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Development of rye embryo. I. Preliminary investigations on ultrastructure of 7-day-old proembryo

BARBARA STEFANIAK

Department of General Botany, Institute of Biology, A. Mickiewicz University, Stalingradzka 14, 61-713 Poznań, Poland

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Abstract

Ultrastructural examination of 7-day-old rye proembryos did not reveal distinct differences in the cell structure of its particular parts. It was noted, however, that in the cells of the apical part vacuoles and multivesicular bodies were more numerous than in the basal part. These specific features of the cells of the apical part of the proembryo (together with data of other authors) seem to indicate an early stage of development of the embryonal scutellum.

Key words: embryo, scutellum, Secale cereale

INTRODUCTION

There is in the development of the embryo a critical stage which determines its further progress. This stage has been hypothetically defined as the moment when the embryo acquires a certain independence from the parent organism, and it appears among other things in the ability of independent utilisation of nutrient substances (Batygina and Vasilyeva 1981). It has been established, above all on the basis of morphological observations performed under conditions of *in vitro* culture, that, in the embryogenesis of cereals it is the proembryo at the moment preceding the appearance of the axial organs that constitutes the critical stage (Norstog 1965, Batygina and Vasilyeva 1981). Unfortunately, in spite of the existence of relatively numerous and well documented papers devoted to cereal embryogenesis (among them: Norstog 1972, 1974, Poddubnaya-Arnoldi 1976, Cebrat 1979, Smart and O'Brian 1983), the early stages of development of the axial organs in the proembryo are still little known. Data

are, therefore, lacking which would allow to determine the moment when the proembryo is in the critical stage, and this in turn, is an obstacle in the culture *in vitro* of isolated cereal proembryos (Norstog 1965, Batygina and Vasilyeva 1981).

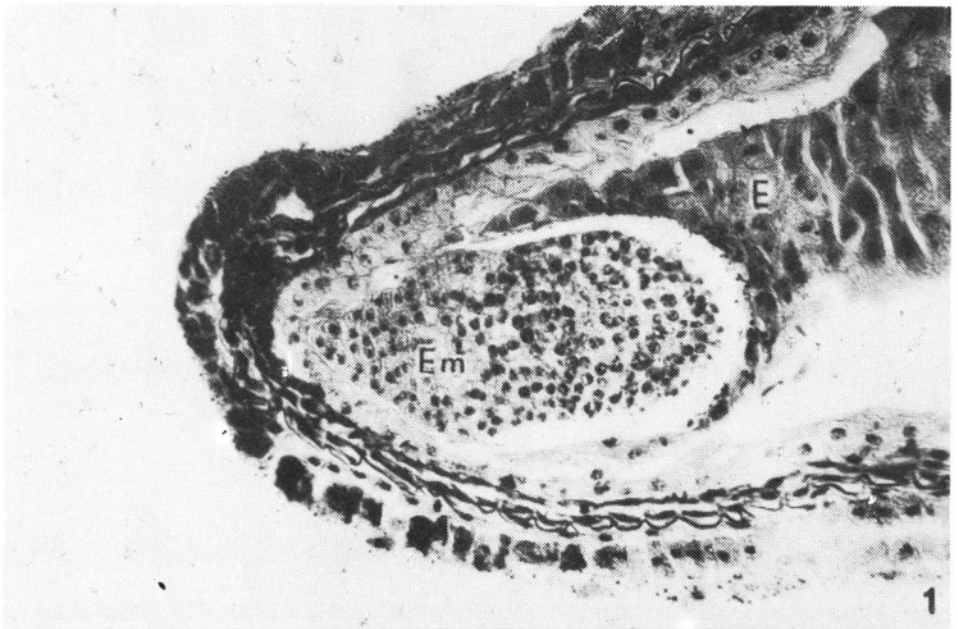
In the present paper the structure of a 7-day rye embryo is analysed with the aim of establishing the first ultrastructural symptoms of cell differentiation towards axial organs. The periphery cells on the proembryo were also inspected in search for a structure(s) which might mediate transport of nutrient substances from the endosperm to the embryo. A 7-day-old proembryo was chosen because it is the initial stage in the presently carried on experiments in the Department of General Botany on culture *in vitro* of isolated immature rye embryos.

MATERIAL AND METHODS

Secale cereale L. cv. Strzekęcińskie was used for the investigations. Ears from which the pistils had been removed were placed in closed parchment paper bags, and when the stigmas had developed (plumes) they were pollinated with pollen of the same species and again isolated. Material was taken 7 days after pollination only from the central part of the ear. For cytological observations fragments of the ovary were fixed first in Karnovsky's fixative (1965) in cacodylate buffer, pH 7.2 and then in 2 per cent OsO_4 in the same buffer. The material was dehydrated in an aqueous ascending acetone solution gradient and embedded in Epon of low viscosity according to Spurr (1969). Semithin (1-3 μm) and ultrathin sections were prepared on a LKB — "Ultratome III". For observation in the light microscope the sections were stained with toluidine blue after Kay (1965). Photographs were taken with the use of a type "Amplival" microscope. The ultrathin sections were first contrasted with uranyl acetate and lead citrate and then inspected and photographed in a transmission electron microscope JEM 7A.

RESULTS

The rye proembryo examined 7 days after pollination is oval in shape tapering towards the micropyle pole (Fig. 1). It lies along the micropylar-chalazal axis of the embryo, its length in this plane amounting to about 150 μm. The proembryo consists of minute thinwalled and frequently polygonal cells. All the cells tightly adhere to one another and as a rule are joined by a large number of plasmodesmata. Most of the volume of the proembryonal cells is occupied by the centrally situated large nucleus mostly circular on the cross sections (Fig. 2). It generally contains one nucleolus in the centre of which frequently minute "nucleo-



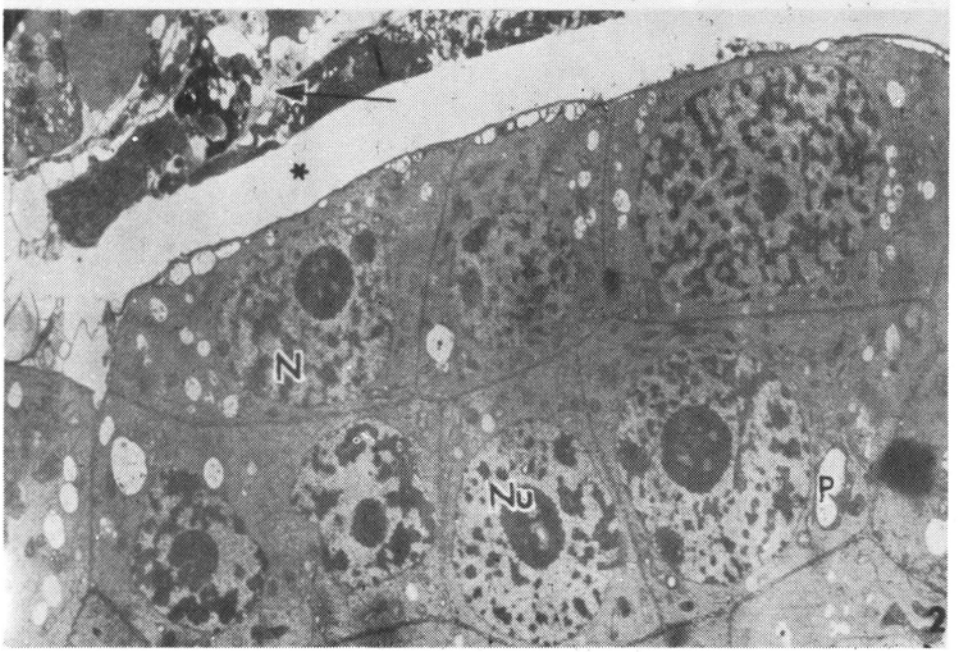
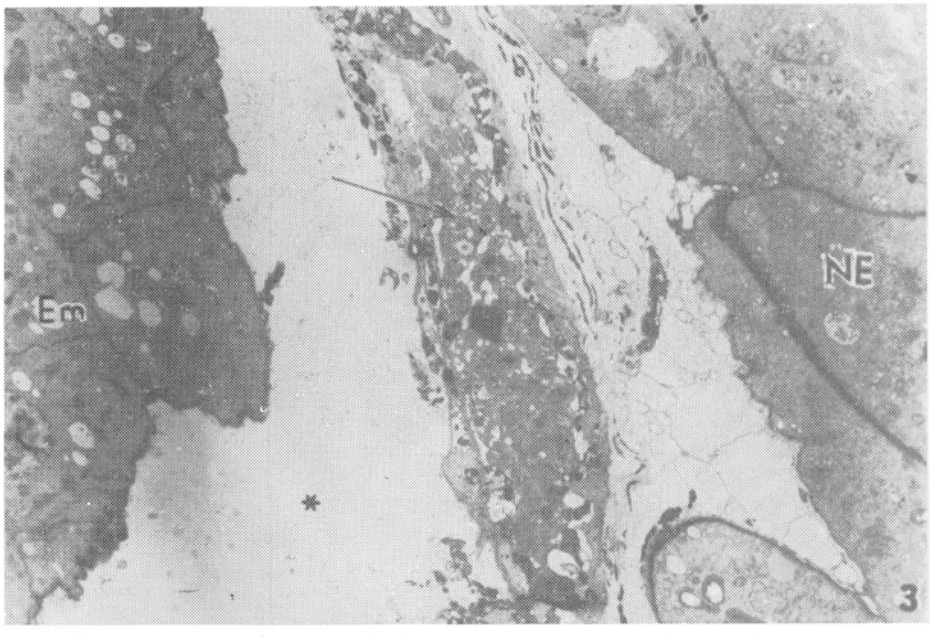


Fig. 1. Oval 7-day-old proembryo. Em — embryo, E — endosperm. X 400
Fig. 2. Basal part of rye proembryo. N — nucleus, Nu — nucleolus, P — proplastid, arrow — degenerated nucellar cells, asterisk — electron-translucent space. X 3500



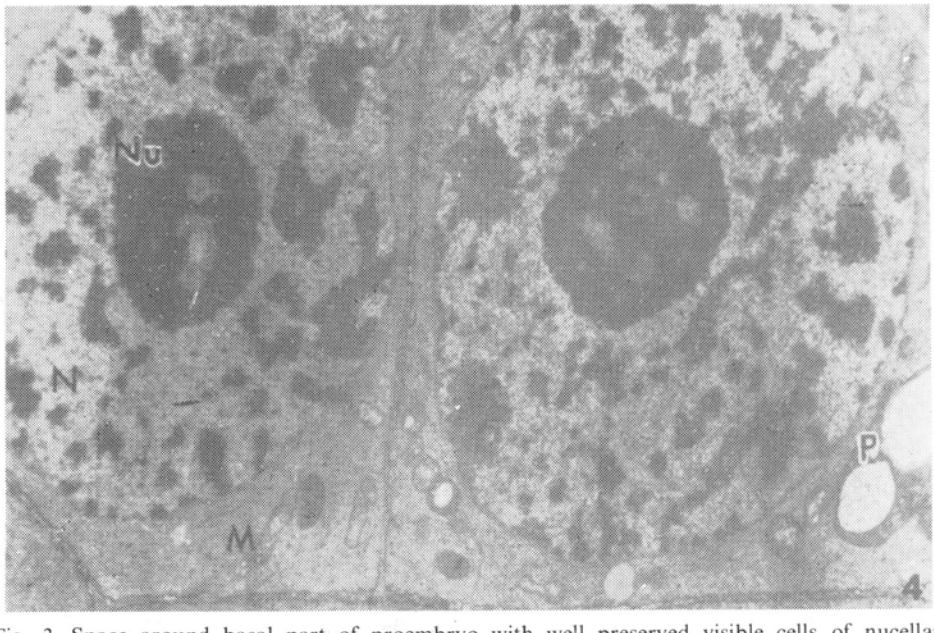
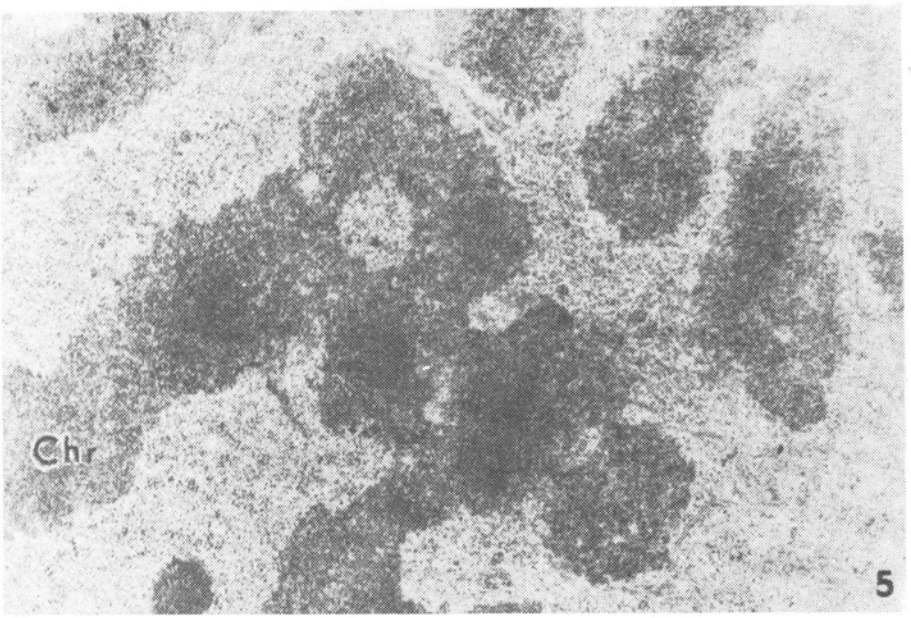


Fig. 3. Space around basal part of proembryo with well preserved visible cells of nucellar epidermis. Em — embryo, NE — nucellar epidermis, arrow — degenerated nucellar cells, asterisk — electron-translucent space. X 3000

Fig. 4. Minute nucleolar vacuoles in nucleoli of proembryo cells. M — mitochondrion, N — nucleus, Nu — nucleolus, P — proplastid. X 10 000



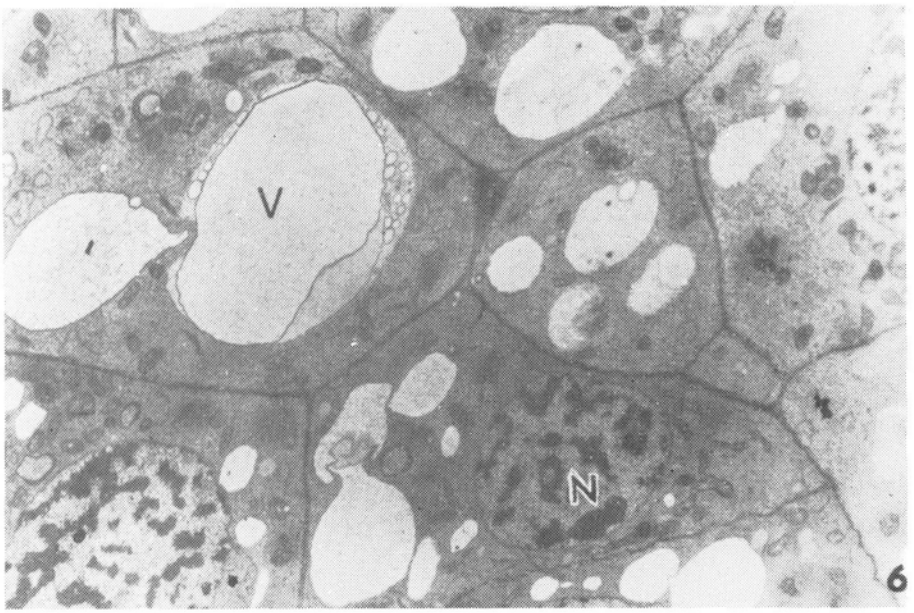


Fig. 5. Fragment of dividing nucleus of proembryo cell. Chr — chromosome. X 16 000
Fig. 6. Cells of apical part of proembryo with visible numerous vacuoles. N — nucleus, V — vacuole. X 4500

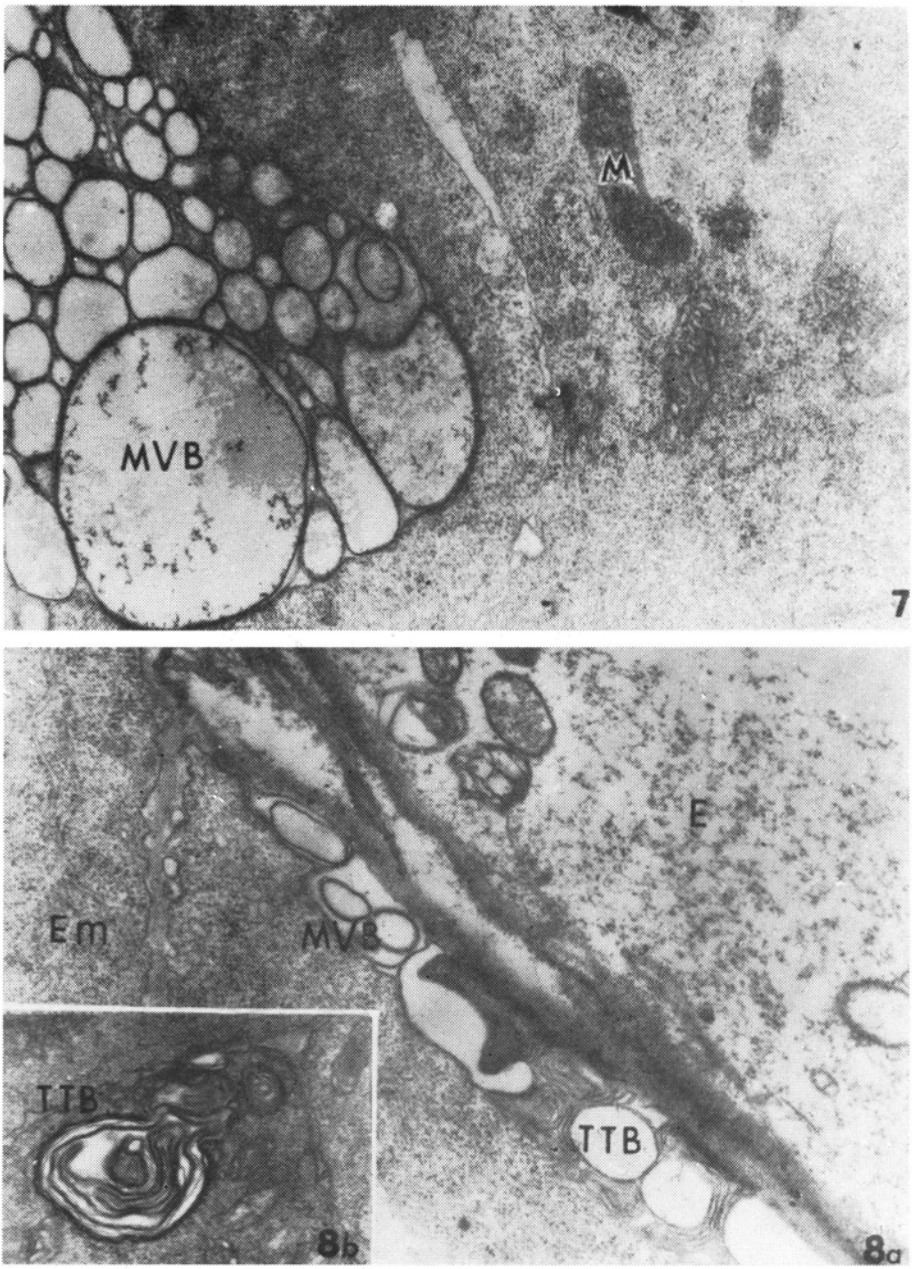


Fig. 7. Multivesicular body at cell wall in apical part of proembryo. M — mitochondria, MVB — multivesicular body. X 19 000

Fig. 8. Multivesicular bodies and tubular-type bodies edhering to cell walls in apical part of proembryo. Em — embryo, E — endosperm, MVB — multivesicular body, TTB — tubular-type body. 8a — X 22 000, 8b — X 16 000

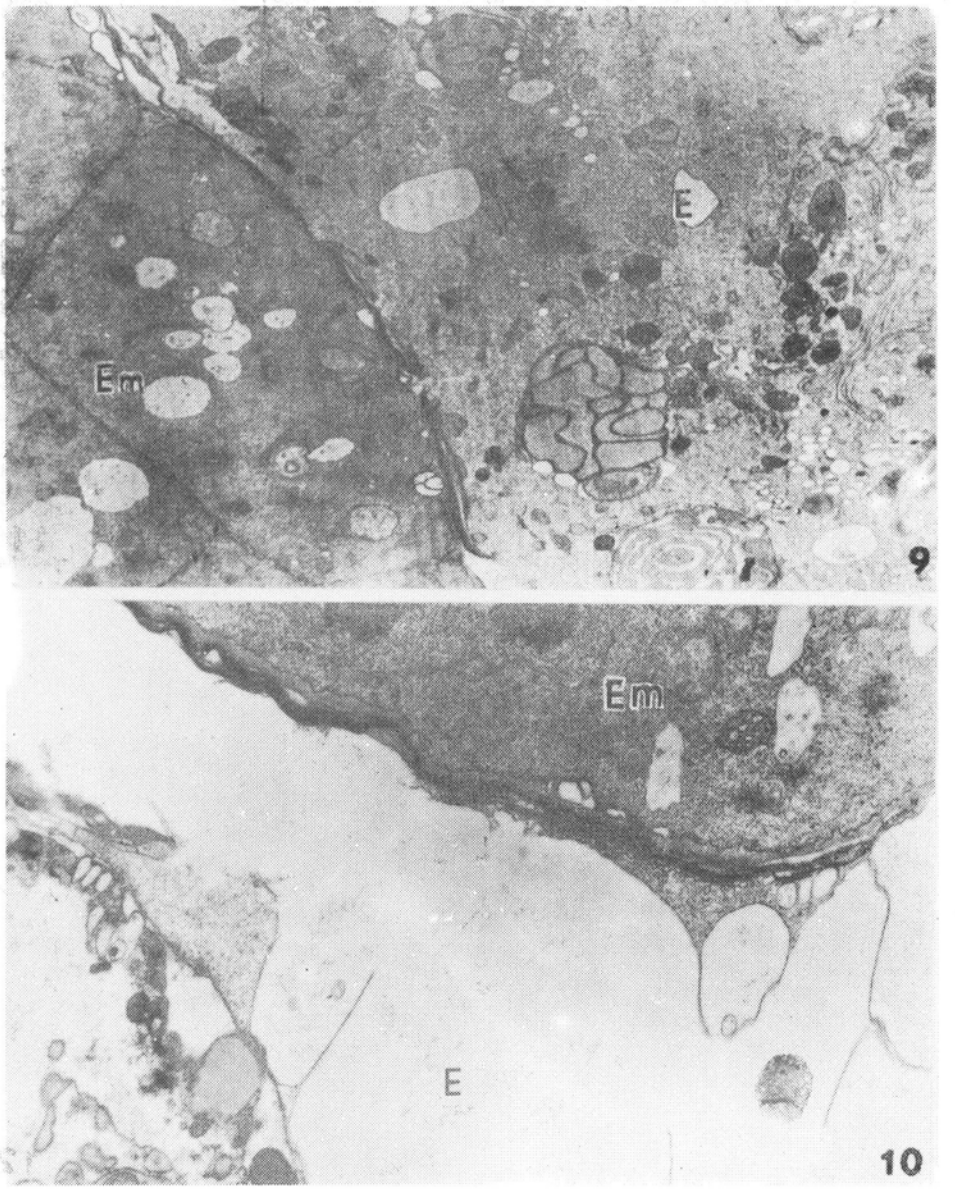


Fig. 9. Degenerated endosperm cells around apical part of proembryo. Em — embryo, E — endosperm. X 6000

Fig. 10. Hydrolysed endosperm cell walls adhering to apical part of proembryo. Em — embryo, E — endosperm. X 10 000

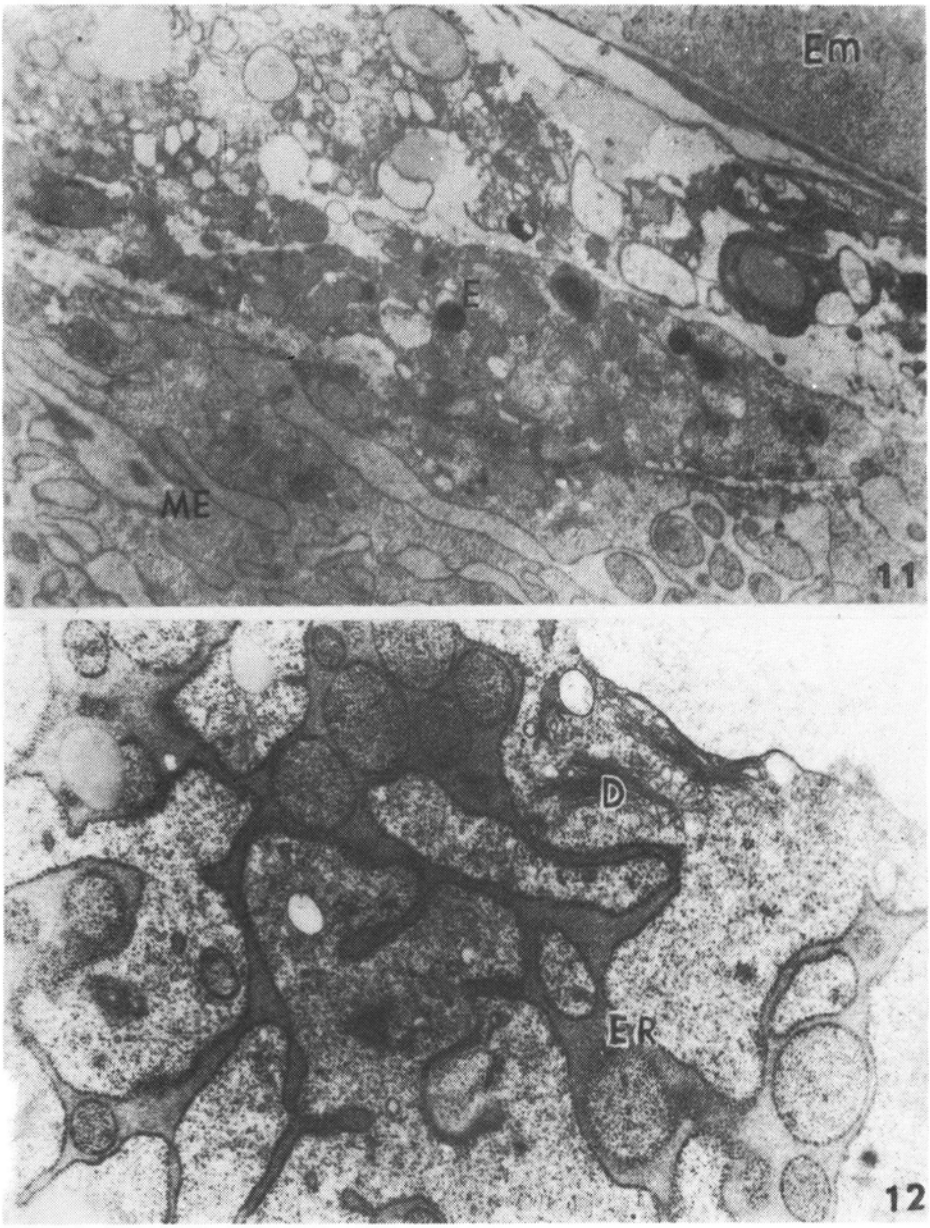


Fig. 11. Region around apical part of proembryo. Fragments of degenerated endosperm cells and of "modified" endosperm are visible. Em — embryo, E — endosperm, ME — modified endosperm. X 12 500

Fig. 12. Fragment of modified endosperm. D — dictyosome, ER — endoplasmic reticulum. X 24 000

lar vacuoles" can be seen (Figs. 2 and 4). Within the whole proembryo pictures were frequently observed indicating division of nuclei (Fig. 5). The formation of cell plates was evidence of cell division. In the electron-dense cytoplasm numerous free ribosomes were noted in the first place. Often dictyosomes and mitochondria with a developed system of cristae were also observed. The endoplasmic reticulum is relatively little developed. Protoplasts have an electron-dense stroma and contain small starch grains. All cells of the proembryo contain minute vacuoles (Fig. 2). It was noted, however, that with increasing distance from the basal part of the proembryo, the number and size of the latter increases (Fig. 6). Within most vacuoles multivesicular bodies are present (Fig. 6). Bodies of a similar character were also found close to the cell walls, mainly in the apical part of the proembryo (Figs. 7 and 8a, b). The proembryo in the studies developmental stage is accompanied by endosperm. As seen in the pictures obtained, the latter does not adhere directly to the proembryo cells. Around the basal part of the latter and electron-translucent space is observed containing remains of degenerated cells of the nucellus (Figs. 2 and 3). Only the epidermis-forming cells of the nucellus are preserved without noticeable changes. Around this part of the proembryo endosperm was not noticed. The central and apical parts of the 7-day rye proembryo were surrounded by a rather small electron-translucent space in which two types of endosperm cells were present. One kind were undergoing degenerative processes of various degrees up to hydrolysis of the cell walls inclusive (Figs. 9 and 10). The other, however, exhibited an electron-dense cytoplasm, abundant granular endoplasmic reticulum, dictyosomes and a relatively small number of mitochondria and proplastids (Figs. 11 and 12). These cells greatly exceeded in size the proembryo cells. The remaining part of the endosperm beyond this zone showed a structure of typical cellular endosperm. It should be added that, on the whole examined periphery of the embryo, plasmodesma junctions were not found between the proembryo cells and the endosperm ones.

DISCUSSION

It results from the data here presented that the 7-day rye proembryo consists still of cells of meristematic character exclusively. The extensive and varied activity of the proembryo cells is indicated not only by the division of nuclei, but also by the pictures of other structures (among them nucleoli, mitochondria and dictyosomes). Although there are no distinct morphological differences between the basal and the apical part of the proembryo, certain different features were observed in the ultrastructure of the cells of both these parts. These differences mainly con-

cerned an increased number of vacuoles and multivesicular bodies in the apical part of the proembryo. It is known from the literature that in the course of embryogenesis a certain group of cells of the proembryo can be distinguished which mediate its nutrition. The characteristic feature of such cells is among other things a large number of vacuoles in them (Hejnowicz 1980). Rodkiewicz (1973), on the other hand, attributes the presence of numerous vacuoles in some of the proembryo cells to the temporary storage in them of nutrient substances supplied by the endosperm. The observations of both these authors thus indicate that the proembryo cells containing numerous vacuoles may be intended for mediation functions in nutrition. Hejnowicz (1980) also mentions that the above mentioned cells are larger than the remaining ones of the proembryo. The cells observed in the present study in the apical part of the proembryo contained, it is true, numerous vacuoles, their dimensions, however, did not differ markedly from other cells, but they were found to contain numerous multivesicular bodies. The latter, according to Gillespie and Hamilton (1977), may serve as a store of cytoplasmic membranes utilised later in the bulding of new cellular structures. The same authors attribute the occurrence of such bodies to cells which in the course of further development exhibit intensive growth. It would seem, therefore, that the specific features of cell structure in the apical part of the 7-day rye proembryo (accumulation of vacuoles and multivesicular bodies) may be indicative of early stages of cell differentiation towards development of the scutellum. This organ, namely, fulfills for the proembryo and embryo of cereals intermediary functions in the transmission of nutrient substances, and its development takes place in the apical part of the proembryo. A problem by itself is the mechanism of transmission of nutrient substances. Neither literature data nor the present investigations reveal the presence of plasmodesmata between the proembryo and the endosperm. There must, therefore, exist other pathways of nutrient substance transport to the proembryo cells. Harris et al. (1982) described in the scutellar epithelial cells of the barley proembryo tubular invaginations of the plasmalemma which they termed plasmatubules. These structures increase the surface area of the membrane in the region of contact between apoplastic and symplastic transport. Harris et al. (1982) compare the function of the plasmatubules to the function of modified microvilli in animal cells which intensively take up solutions. Plasmatubules appeared always where solution flow was intensive. In the case of rye the region of contact of apo- and symplastic transport may involve peripheral and apical cells of the proembryo and the electron-translucent space around the apical part which, according to Smart and O'Brien (1983) is filled with liquid in unfixed material. In the present study both the electron-translucent space and structures resembling somewhat the above described plasma-

tubules were seen. It is possible that these structures are involved in the process of nutrient substances transport from the endosperm to the embryo, the solution of this problem, however, requires further investigations. It should be added that the relatively intensive flow of nutrient substances in this region is also indicated indirectly by observation of the endosperm cells. Smart and O'Brien (1983) noticed around the developing scutellum of the wheat embryo an area of what they called "modified" endosperm. According to these authors, the main function of the modified cells of the endosperm is synthesis and transmission of nutrient substances from more distant parts of the endosperm to the proembryo. It was demonstrated in the present investigations that modified endosperm surrounds the apical part of the proembryo, and the ultrastructure of cells in this region, particularly the considerable amounts of granular endoplasmic reticulum and active dictyosomes are evidence of their involvement in transport processes.

The here presented results seem to confirm that the development of the axial organs in the rye proembryo starts at an earlier stage than reported by Cebrat (1979). This finding requires, however, further detailed studies, especially in the developmental aspect.

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Rozwój zarodka żyta. I. Wstępne badania ultrastrukturalnej budowy 7-dniowego prazarodka

Streszczenie

Badania ultrastrukturowe 7-dniowego prazarodka żyta nie wykazały wyraźnych różnic w budowie komórek, znajdujących się w poszczególnych jego częściach. Zaobserwowano jednak, że w komórkach części apikalnej występowały liczniejsze wakuole i ciała wielopęcherzykowate niż w komórkach części bazalnej. Wymienione wyżej specyficzne cechy budowy komórek apikalnej części prazarodka (w powiązaniu z danymi innych autorów) wydają się wskazywać na wczesny etap wykształcania się tarczki zarodkowej.