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Fine structure of plastids during androgenesis in *Hordeum vulgare* L.

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Abstract

The fine structure of plastids was studied in the course of androgenesis in in the pollen of *Hordeum vulgare* L. It was found that these organelles occur in all stages of androgenesis. Their structure was simple and was frequently manifested on the cross section only by the presence of the envelope and matrix of different degree of density. Single thylakoids, nucleoid-like regions and starch grains were, however, also noted. The structure of plastids in embryoids formed from microspores of barley was compared with embryos developed from fertilized egg cell, and we did not found any fundamental differences between them. However, only plastid ribosomes were difficult to identify on ultrathin sections in embryoids and in the embryos.

INTRODUCTION

One of the aims of contemporary experimental embryology is the obtention of haploid plants. This fact is of great importance for further genetic-breeding practices tending to the production of homozygotic plants and further, by way of crossing, economically useful plants. This purpose can be reached by several methods. In the present studies the technique of culture of isolated anthers at the stage of uninucleate microspore was used. By appropriate conditions of *in vitro* culture the development of the microspores was stimulated towards androgenesis. In this way haploid plants, though mainly albinotic, were obtained. Green haploid plants were an exception (Zenkteler, 1976).

The trait of greenness of the plants in normal embryo developments is conditioned by the presence of plastids transmitted to the zygote mainly or solely by the egg cell cytoplasm. In the androgenic development of

embryoids the microspore cytoplasm is the only source of plastids. Hence the frequent albinotism occurring in haploid plants obtained from microspores may be due to the incomplete development of plastids. Knowledge of the causes of albinotism and its prevention in haploid plants would be of great practical importance. The aim of the present study was the identification of barley microspore plastids, in order to gain a knowledge of their fine structure in the particular stages of androgenesis, and comparison of the ultrastructure of plastids from embryoids with that of embryos obtained by sexual reproduction.

MATERIAL AND METHODS

Hordeum vulgare var. Alsa anthers were taken from plants cultivated in the greenhouse. Part of the anthers containing pollen in the stage of a uninucleate microspore were fixed immediately after being cut off as outset material. The remaining part was cultured on Murashige and Skoog medium (1962) with 8-12 per cent sucrose added for inducing the process of androgenesis.

After 3 days of culture the anthers contained pollen in the stage of a uni- or bi-cellular microspore. After a longer period of culture the pollen grains developed to embryoids of several or a dozen or so cells. Fragments of anthers from their central part were fixed in 6 per cent glutaraldehyde buffer with 0.1 M cacodylate, pH 6.8 for 18 h at 4° C. The anthers were then washed for 1 h with four changes of 0.1 M cacodylate buffer and postfixed with 2 per cent OsO_4 in cacodylate buffer of the same molarity for 2 h. After postifixing and washing with buffer the material was stained with 2 per cent uranyl acetate aqueous solution for 1 h, then dehydrated in an ethanol gradient, acetone and propylene oxide and embedded in Epon 812.

The anthers were then cut on an LKB ultramicrotome and the ultrathin sections were counterstained with uranyl acetate 2 per cent solution and lead citrate (Reynolds, 1963; Venable, Coggshall, 1965). The sections were examined in a JEOLCO type 7A electron microscope.

Embryos were obtained from fertilize *Hordeum vulgare* × *Hordeum vulgare* cultivated in the greenhouse. All technical manipulations were the same as for the anthers.

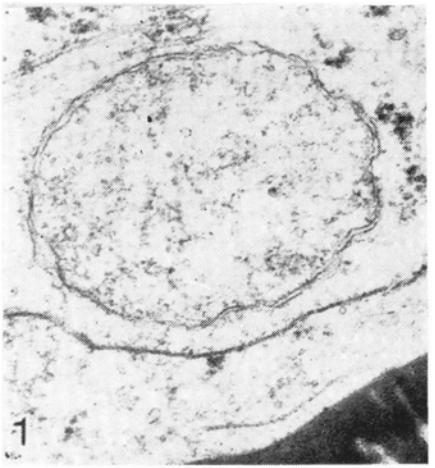
Figs 1 and 2. Microspore plastids (outset material). Fig. 1 — × 44 000, Fig. 2 — × 44 000

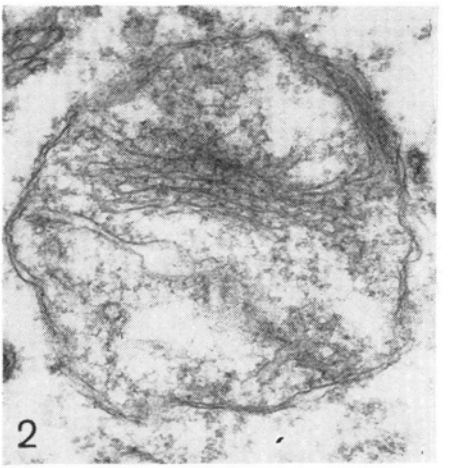
Fig. 3. Plastid of generative cell. × 33 000

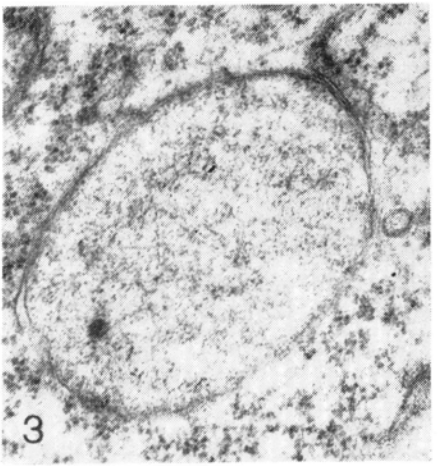
Fig. 4. Plastids of vegetative cell. × 22 500

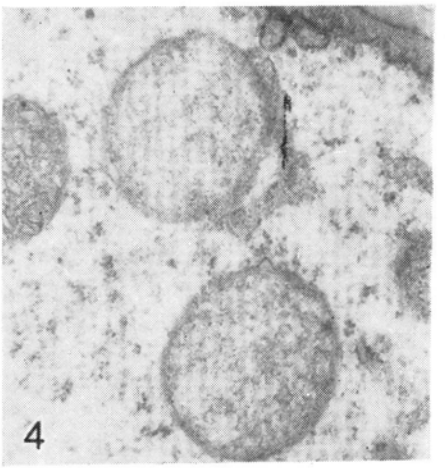
Figs 5 and 6. Plastids with dense matrix and vesicular thylakoids at various cross section planes in vegetative cell. At the point of contact of the vesicles stripes are visible. Fig. 5 — × 42 000, Fig. 6 — × 56 000

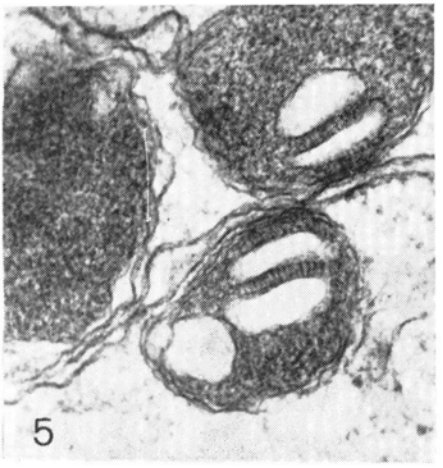
PLATE I

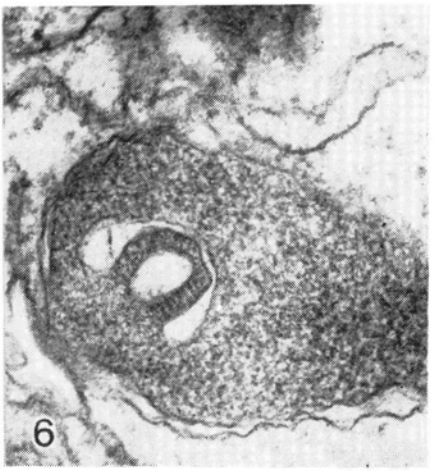


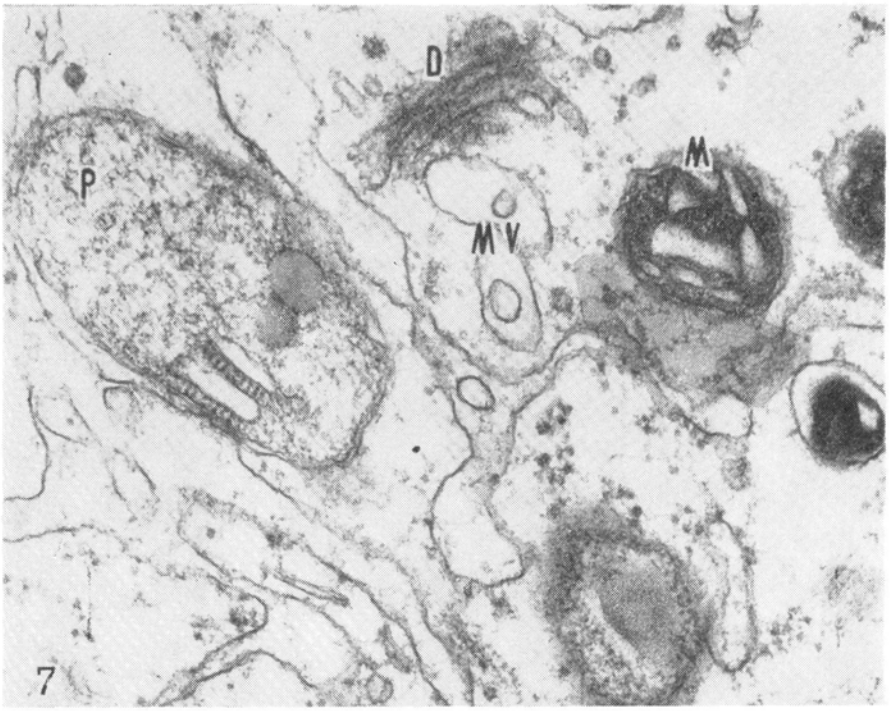








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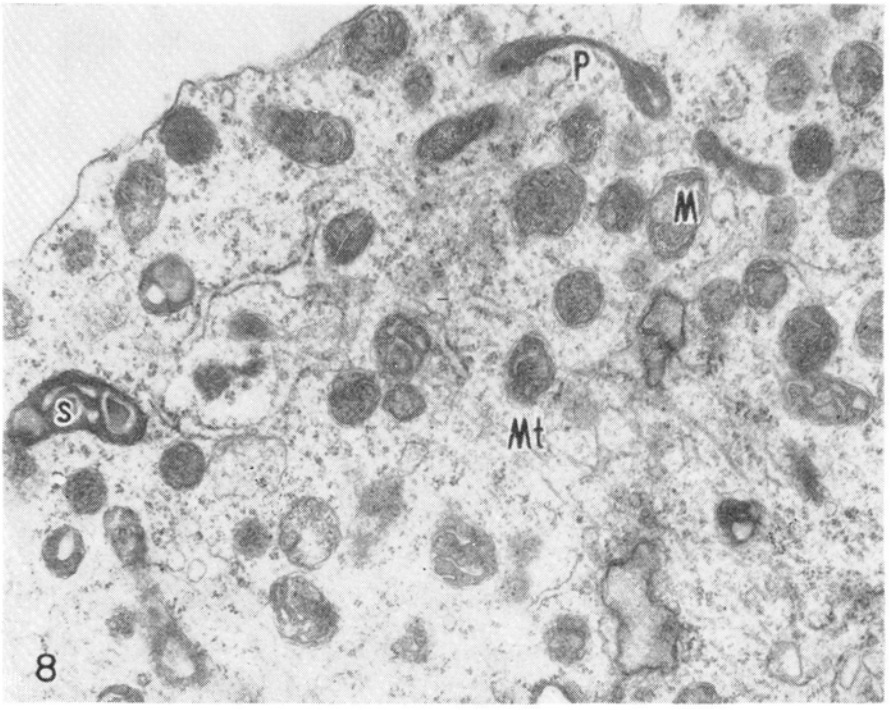
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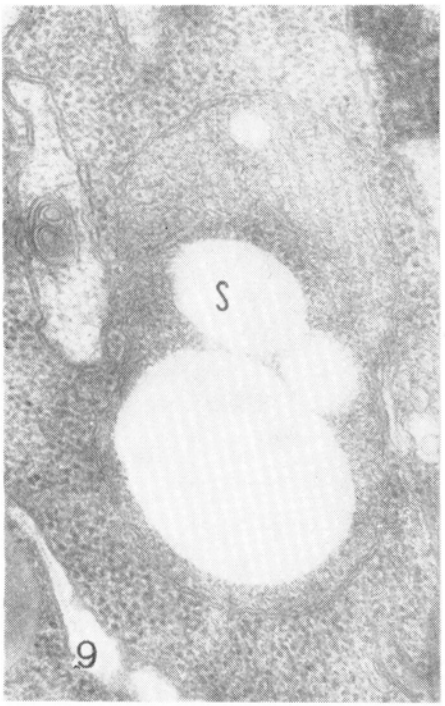
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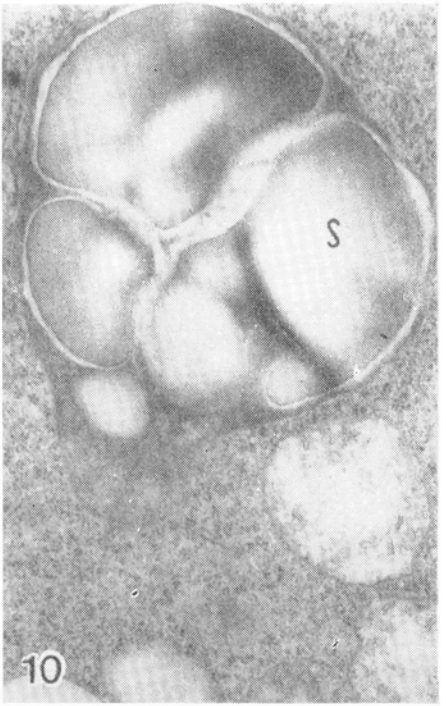
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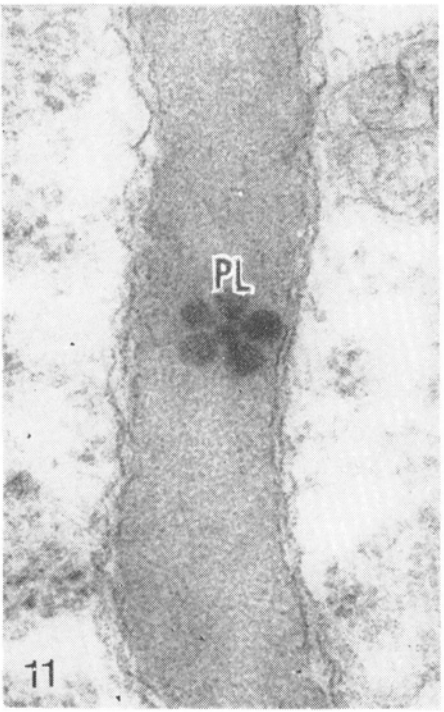
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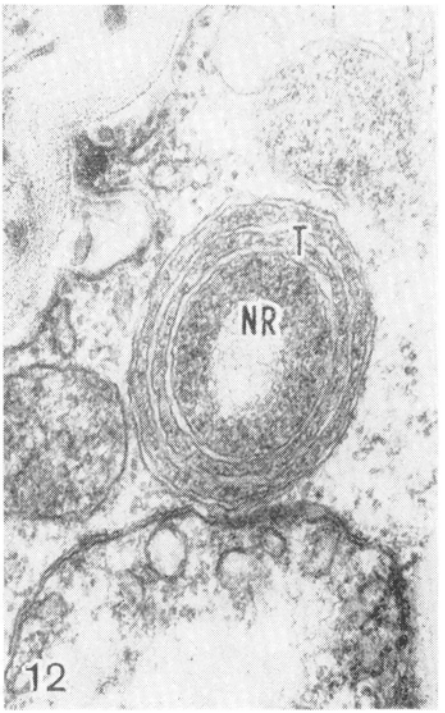
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RESULTS AND DISCUSSION

At the moment when the detached anthers were placed on the medium the microspores contained few plastids with a simple structure (Młodzianowski, Idzikowska, 1978; Fig. 1). Nevertheless, beside plastids which on the cross section showed only an envelope and fine-grained matrix, plastid profiles with thylakoids were also observed (Fig. 2). It was characteristic for microspore plastids that starch was completely absent in them.

After 3 days of culture, when the microspore developed normally, that is if by way of asymmetric division two cells arose: a smaller generative and a larger vegetative one, plastids were present in both cells, their structure resembling that of microspore plastids (Figs 3, 4). These plastids also did not contain starch. Plastids were more numerous in the vegetative cell where some of their forms indicated their division by way of constriction. In some plastids of this stage vesicular thylakoids could be seen, usually two on one cross section. At the point of their contact osmophilic regular stripes were deposited (Figs 5, 6). The origin of these stripes and their significance are unclear. The matrix of plastids with abnormal structure showed various degrees of density (Figs 6, 7).

A number of cross sections through the vegetative cell indicated symptoms of cytoplasm breakdown (Fig. 7). The ground cytoplasm was electron-clear, and vesicular structures, multivesicular ones as well as numerous dictyosomes were visible. On account of the highly heterogeneous pollen population in the anther, it is difficult to guess whether these were stages leading to their irreversible degeneration or whether degeneration of some cell components preceded only the renewal of the organelles which, according to certain authors, is necessary to set in operation mechanisms preventing the continuation of gametophytic pollen development (Dunwell, Sunderland, 1974; Raghavan, 1976). It results, namely, from our preceding paper (Idzikowska et al., 1979) that the beginning of embryogenic development of *Hordeum* pollen may be referred either to the vegetative nucleus division in bicellular pollen or earlier to the first mitosis of the microspore after which asymmetric formation of the generative cell does not occur.

During the first symmetric division, thus the embryogenic division of the microspores, the plastids contained starch (Fig. 8). Starch was

Fig. 7. Fragment of cross section through vegetative cell with symptoms of degeneration. M — mitochondrium, P — plastid, D — dictyosome, MV — multivesicular structures. × 45 500

Fig. 8. Dense cytoplasm with numerous mitochondria (M) and dividing plastids (P) during first symmetric division of microspore. In plastids starch (S) is visible Mt — microtubule. × 20 000

also present, sometimes in very large quantity, in the embryoid plastids (Fig. 9). It was believed that the large starch grains synthesised from sugars, the level of which is often very high in the medium (up to 12%), are the main cause of underdevelopment of the chloroplasts. It appeared, however, that the plastids of barley embroys formed by sexual reproduction also contained much starch (Fig. 10).

The plastids of embryoids in the areas deprived of starch showed on the cross section single, frequently concentric thylakoids (Fig. 12) similar to those in typical proplastids (Młodzianowski, 1974). As regards the number of thylakoids, the only chlorophyll carriers in plants, and their configuration, no differences were noted between embryoids and barley embryos. The not numerous and minute plastoglobules which in proplastids are considered as reservoirs of certain material for building the photosynthetic apparatus (Lichtenthaler, 1968, Gunning, Steer, 1975) were present on the cross sections of embryoids and embryos. The complex of plastoglobules with a regular arrangement was an exception in the embryoid plastids (Fig. 11).

A very important matter — the existence of ribosomes in embryoid plastids remains unsolved. The technique of ultrathin sections does not allow to establish their presence unequivocally. In our preparations distinct contours of plastid ribosomes could not be demonstrated both in embryoid and embryo plastids. We consider this question as open and perhaps dependent on the technique used in fixation and staining of material.

Nucleoid-like areas with DNA filaments were observed in embryoid plastids (Fig. 12).

Since no differences could be found at the ultrastructure level between the plastids of the multicellular androgenic barely embryoids and those of embryos of the same plant obtained by sexual reproduction, it may be supposed that: (a) no differences exist either at the genetic-biochemical level; the causes of albinotism may then be searched for at later stages of chloroplast ontogenesis in extragenetic factors; or (b) the differences are of genetic nature, but their consequences are not yet discernible in the ultrastructure and may appear later.

It is only if the former supposition is true that there may be some hope of finding factors inducing chloroplast development in haploids under modified culture conditions. Independently, however, of the sup-

Fig. 9. Plastid with starch grains (S) in several-celled embryoid. 45 000 ×
Fig. 10. Plastid with starch grains (S) in several-celled embryo obtained from fertilize *Hordeum vulgare* × *Hordeum vulgare*. 17 000 ×
Fig. 11. Plastoglobule complex (PL) in plastid of vegetative cell. 69 500 ×
Fig. 12. Cross section through embryoid plastid. Concentrically arranged thylakoids (T) and the nucleoid-like region (NR) are visible. 50 000 ×

posed conditioning of albinotism, we believe that noticeable differences in the ontogenesis of plastids in normally developing plants and those induced from microspores may be expected at the moment of differentiation of proplastids to chloroplasts.

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Ultrastruktura plastydów w czasie androgenezy u Hordeum vulgare L.

Streszczenie

Prześledzono ultrastrukturę plastydów w czasie androgenezy pyłku *Hordeum vulgare* L. oraz porównano budowę plastydów wielokomórkowych embrioidów powstałych z mikrospor z plastydami zarodka jęczmienia otrzymanymi na drodze

płciowej. Stwierdzono, że plastydy występują we wszystkich stadiach androgenezy. Ich ultrastruktura była prosta i wyrażała się często na przekrojach jedynie obecnością otoczki i matriks o różnym stopniu zagęszczenia. Obserwowano jednak również proste tylakoidy, obszary nukleoidopodobne i ziarna skrobi. Bardzo dużo skrobi było w wielokomórkowych embrioidach. Pod względem wymienionych cech plastydy embrioidów jęczmienia nie różniły się od plastydów zarodków otrzymanych z zapłodnionej komórki jajowej tej samej rośliny. Rybosomy plastydów były trudne do zidentyfikowania na ultracienkich skrawkach zarówno w embrioidach jak w zarodkach.