (1976) described guard cell plastids in *Oryza sativa* as amylo-

plast-like in configuration. On the other hand, Singh and Srivastava (1973) have found plastids with typical grana and stroma thylakoids within guard cells of *Pisum sativum*.

The sequences of ultrastructural and functional changes described in the present paper were found to accompany both cellular development and differentiation; they stop after cells achieve full maturity. Similar opinion was presented by Whatley (1979).

It seem extremely interesting that in adjacent epidermal and guard cells which develop under the same conditions, the differentiation of plastids derived from morphologically equal proplastids proceeds differently (Fig. 17). The alterations concern not only the structure but also the function of plastids. Epidermal plastids accumulate starch, protein and plastoglobuli concurrently, and undergo conversion from amyloplasts into proteoplasts. Guard cell plastids store starch, solely, but also form thylakoids of grana and stroma. This capacity is not present in epidermal plastids, though they also develop at light.

A conclusion can be drawn from the results obtained that both the developmental sequence and function of plastids are cell-type-dependent and determined by the stage of differentiation as well as by the exact physiological state of the cell.

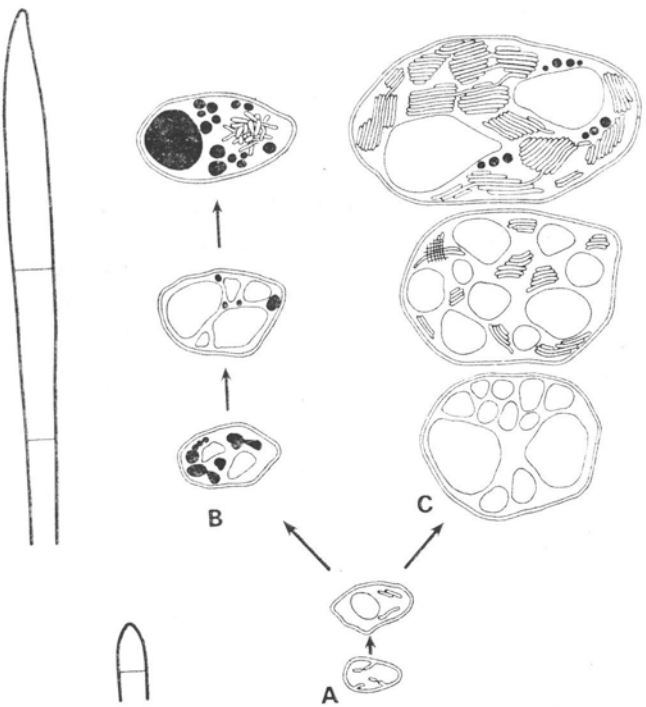


Fig. 17. Developmental sequences of epidermal plastids (B) and guard cell plastids (C) derived from common precursors (A)

Acknowledgments

This work was supported by Polish Academy of Sciences, II Department, Botanical Committee.

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Polimorfizm strukturalny i funkcjonalny plastydów liści Clivia miniata Rgl.

I. Ontogeneza plastydów epidermalnych i plastydów komórek zamykających

Streszczenie

Różne strukturalnie i funkcjonalnie plastydy komórek skórki i komórek zamykających liści *Clivia miniata* Rgl. różnicują się z morfologicznie jednakowych proplastydów. Różnicowanie plastydów przebiega równolegle z różnicowaniem komórek i zostaje zakończone w komórkach dojrzałych. Sugeruje się, że sekwencja rozwojowa plastydów jest utrwalona w komórkach i znajduje się pod kontrolą mechanizmów wewnętrznych.