

Plant Embryology

ULTRASTRUCTURAL ASPECTS OF FORMATION OF SOME STORAGE SUBSTANCES IN THE EMBRYO OF ANGIOSPERMS

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The plastid apparatus of embryos of angiosperms is represented by chloroplasts and leucoplasts. The presence of chloroplasts in different organs of embryo is characteristic only of some plants, the so-called chloroembryophytes. On the basis of comparative morphological analysis of ultrastructure of chloroplasts of such chloroembryophytes as *Nelumbo nucifera*, *Alcea rosea* and others it was concluded that these organelles are peculiar as compared to a typical mesophyll chloroplast of most flowering plants. Chloroplasts of embryos combine the properties of chloroplasts, and some peculiarly organized leucoplasts. Like leucoplasts, chloroplasts of embryos are specialized not in the synthesis of primary starch but in the synthesis of various storage substances, mainly secondary starch and storage protein. In other words, the presence of chloroplasts in embryo does not necessarily mean its autonomy. The division of angiosperms into chloro- and leucoembryophytes is, therefore, connected not with autonomy of a green embryo as opposed to a colourless one, but with the presence or absence at angiosperms of the specific enzymatic systems which provide for chlorophyll synthesis in embryos even in situ under the seed coat and pericarp (with deficiency or absence of light).

Electron microscopic study showed the polymorphism of plastids of various embryonal organs, both at the specific and tissue levels. Ultrastructural transformations of plastids in the course of embryogenesis, as well as polymorphism of plastids within the embryo sac and ovular tissues testify to separation of various stages in the synthesis of storage substances in the seed.

INTRODUCTION

One of the objectives of plant embryology is to determine the formation of storage substances in embryo which is of theoretical as well as practical importance. The main storage substances in the majority of angiosperm embryos are known to be oils, proteins and starch. Resinous substances, oil globules, and aleurone grains have been observed in the embryos of *Meliaceae* and *Ochnaceae* (Netolitzky, 1926). In some families, together with ordinary storage substances embryos also accumulate tannins, alkaloids, glucosides, essential oils, various pigments, and other substances.

All this confirms that extremely complex and different processes of metabolism underlie the growth, formation of embryo, and accumulation of storage substances. The study of subcellular organization of these processes may contribute largely to the knowledge of peculiarities in the formation of storage substances in various plants. Of special interest is the study of ultrastructure of plastid apparatus of embryo and adjoining tissues. The occurrence of plastids is

one of the specific characters of plant cell organization, as compared with the animal cell. Many synthetic processes are referred to these organelles.

MATERIALS AND METHODS

Materials studied with the electron microscope were prepared by dissecting out individual ovules, or embryos, and immediately placing them into fixative. Data on the investigation of embryos, of *Nelumbo nucifera* Gaertn. and *Tilia platyphyllos* Scop., and details on their preparation for electron microscopy have been reported earlier (Zhukova, 1972, 1974, 1975; Yakovlev & Zhukova, 1973). Ovules of *Alcea rosea* L. were collected from plants growing in the Botanical Garden of Komarov Botanical Institute (Leningrad).

The material was fixed by two methods: 3% glutaraldehyde in a phosphate buffer (pH 7.2) for about 6 hr at 4°C, followed by 2% aqueous osmium tetroxide (same buffer and pH) at 4°C overnight; 3% glutaraldehyde in a phosphate buffer (pH 7.2) for about 6 hr at 4°C, followed by 5% unbuffered potassium permanganate for 0.5 hr at 4°C. The ovules and embryos were dehydrated in a graded ethanol and acetone series, and embedded in Epon. Ultrathin sections were cut with a glass knife on an LKB ultramicrotome, stained with lead citrate (1% uranyl acetate was also used at the ethanol dehydration), and examined with a JEM-7A electron microscope.

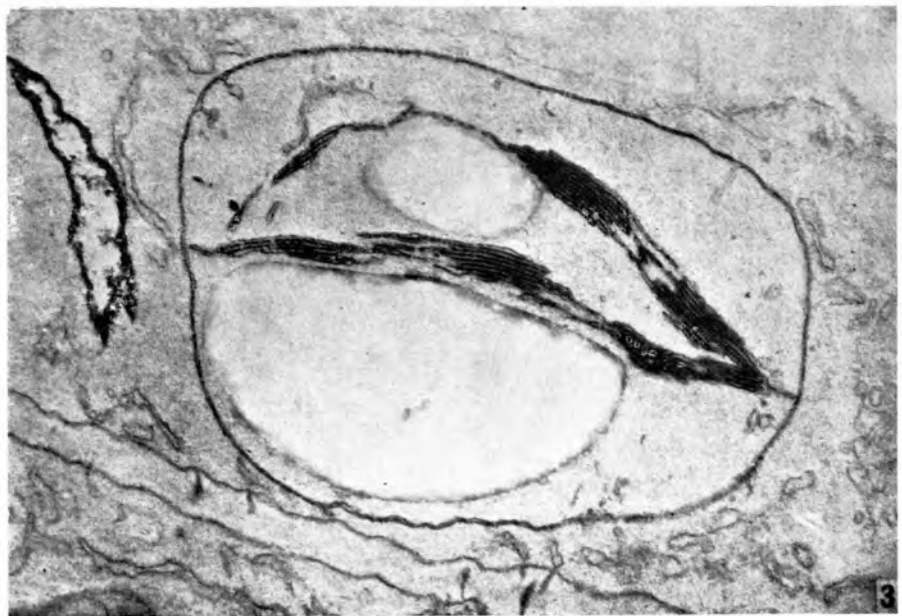
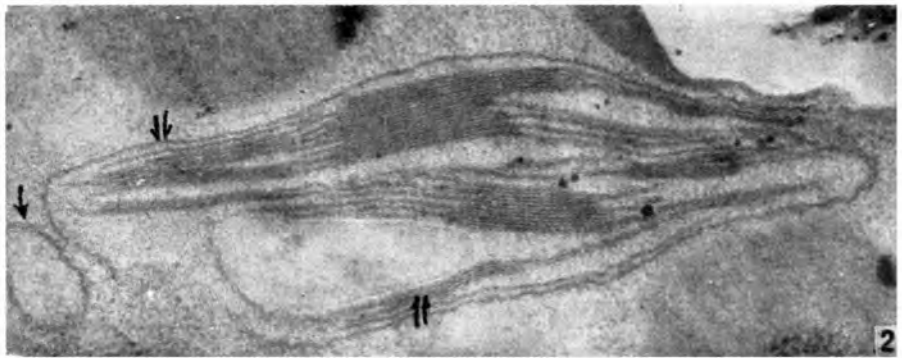
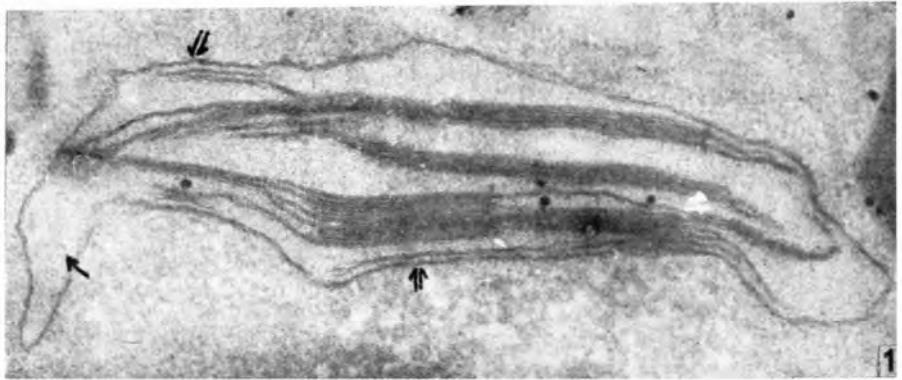
RESULTS AND DISCUSSION

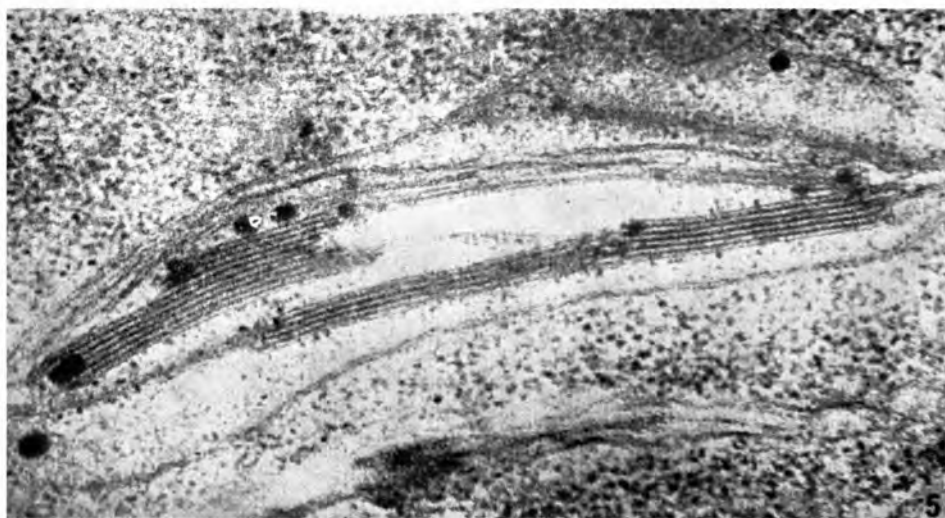
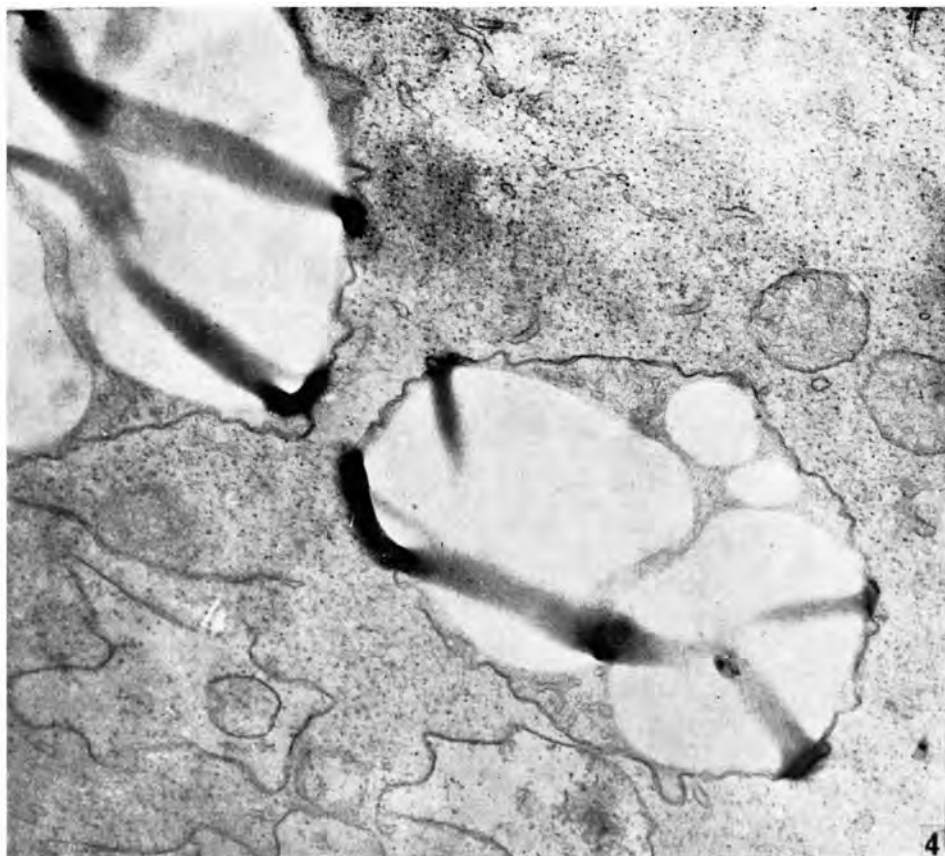
The plastid apparatus of angiosperm embryos is represented by leucoplasts and chloroplasts. The occurrence of chloroplasts in the embryo is known only in certain plants generally called chloroembryophytes (Yakovlev, 1969; Yakovlev & Zhukova, 1973, 1975).

The comparative morphological analysis of chloroplast ultrastructure in embryos (in about 12 species) enables us to make the following conclusion. Chloroplasts of embryos have many similar features with the ultrastructure of mesophyll chloroplasts of most flowering plants. They possess a double membrane envelope, and sufficiently developed internal membrane system. In their stroma starch grains, osmiophylic plastoglobules, and ribosomes have also been observed.

However, the chloroplasts in embryos have a number of specific features. They concern all structural elements of chloroplast, involving its internal membrane system. One can observe here a series of specific features which are common for

FIGS 1-3, Chloroplast profile in a fresh embryo mesophyll cell. Note the evagination of chloroplast peristrome (arrow). The subdivision of internal membrane system into central and peripheral parts (double arrow) can be easily followed (see also Fig. 2) $\times 40000$; 2, Section through a chloroplast of a mesophyll cell in a fresh embryo of *Alcea rosea*. In this section the evagination of chloroplast peristrome (arrow) can be taken for independent structure $\times 38000$; 3, Electron micrograph of chloroplast of *Alcea rosea* embryo; the material was post-fixed in a solution of 5% KMnO_4 after fixation in a solution of 3% glutaraldehyde (compare Fig. 3 with Figs. 1 and 2—post-fixation in a solution of 2% osmium tetroxide) $\times 29800$. \rightarrow





embryo chloroplasts of all species studied and which distinguish them, in a certain degree, from the typical mesophyll chloroplasts.

First of all, these organelles have no regular lens-like shape which is characteristic of a typical mesophyll chloroplast (Figs. 1, 2; see also Zhukova, 1972, 1975; Yakovlev & Zhukova, 1973). Due to the folding of the envelope and its numerous invaginations into the body of plastids the shape of chloroplasts of the embryo has amoeboid appearance. This phenomenon results in considerable increase of the surface of external membranes of chloroplasts as well as in the expansion of the area of their contacts with surrounding cytoplasm.

There is additional extension of the internal membrane surface of their envelope due to invaginations of a different kind.

Even in the inner membrane system of chloroplasts of the embryo there are some specific features. In the majority of species studied it does not principally differ from a typical one, and consists of grana and intergrana lamellae (Fig. 1-3). However, in some of species studied very peculiar lamellar system was observed. In the embryo of *Nelumbo nucifera* (Yakovlev & Zhukova, 1973), *Acer pseudoplatanus* (Pinfield *et al.*, 1973) and *Citrus* species (Orsenigo, 1964), in contrast to the typical mesophyll chloroplasts of flowering plants including the adult leaf of the above species, the internal membrane system of chloroplasts consists of large stacks of discs as in some green algae. According to our data on the embryo of *Nelumbo nucifera* there may be one or several such stacks in each chloroplast. The number of discs in each stack may reach 30 or more. Like grana of leaf chloroplasts they are characterized by close contact between membranes.

In embryo the chloroplasts of a number of species are associated with the elements of endoplasmic reticulum. The latter often surround chloroplasts forming a kind of a sheath around them. There can be several layers of ER membranes in such a sheath (Yakovlev & Zhukova, 1973). The profiles of these sheaths sometimes completely surround plastid sections.

In *Tilia platyphyllos* embryo a "tubular complex" was observed as a component of the inner membrane system of the chloroplasts (Zhukova, 1975). A similar complex is characteristic of multifunctional or protein-synthesizing leucoplasts in some plant tissues. In a number of species *Alcea* (*Althaea*) *rosea*, *Nelumbo nucifera* and *Tilia platyphyllos*, there are distinct subdivisions of the inner membrane system into central and peripheral parts (Figs. 1,2).

Of particular interest are such modifications as the occurrence of different tubules with definite arrangement in chloroplast peristrome of the embryo, (Zhukova, 1975), also of vesicles with various diameter and with the contents

FIGS. 4-5, Amyloplasts of an *Alcea rosea* globular proembryo containing starch grains, but devoid of internal membrane systems. Note the presence of tubules in their peristrome $\times 19000$; 5, Chloroplast profile in a heart-shaped embryo of *Alcea rosea*. The embryo plastids at this stage of embryogenesis are observed as ovoid or elongated organelles, which do not contain starch grains, or extremely small ones $\times 55000$.

of different electron density, as well as of phytoferritine and of specific peristrome evaginations, containing peripheral reticulum (Figs. 1, 2).

A question arises as to what function do the chloroplasts in the embryo perform. Since the main function of chloroplasts is to provide photosynthetic reactions, several workers are inclined to consider embryo-greening as an indication of its photosynthetic activity. However, the electron microscopic study carried out by the author does not support evidence in favour of such an assumption. This is because the light conditions in the embryonal tissues are not always favourable for photosynthesis. Such features as difference in the pigment composition and the ultrastructure of chloroplasts of the embryo especially at the occurrence of a number of specific features unusual for a typical photosynthesizing mesophyll chloroplast and the lack of correct orientation of chloroplasts characteristic of cells of photosynthesizing tissue, question the photosynthetic activity in the tissues of developing embryo.

Specialization of another kind is observed in the chloroplasts of the embryo. They have many features of leucoplasts, the very polymorphic group of plastids which specialize in various syntheses: synthesis of secondary starch, synthesis of storage proteins, lipids, phenolic compounds, phytoferritine, etc.

Let us turn to examples. In the chloroplasts of the plumule of embryo of *Nelumbo nucifera* large starch grains (Yakovlev & Zhukova, 1973) were observed at different stages of differentiation. They became especially large approximately in 27 days after opening of the flower. It should be emphasized that this object is of special interest due to the fact that the woody pericarp of monospermous fruitlet of lotus together with massive cotyledons surrounding the plumule is, as it was shown by special studies (Toyada, 1960), not transparent already at the first stages of seed formation and absolutely light-proof at later stages. Thus, in this case the formation of chloroplasts as well as their preservation takes place in the conditions of almost complete absence of light. The light deficiency questions the possibility of photosynthesis in chloroplasts of plumule of the embryo of Lotus. Besides, starch grains were observed in these chloroplasts already at the first stages of their differentiation, before transformation of prolamellar bodies in the internal membrane system. These data allow us to consider the starch of chloroplasts of lotus embryo as the product not of primary but of secondary synthesis.

The following fact is of interest in connection with the problem of functional specialization of chloroplasts of the embryo. In chloroplasts of the embryo of *Citrus nobilis* × *C. aurantium amara pumila* the starch does not accumulate at all (Orsenigo, 1964). In other words, these chloroplasts do not perform not only primary but even secondary starch synthesis. Therefore the function of chloroplasts of the embryo of *Citrus* remains obscure.

Throughout the embryogenesis of chloroembryophyte *Alcea rosea* the following transformation of plastids was observed. In the globular, still colourless proembryo, the plastids are represented by leucoplasts or, to be more exact, by amyloplasts since they contain starch grains (Fig. 4). At the heart-shaped stage of

development only chloroplasts are found (Fig. 5). These are small ellipsoidal plastids containing internal membrane system, and insignificant amount of starch. In the course of further differentiation of embryo the chloroplasts obtain the features of structural organization described above (Figs. 1-3). Accumulation of starch was observed in them. On the basis of ultrastructural peculiarities of these chloroplasts, we think it doubtful to consider their starch to be primary in contrast to the starch of leucoplasts at the globular stage of embryonal development.

The data obtained allow us to suggest the participation of the chloroplasts of embryo not only in the carbohydrate metabolism, i. e. in the synthesis of secondary starch and its hydrolysis in the course of embryo formation, but also in the synthesis of storage protein. The latter is referred to the chloroplasts of the embryo of *Tilia platyphyllos* (Zhukova, 1974, 1975). The occurrence of protein-like bodies in the invaginations of these chloroplasts as well as peculiar structure of their internal membrane system consisting of lamellar and tubular elements underlies such an assumption. The tubular elements resemble the tubular complex characteristic of protein-synthesizing plastids of root apices of bean (Newcomb, 1967). Particularly noteworthy is the fact that similar tubular complex was observed in the plastids of cells of digestive glands, secreting proteolytic enzymes (Schwab *et al.*, 1969).

The above data show that chloroplasts of the embryo combine the features of chloroplasts, and some specifically organized leucoplasts. Like leucoplasts chloroplasts of the embryo are involved in the synthesis of some metabolites, primarily stored substances rather than photosynthesis. Some features of ultrastructure of chloroplasts in *Tilia platyphyllos* embryo may be considered as an indication of their involvement in the synthesis of a stored protein. In other words, the occurrence of chloroplasts in embryo does not mean its autonomy. It is difficult to suggest sufficient photosynthetic activity of chloroplasts of the embryo *in situ* under cover of seed coat and pericarp (due to deficiency or absence of light). The occurrence of chloro- and leucoembryophytes does not seem to imply the autonomy of green embryos in contrast to achlorophyllous ones. This phenomenon seems to be linked with the presence or lack of specific enzymatic systems providing chlorophyll synthesis in embryo even without photochemical reaction.

Leucoembryophytes are not capable of differentiation of chloroplasts in embryonal tissues though one might suggest with equal measure the probability of light penetration to colourless embryos without causing in them chlorophyll formation. The facts of the so-called metamorphosis of leucoplasts into chloroplasts at the illumination of the former are known. However, the earlier data show that in all plant tissues leucoplasts are not capable of such transformation. Of special interest is the following example. In the endosperm of Cruciferae (Yoffe 1952, 1957) chloroplasts are differentiated under natural conditions, i. e. under cover of surrounding tissues. The endosperm of *Ricinus communis*

(Orsenigo, 1964) does not turn green even at the optimal light conditions *in vitro*.

In the same embryo sac of *Pisum sativum*, under the same light conditions, chloroplasts are differentiated in the cells of embryo proper and leucoplasts in the suspensor cells with a specific membrane system like tubular complex (Marinos, 1970).

In conclusion it should be emphasized that chloroplasts of the embryo, polymorphic-like leucoplasts, may take part in very different metabolic reactions in developing embryos. Ultrastructural transformations of plastids in the course of embryogenesis, as well as polymorphism of plastids within embryo sac and ovule tissues, testify to separation of various stages in the synthesis of storage substances in the seed.

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