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The ultrastructure of anther wall and pollen of *Hordeum vulgare* at the microspore stage

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Abstract

The ultrastructure of anther wall and pollen of *Hordeum vulgare* was described at the microspore stage. In future studies this stage will constitute an initial material in experiments for the inducement of androgenesis.

INTRODUCTION

Many ultrastructural and biochemical studies are being carried out on bi-nucleate pollen (Mascarenhas 1975). On the other hand investigations on the ultrastructure of the anthers and the process of microsporogenesis in *Graminae* are not frequent (Rowley 1964; Skvarla and Larson 1966; de Vries and Ie 1970).

Although Cass and Karas (1975) have discussed the structure of barley pollen grains and the development of sperms the present work was undertaken also to this problem in order to carry out a thorough study of the anther wall, as well as spores, which constitue an initial material for future experiments on androgenesis.

MATERIAL AND METHODS

Anthers of *Hordeum vulgare* cv. Alsa originated from plants growing in greenhouse contained microspores either just before, or just after the formation of vacuole. Anthers at this stage of development were cultured *in vitro* in order to induce the process of androgenesis. In the presnt work anthers were analyzed only as an initial material for such studies. Fragments of anthers from their middle part, were fixed in 6% glutaraldehyde in 0.1 M cacodylate buffer at pH 6.8 for 18 hours, in

the temperature of 4°C. Additional fixing was performed with 2% OsO_4 in cacodylate buffer for 2 hours. After washing with the buffer, the preparations were stained with 2% solution of uranyl acetate for 1 hour, dehydrated with ethanol, aceton, and propylene oxide, and immersed in a mixture of Epon. These preparations were cut on an LKB ultramicrotome and the sections were contrasted with uranyl acetate and lead citrate (Reynolds 1963; Venable et al. 1965). Electron micrographs were made on JELCO microscope, type 7A.

RESULTS AND DISCUSSION

The structure of anthers of barley, on the cross-sections was alike other representatives of *Gramineae* with four embryo sacs and connective tissue with the central vascular bundle. Microspores were arranged in one layer at embryo sac circumference, usually at the number of 8-12 per one section (Fig. 1 and 2). Microspore profiles were not always spherical or oval in shape (Fig. 1), but sometimes ringshaped (Fig. 15), pointing in this case to rather deep sporoderm pockets. As a rule, although not always, microspores were arranged in such a way that these poles in which nucleus was presented were oriented toward the anther cavity, while poles with poruses were found at the tapetum side (Fig. 8, 15). Such type of orientation was also typical for other species of *Gramineae* (Romanov 1971; Christensen, Horner 1974; Huynh 1975; Mostowska, Charzyńska 1977). Anther wall consisted of four layers (Fig. 3). Its epidermis had oval cells, with large, centrally located vacuole, and cytoplasm arranged close to the cell wall. Cells of the endothecium were tangentially elongated, contained only small vacuoles and dense cytoplasm, rich in various structures. Nucleus was usually found in the centre of the cell and possessed large areas of condensed chromatin. Endothecium cells also contained well developped chloroplasts with high grana (Fig. 4). Very frequently in chloroplasts large amounts of starch were present. Ultrastructure of this layer shows to of a high metabolic activity. Under the endothecium there was an unicellular intermediate layer — the cells of which were wedge-shaped, overlaped each other, and were highly vacuolized (Fig. 3). On some sections it was possible to observe degeneration of the secretory tapetum (Fig. 5), but well preserved cells, with cytoplasm filled with ribosomes and endoplasmatic reticulum, were also found. Both the endoplasmatic reticulum, Golgi apparatus, mitochondria and plastids (Fig. 7), of these cells contained osmophilic material which according to several authors (Gabara and the references 1976) — takes part in the exine formation or in the formation of exine coatings, such as pollenkit or tryphine. Pollenkit has so far been described only for entomophilous plants, but

tryphine was found also in anemophilous ones (Echlin and the references 1971), although according to Dickinson (1973) both these substances are synthetized in the plastids of tapetum. Many Ubisch bodies were found on the edges of tapetum cells, as also between the tapetum and microspores (Fig. 5 and 6). This fact would show either to the still continouing process of the formation of pollen wall, or to a certain excess of sporopollenin (Rowley and the references, 1964), since the two-layered exine was already quite well visible, and the sexine possessed its characteristic pattern.

The problem of pollen wall origin is extremely interesting and still rather unclear. However, in the case of *Hordeum* its explanation necessitates further, separate studies.

In barley pollen, similary as in pollen grains of all *Gramineae*, there is a single, ring-shaped pore, which contains all parts described previously for *Poa annua* (Rowley 1964) or *Triticum sativum* (de Vries 1970).

Upon the section the layer of intine was plicated, rather thick, and fine-grained (Fig. 9). At this stage the structure of barley intine closely resembled the intine of young microspores of *Lilium Henryi* (Helsop-Harrison 1968). Its thickening was noticeable in the pore area, where was also a bulge described in the literature as "Zwischenkörper" (Fig. 8, Rowley 1964 and the references). On some sections operculum and well defined annulus lamelle were also visible (Fig. 9). In the intine of barley microspores there were no dark cytoplasmatic strands, running radially from the cytoplasm to the exine (de Vries and Ie 1970), although some membranoeus components or microtubules (Fig. 9) were distinguishable. Microtubules in the area of pollen wall have already been observed by other authors (Flynn 1971).

Cytoplasm of microspores showed varying degree of vacuolization. There were sections of microspores without vacuole (Fig. 12), with small vacuole (Fig. 13), as also with large, central vacuole thrusting the nucleus aside, to the pollen wall (Fig. 14). According to Cass and Karas (1975) vacuoles in barley microspore are formed only after the first mitosis. In our material formation of vacuoles took place before the first mitotic division.

The amount of ribosomes in the cytoplasm, or the density of cytoplasmatic matrix was rather differentiated. Special attention should be given to membrane complexes occurring in the cytoplasm, sometimes close to the nucleus but most frequently distant from it, in the number of 1-2 per one microspore section (Fig. 12). However, these membranes were not packed in stacks such as, for instance, the "nuclear caps" found in *Saintpaulia ionatha* (Ledbetter and Porter 1970), nor formed myelin-like structure polarizing the cytoplasma as in case of *Tradescantia* microspores (Mephan and Lane 1970), but rather resembled the

reticulum complex of *Beta vulgaris* pollen (Hoefert 1969). However, contrary to the latter, in barley these membranes did not contact directly the superficial membrane of the nucleus nor the plasmalemma. In barley each such membrane complex possessed 2-4 centres, from which the membranes run radially (Fig. 16). These centres formed common zones with cisterns enclosed by the membranes — in our opinion they represent the endoplasmatic reticulum. They can ramify or form bridges within one complex. The origin of these complexes is still unknown. It is assumed that they constitute a certain pool of endoplasmatic reticulum, or else the centres from which it originates, the more so that it was also possible to observe significant loosening of these complexes into typical endoplasmatic reticulum (Fig. 17).

The nucleus was most frequently spherical, with slightly visible chromatin area (Fig. 12). Nucleoli had a compact structure and possessed very small "vacuoles" (Fig. 11). Mitochondria were numerous, of a condensed type (Fig. 16—17). According to Hackenbrock et al. (1971) transformation of mitochondria from orthodox to condensed state is connected with the induction of phosphodylation, and thus reflects their active state.

There were only few plastids on the section of the microspore. They did not contain starch and internal membranes were rarely present (Fig. 10). The Golgi apparatus was also scarce, most frequently close to the wall, and consisted of about 3-6 cisterns (Fig. 18).

The ultrastructure of barley microspores presented in this work constitutes a starting material for the observations on changes taking place in cultures *in vitro*, leading to the formation of embryoids.

SUMMARY

The ultrastructure of *Hordeum vulgare* anther wall and pollen grains at a uni-nuclear stage has been described. In future studies this stage will constitute an initial material for inducing the process of androgenesis.

Most frequently the microspores were vacuolized and arranged with nuclear pole toward the cavity of the anther, and porus close to the tapetum. Porus, as also pollen wall, were typical for *Gramineae*. The problem of sporopollenin formation, and the role of tapetum in this process are briefly discussed. They will be analysed in detail in another paper. Areas of chromatin were relatively small. As regards the structure of cytoplasm attention should be given to the complexes of membranes of endoplasmatic reticulum. Plastids were scarce, did not contain starch, and possessed poorly developed system of internal membranes. Mitochondria were of a condensed type.

Acknowledgments

The authors wish to express their gratitude to prof. Maciej Zenkteler — the consultant of the Institute of Plant Genetics of the Polish Academy of Sciences

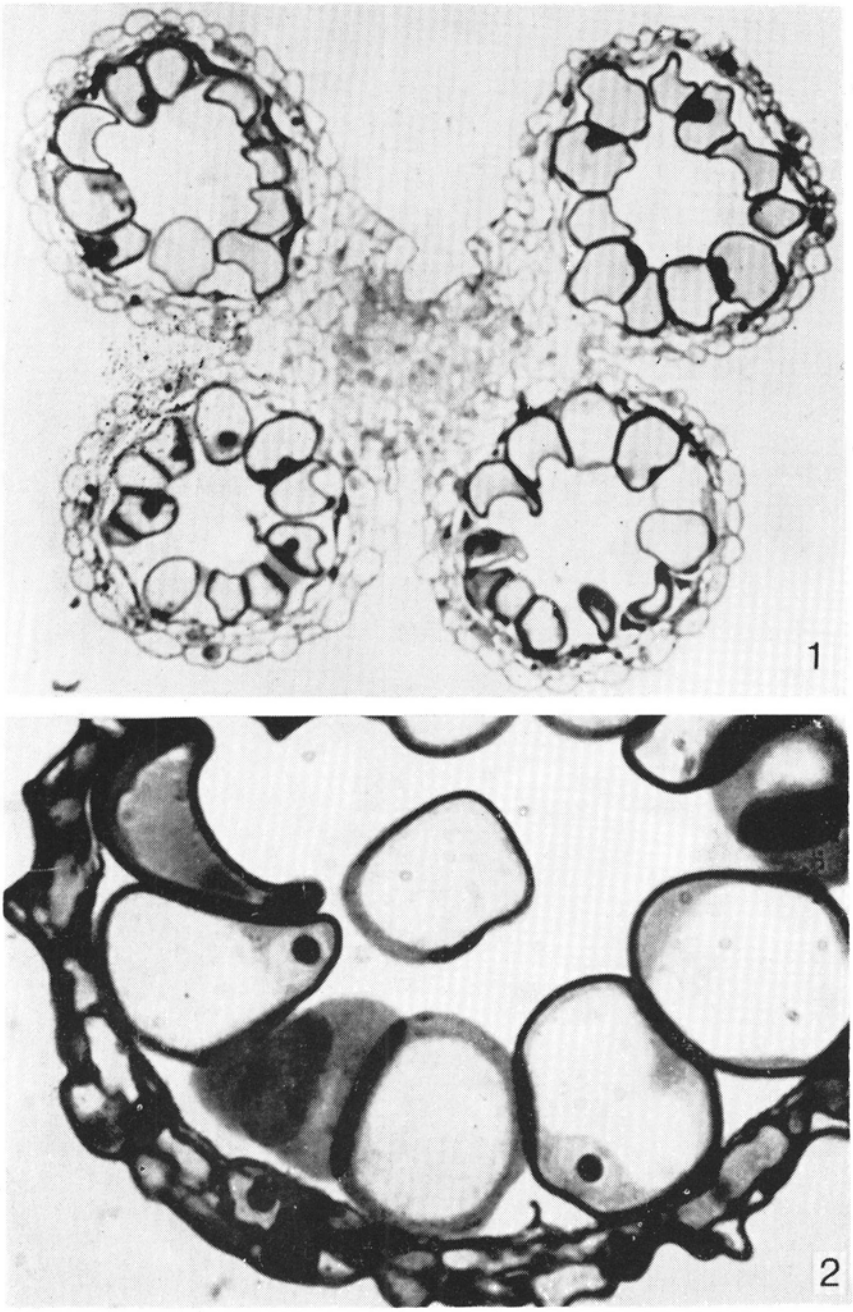

Fig. 1. Cross-section of anther with annular pattern of microspores arrangement. Epon sections stained with toluidine blue. Fig. 2. Fragment of embryo sac. Epon section stained with toluidine blue

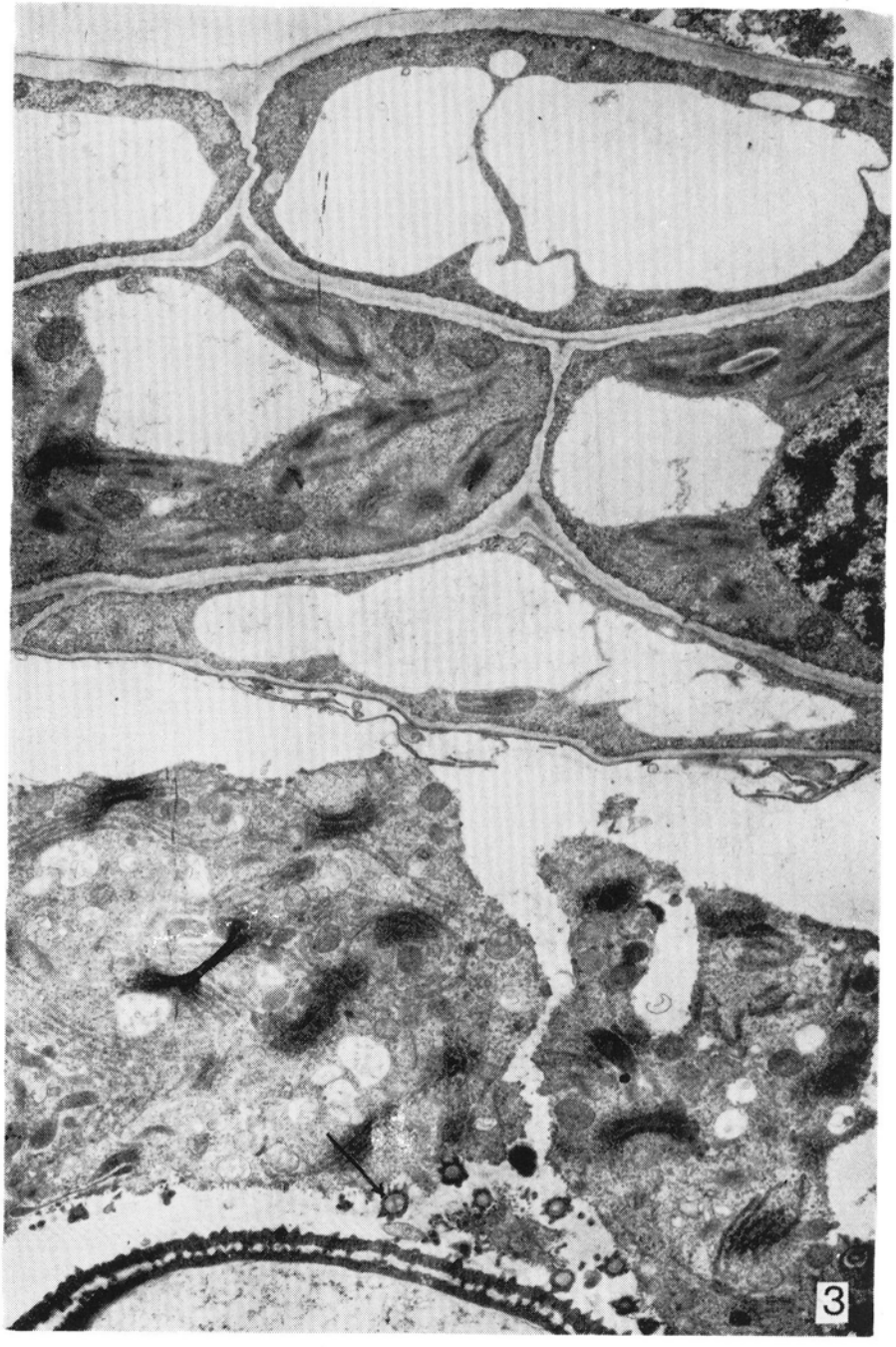

Fig. 3. Cross-section of anther wall. From top to bottom: epiderm, endothecium, intermediate layer, tapetum, part of microspore. Ubisch bodies (indicated by an arrow) visible between tapetum and microspore. Magn. 8700 ×

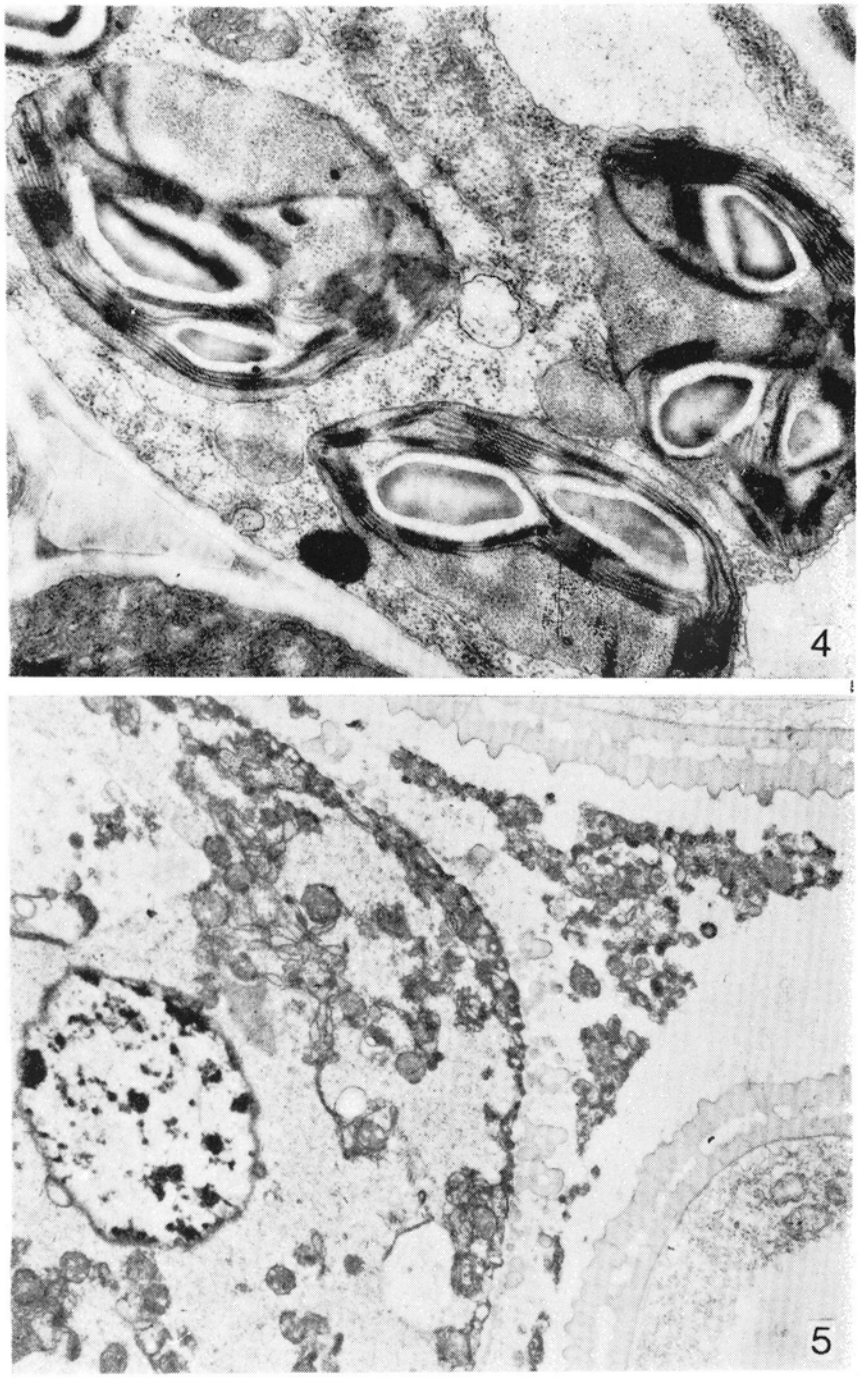

Fig 4. Fragment of endothecium cell with well developed chloroplasts. Magn. 17 000 ×

Fig. 5. Degenerating tapetum cell. On the circumference Ubisch bodies and osmophilic material between microspores. Magn. 11 000 ×

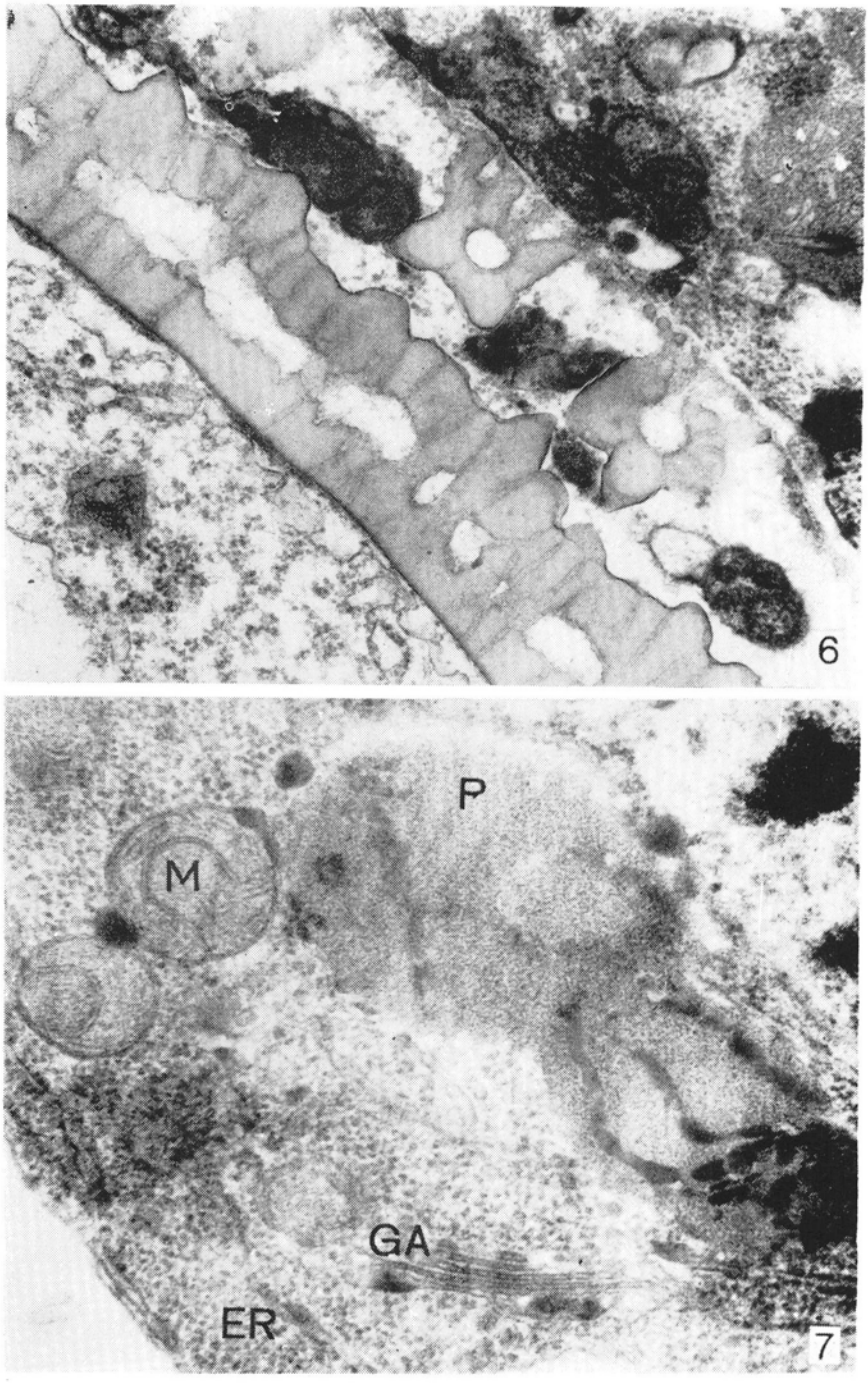

Fig. 6. Ubisch bodies and osmophilic substance in the space between tapetum cell and miscrospore wall. Magn. 32 000 ×

Fig. 7. Osmophilic material in plastid (P), mitochondria (M), edoplasmatic reticulum (ER), and Golgi apparatus (GA) of a tapetum cell. Magn. 45 000 ×

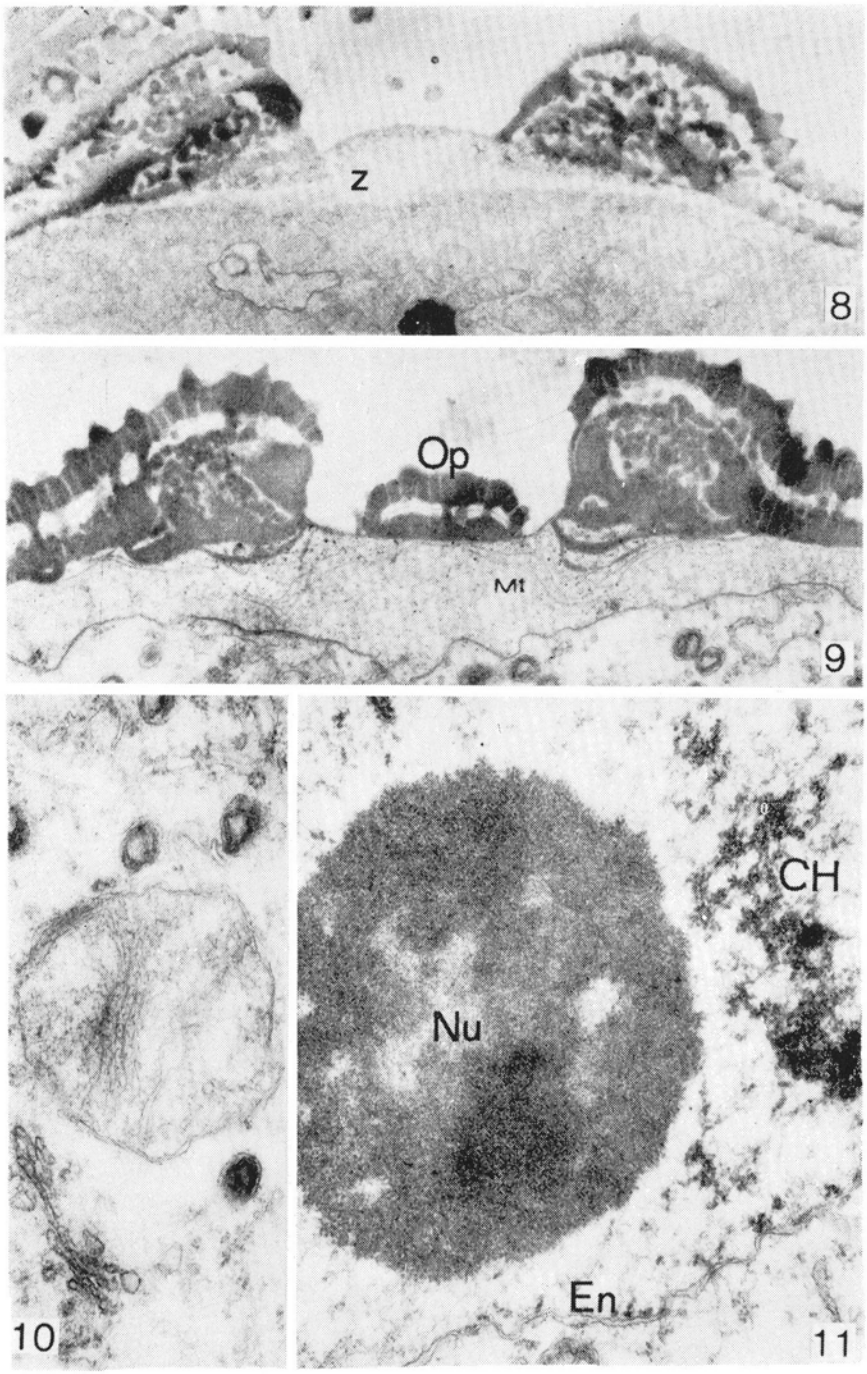

Fig. 8—9. Cross sections through pore. Visible rich carvings on exine sides. Within the porus intine forms a continous layer, frequently with visible — "Zwischenkörper" (Z). Various microtubul-like structures (Mt) present in the intine. Op — operculum. Magn.: Fig. 8 — 11 000 ×, Fig. 9 — 12 000 ×. Fig. 10. Typical microspore plastid with poorly developed membrane system. Magn. 30 000 ×. Fig. 11. Fragment of microspore nucleus with nucleolus (Nu), chromatin (Ch), and nucleus envelope (En). Magn. 11 000 ×

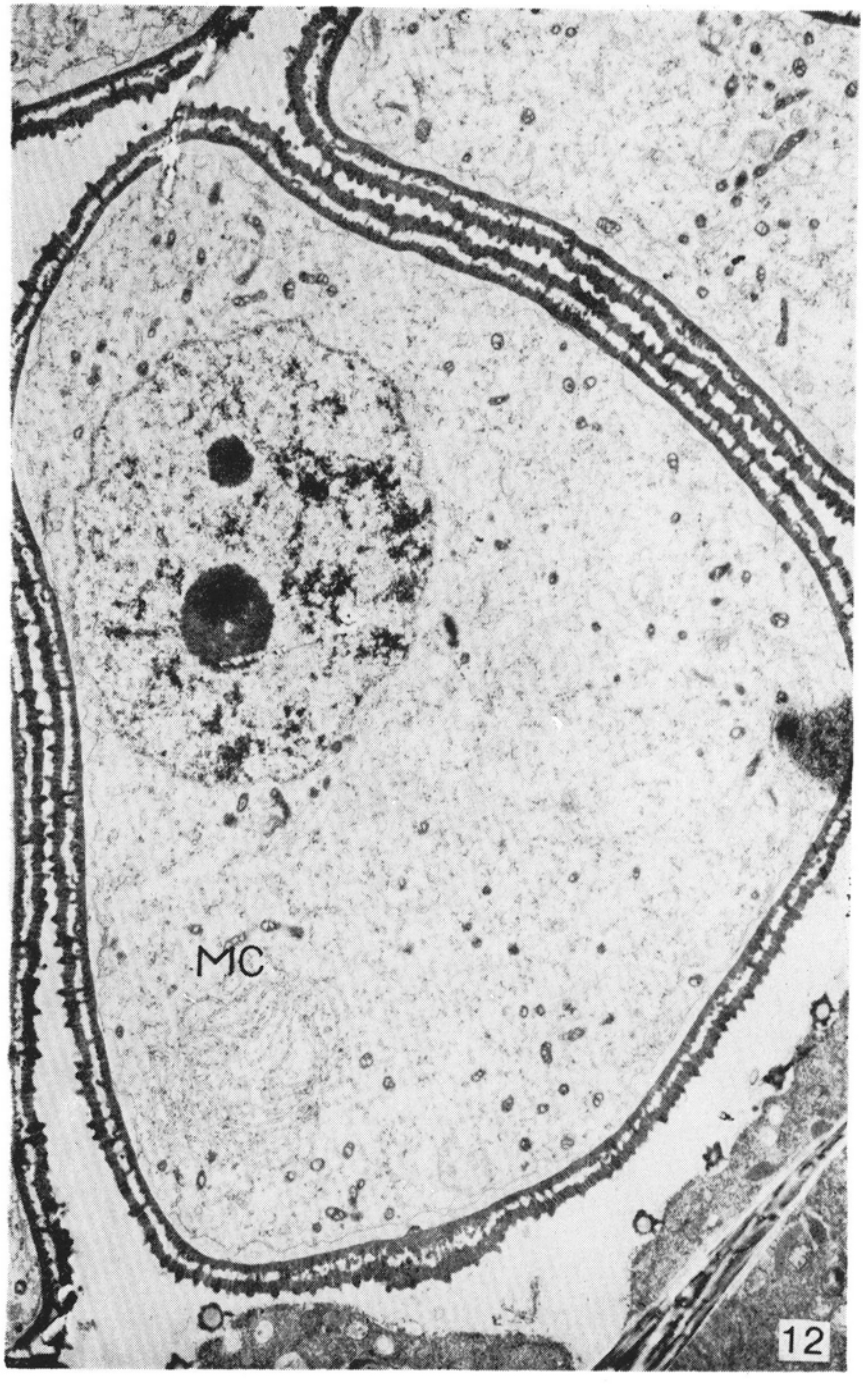

Fig. 12. Section of microspore at the level of the nucleus and membrane complex (MC). Magn. 7500 ×

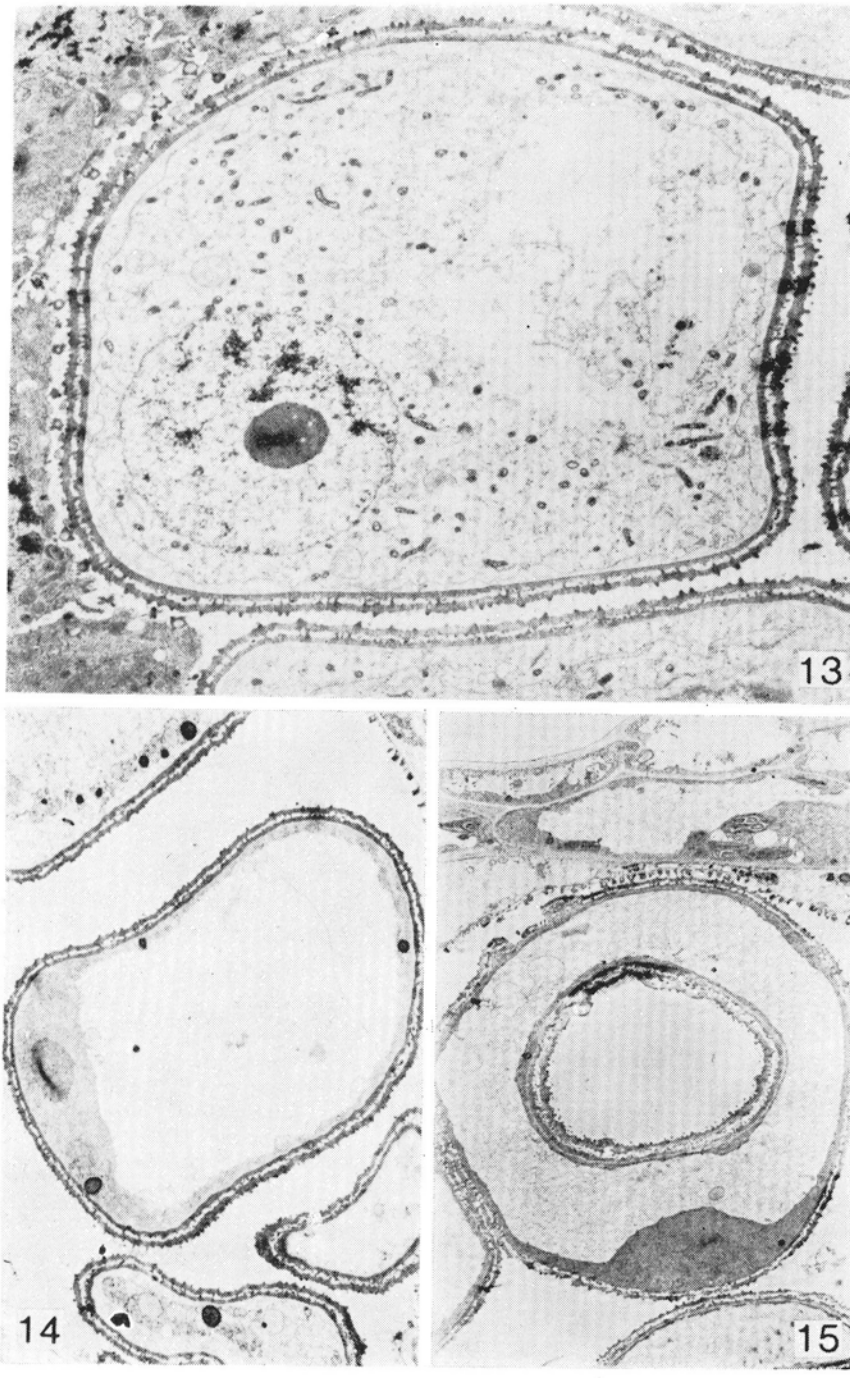

Fig. 13—15. Sections of microspores. Fig. 13 — with small vacuole, Fig. 14, strongly vacuolized, Fig. 15, vacuolized but with sunken wall. This picture presents most typical, polar arrangement of microspore toward tapetum. Magn.: Fig. 13 — 6000 ×, Fig. 14 — 2000 ×, Fig. 15 — 2000 ×

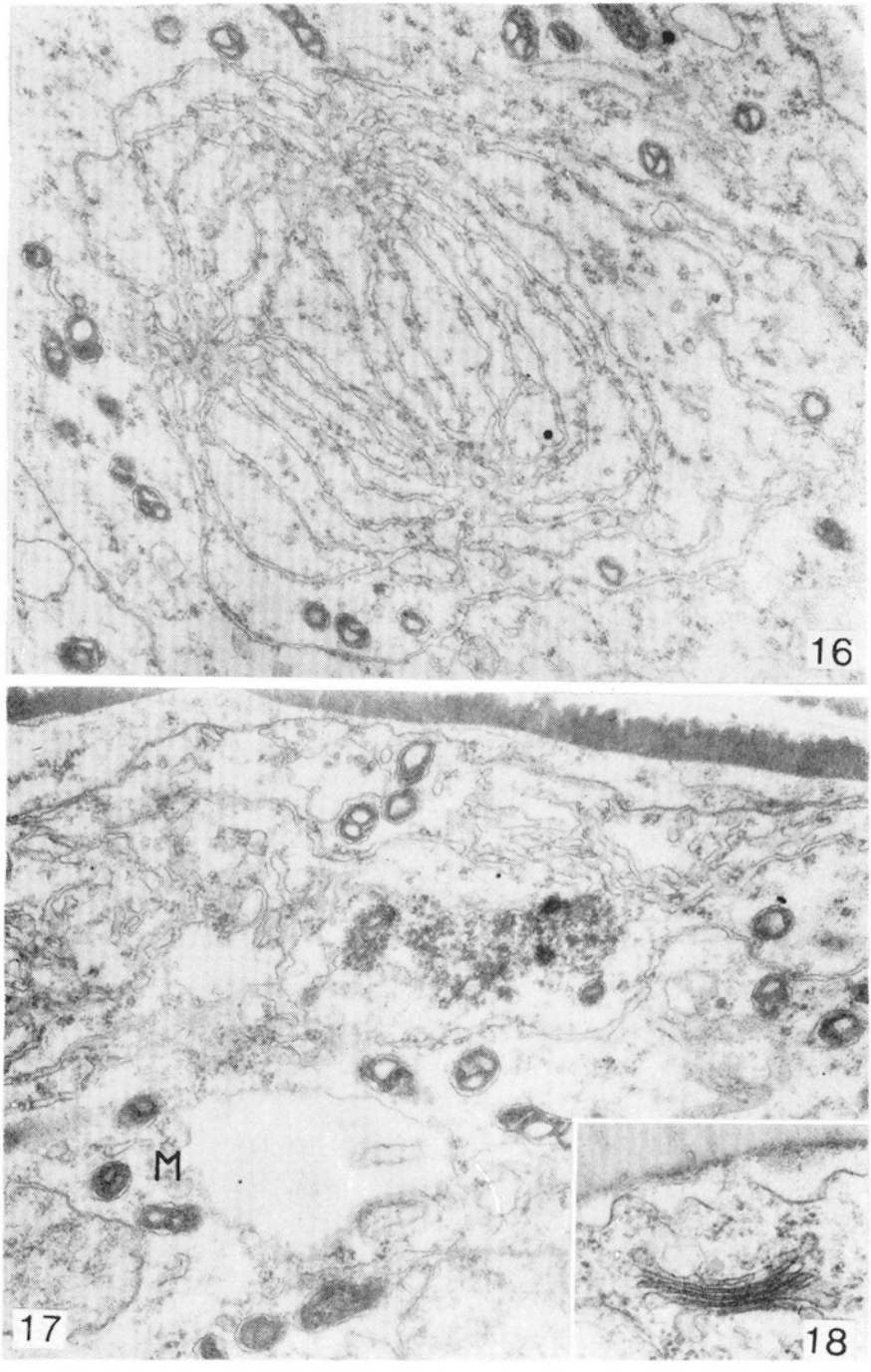

Fig. 16. Complex of membranes of endoplasmatic reticulum with three centres, characteristic for the microspore cytoplasm. Magn. 18 500 ×. Fig. 17. Fragment of microspore cytoplasm with loosened complex of endoplasmatic reticulum. In the stage under study mitochondria (M) were of the condensed type. Magn. 28 000 ×. Fig. 18. Typical Golgi apparatus. Magn. 33 500 ×

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Ultrastruktura ścian pylnika oraz pyłku Hordeum vulgare w stadium mikrospor

Streszczenie

Opisano ultrastrukturę ścian pylnika oraz pyłku *Hordeum vulgare* w stadium jednojądrowym. Stadium to w przyszłych badaniach będzie materiałem wyjściowym do indukowania embrioidów.

Mikrospory były najczęściej zwakuolizowane i ułożone biegunem jądrowym do wnętrza pylnika, a porusem do tapetum. Porus, jak i ścianka pyłku były typowe dla *Gramineae*. Problem tworzenia sporopoleniny i udziału tapetum w tym procesie został przedstawiony pobieżnie i będzie analizowany szczegółowiej w innej pracy. W jądrach było stosunkowo mało obszaru zajętego przez chromatynę. W strukturze cytoplazmy na szczególną uwagę zasługują kompleksy błon retikulum endoplazmatycznego. Plastydów było mało, nie zawierały skrobi, a ich system błon wewnętrznych był słabo rozwinięty. Mitochondria były typu skondensowanego.

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