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ULTRASTRUCTURAL STUDY OF MATURING POLLEN IN *ARABIDOPSIS THALIANA* (L.) HEYNH. (*BRASSICACEAE*)

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ABSTRACT

Ultrastructural changes in *Arabidopsis thaliana* pollen, between late microspore stage and mature pollen stage were described. When the generative cell was peeled off from the intine, it was of spherical shape and had all usual organelles with the exception of plastids. The cytoplasm transformation of the vegetative cell included an increase in the number of mitochondria and changes in the accumulation of starch and lipid bodies. The starch plastids were observed at the bicellular and early tricellular pollen stages and next starch was utilized during the maturation procces. The lipid bodies of the vegetative cell form a very regular sheath around the generative cell and then, around the sperm cells. Before anthesis the lipid bodies were dispersed within the whole vegetative cell cytoplasm.

KEY WORDS: *Arabidopsis thaliana*, vegetative cell, generative cell, lipid bodies, starch, pollen.

INTRODUCTION

The ultrastructure of pollen of different plants has been studied by several authors (Cresti et al. 1988, 1990; Charzynska et al. 1989a; Noguchi and Ueda 1990; Murgia et al. 1991; Polowick and Sawhney 1993). The small generative cell is completely surrounded by the vegetative cell, and these cells are separated by plasma membranes, that are closely parallel (Van Aelst and Van Went 1991; Murgia et al. 1991; Cresti et al. 1992; Southworth 1992; Hess 1993). The vegetative cell is a metabolically active cell and accumulates stored food in organelles, whereas the generative cell lacks storage food. (Charzynska 1984; Bednarska 1988; Polowick and Sawhney 1993). These studies have shown that, among the Angiospermae, the different metabolisms of generative and the vegetative cell are associated with different ultrastructural transformation in each of them (Bednarska 1988).

Similarly to other species of *Brassicaceae*, the mature pollen grains of *Arabidopsis thaliana* are composed of a large vegetative cell and two small sperm cells (Misra 1962, Charzynska et al. 1989b; Regan and Moffat 1990, Dawson et al 1993; Owen and Makaroff 1995).

This paper describes ultrastructural changes in the vegetative and generative cells that accompany the maturation of pollen grain in *Arabidopsis thaliana* ecotype Columbia with a particular reference to accumulation of different storage food.

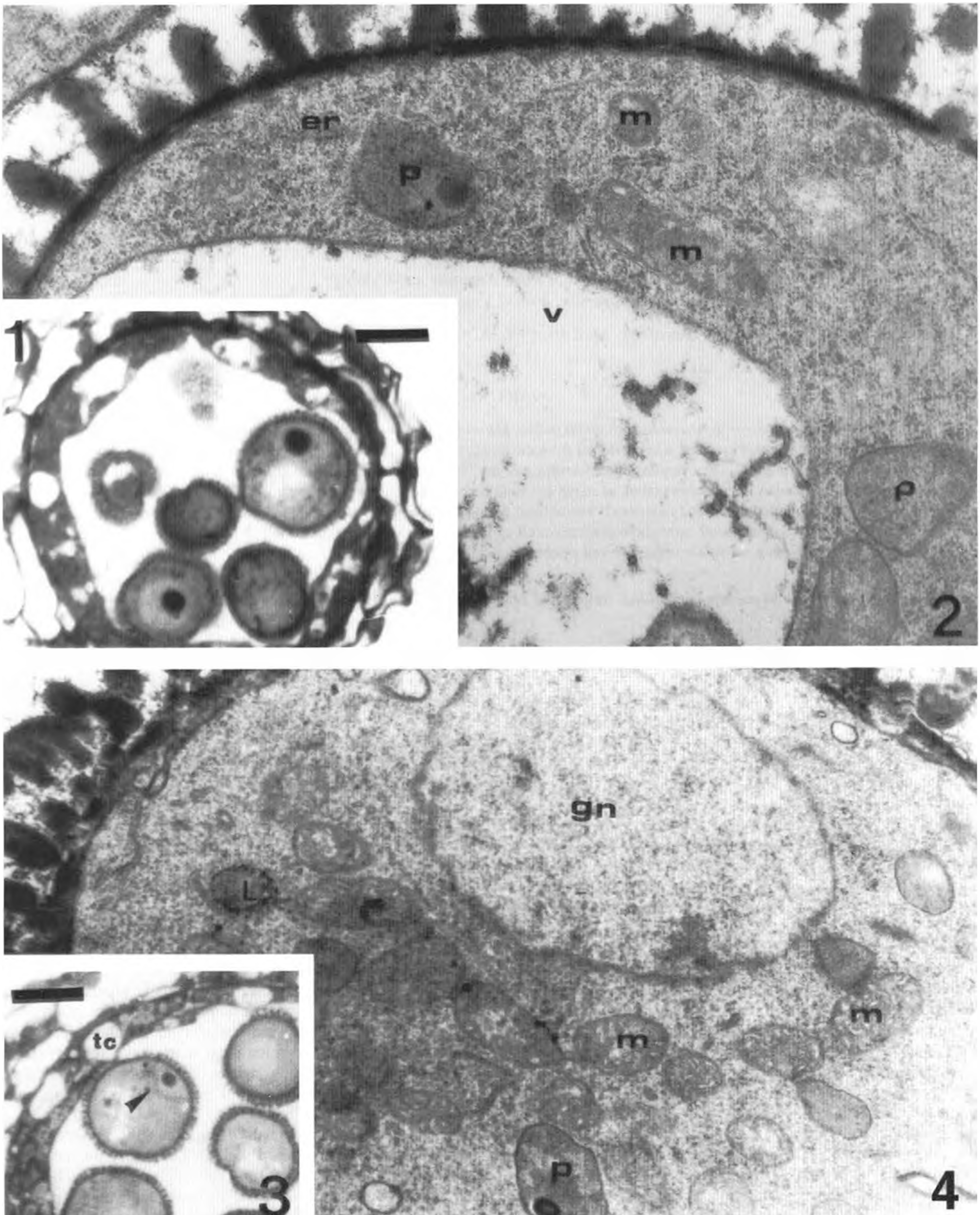
MATERIAL AND METHODS

Plants of *Arabidopsis thaliana* (L.) Heynh var. Columbia were cultivated in sterile conditions in our laboratory. Single flower buds at different development stages were fixed for electron microscopy in 3% glutaraldehyde in 0.1 M phosphate buffer (ph7, 4) at room temprerature for 4 hrs. Then they were rinsed in three changes of buffer and postfixed in 1% OsO_4 in the same buffer for 2 hrs, dehydrated in ethanol, propylene oxide and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and then examined with a JOEL JEM 100 S transmission electron microscope. Semithin sections were stained: 1) with 0.3% Sudan Black B solution in 70% ethanol, according to Bronners method (1975) for detection of lipid bodies, 2) with methyl blue – borax (Postek and Tucker 1976) for morphological analysis.

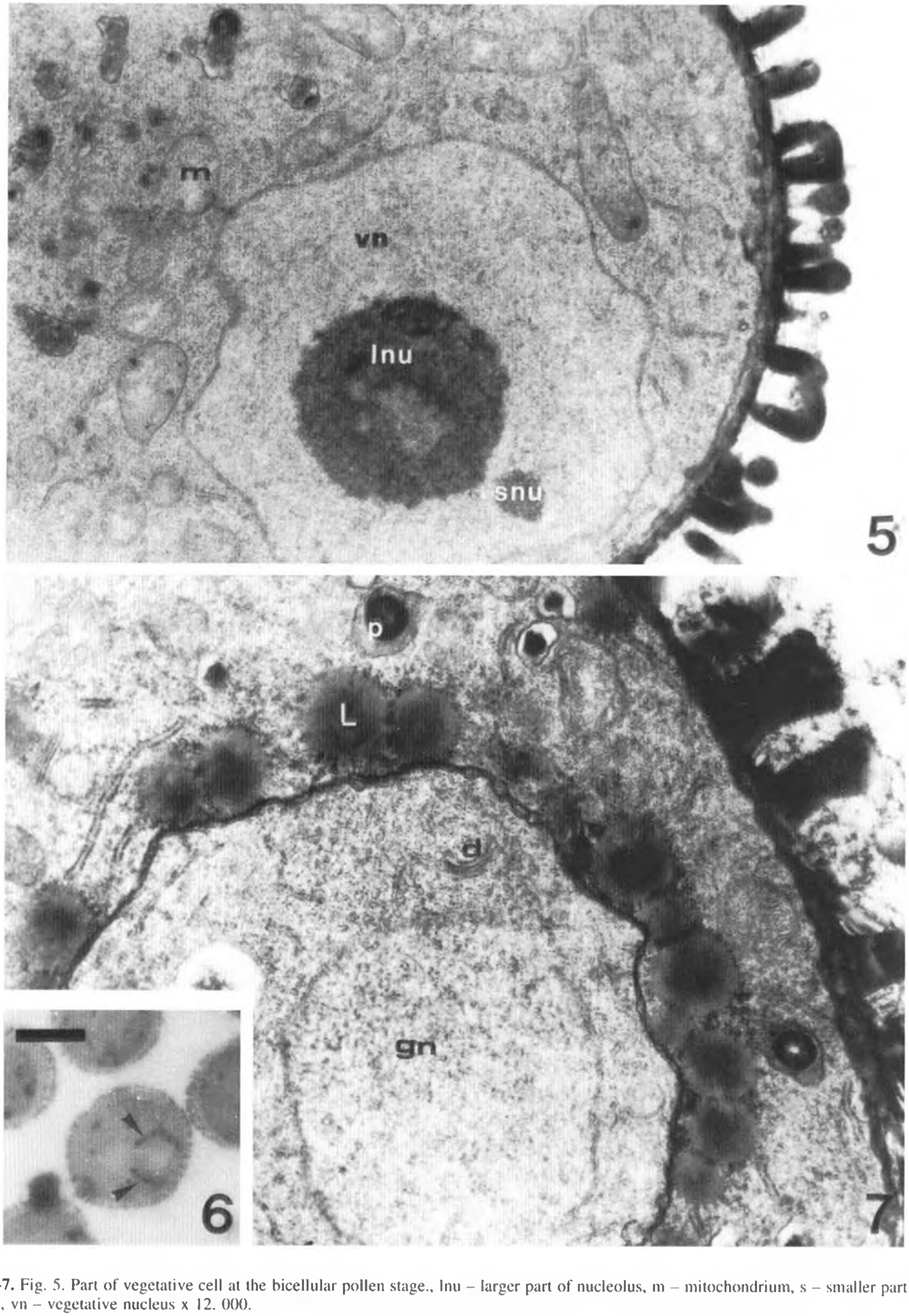
RESULTS

In vacuolated microspore, the nuclus is regular in outline and contains single nucleolus, plastids have a poorly developed internal system and not contain starch grains. Mitochondria of spherical or elongated shape have some enlarged cristae. In addition, short cristae of endoplasmic reticulum and occasionally spherical lipid bodies are present in cytoplasm. (Fig. 1, 2).

After the microspore division, when the generative cell was appressed to the pollen wall (Fig. 3), very numerous mitochondria are aggregated near forming cell plate. The mitochondria exhibit more internal structure than at the previous stage; cristae are tubular and the matrix is electron dense. The endoplasmic reticulum appear in short stretches, but is more abundant than at the previous stages. In addition, in the cytoplasm, between mitochondria single lipid bodies are present. They are contacted with cisternae endoplasmic reticulum and covered by free ribosomes. Plastids contain single starch grains (Fig. 3, 4).



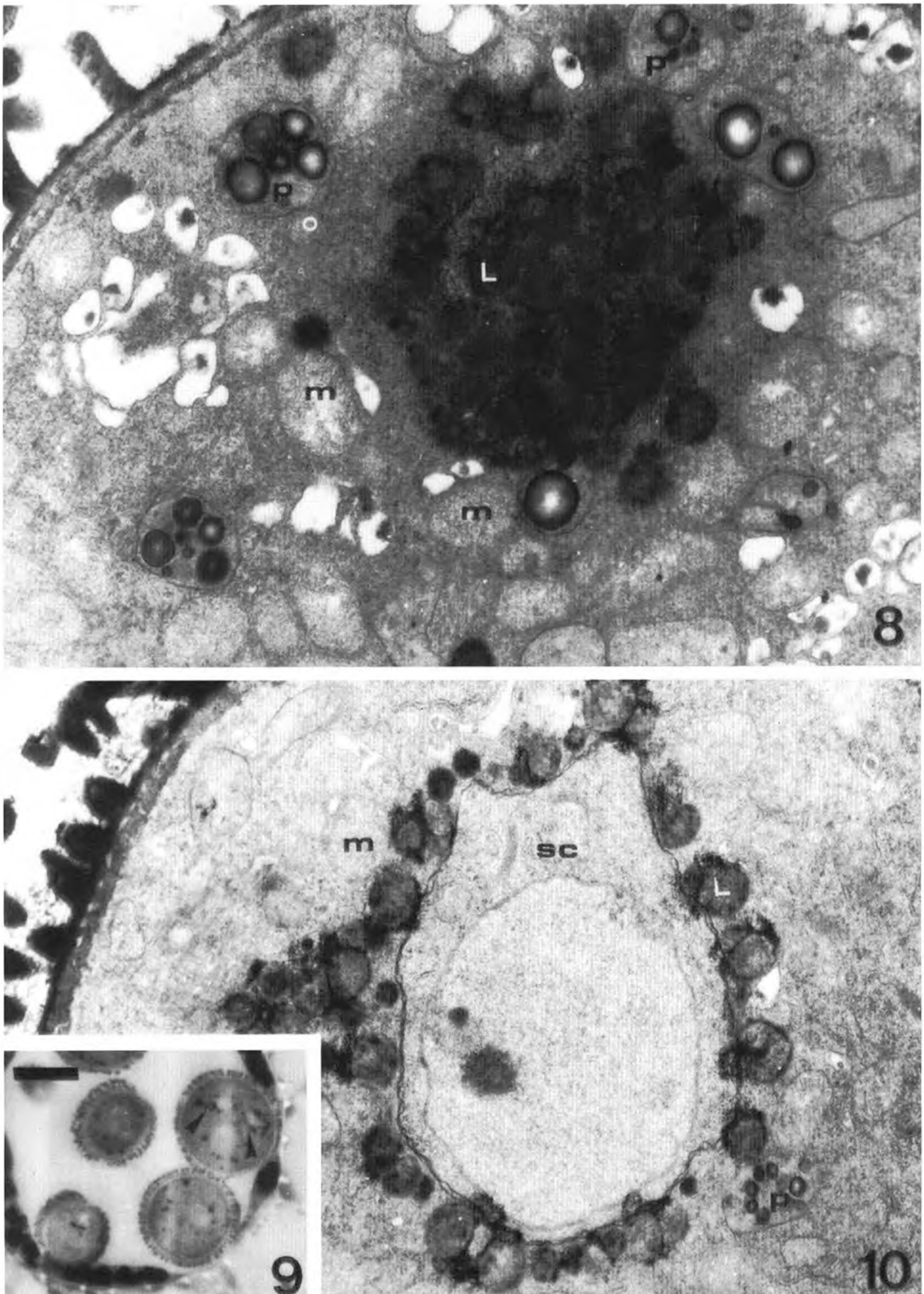
Figs 1-4. Fig. 1. Light micrograph of a cross section of an anther locule at the vacuolate microspore stage, bar – 10 µm.
Fig. 2. Part of the microspore at the vacuolate stage, er – endoplasmic reticulum, m – mitochondrium, p – plastid, v – vacuole x 12. 000.
Fig. 3. Light micrograph of a cross section of an anther locule at the early biccelular pollen stage, arrow – generative cell, tc – tapetal cell, bar – 10 µm.
Fig. 4. Part of bicellular pollen grain with the generative cell attached to the pollen wall. In the vegetative cell, mitochondria are numerous and located near a cell plate. gn – generative nucleus, l – lipid body, m – mitochondrium, p – plastid x 12. 000.



Figs 5-7. Fig. 5. Part of vegetative cell at the bicellular pollen stage., lnu – larger part of nucleolus, m – mitochondrium, s – smaller part of nucleolus, vn – vegetative nucleus x 12. 000.

Fig. 6. Light micrograph of the bicellular pollen grain, Sudan Black B. Arrows – lipid bodies around generative cell, bar – 10 μm.

Fig. 7. Portion of pollen at the same stage in Fig. 6. Sherical lipid bodies deposit in the vegetative cell bordering with the generative cell, d – dictiosome, gn – generative nuclues, l – lipid bodies, p – plastid x 14. 000.



Figs 8-10. Fig. 8. Part of bicellular pollen grain at the same stage in Fig. 7. Lipid bodies (L) covered the generative cell. Plastids (P) with numerous starch grain. m – mitochondrium x 12. 000.

Fig. 9. Light micrograph of a cross section of an anther locule at early tricellular pollen grain stage. Sudan Black B. Arrows – lipid bodies around two sperm cells, bar – 10 µm.

Fig. 10. Part of pollen grain at the same stage in Fig. 9. The sperm cell is surrounded by spherical lipid bodies (L) localized in the vegetative cell, m – mitochondrium, p – plastid sp – sperm cell x 12. 000.

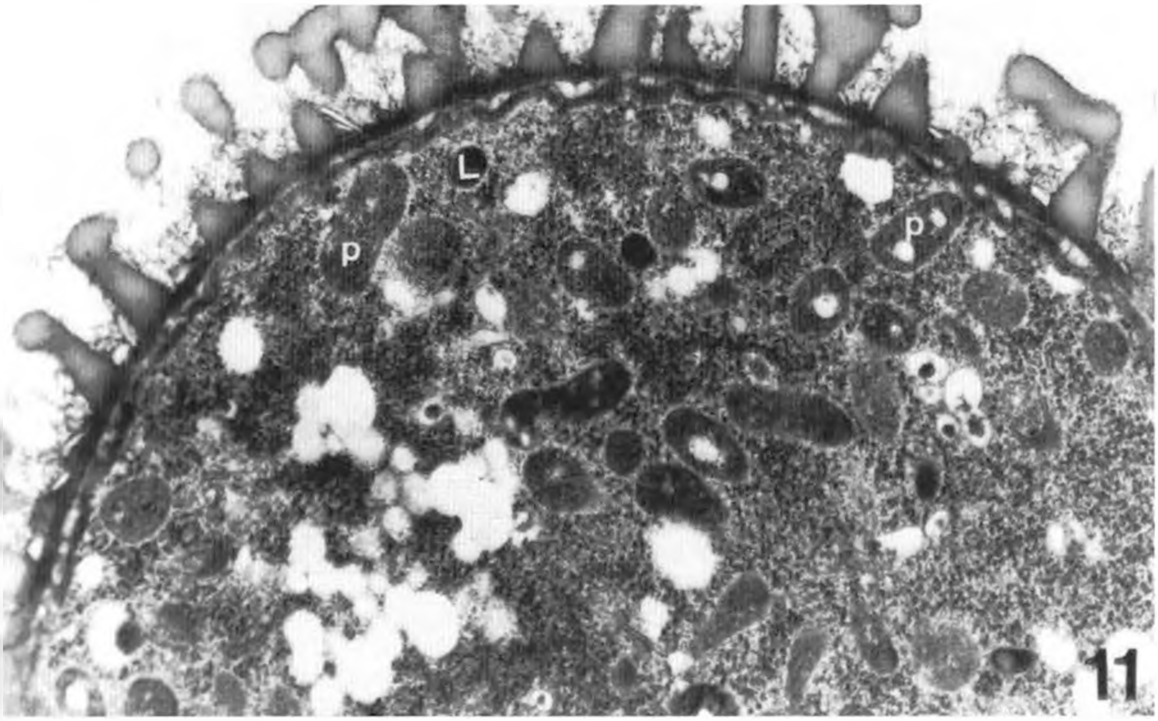


Fig. 11. Part of the vegetative cell in the mature pollen grain. Plastids (p) with dense matrix, devoid of starch grains. The lipid bodies (L) dispersed within whole vegetative cell cytoplasm x 12. 000.

When the generative cell detached from intine and is enclosed by the vegetative cell, the vegetative cell nucleus is of bigger then generative cell nucleus. The vegetative cell nucleus contain two part of nucleolus, differing in size. The bigger part of nucleolus is granular and vacuolate, while the smaller part is fibrilar (Fig. 5). At the LM level, in the pollen grains, small black globules around the generative cell are visible (Fig. 6). Ultrastructurally, the dark globules are the spherical osmophilic bodies distributed in the vegetative cytoplasm bordering with the generative cell (Fig. 7, 8). Each spherical lipid body is surrounded or contacted with cisternae of rough endoplasmic reticulum and covered by free ribosomes. Mitochondria are more numerous than at the previous stage and they tended to gather near lipid bodies. They are generally of spherical or eliptical shape with well developed cristae (Fig. 5, 7, 8). The plastids of a regular outline contain three or more starch grains (Fig. 8).

The vegetative and generative cells are separated by their respective plasma membranes. The electron – translucent space present between two cells is slightly irregular. In the generative cell at this stage, some cristae of endoplasmic reticulum, spherical mitochondria and some dictiosomes are present while plastids are not observed (Fig. 7).

After generative cell division, prior to the final maturation, pollen grain consists of two sperm cells enclosed by vegetative cell. At both the LM and TEM level very densely stained lipid bodies occurred around two sperm cells. As before, spherical lipids are distributed very regularly and form specifical sheath around each of sperm cell (Fig. 9). Similarly to the previous stage, the lipid bodies are covered by free ribosomes but not contacted with cisternae endoplasmic reticulum (Fig. 10).

In mature pollen grain, yust before anthesis, the vegetative cytoplasm contains a dense population of the ribosomes. The plastids, with dense matrix, have irregular shape and are devoided of starch grains. In the cytoplasm bordering with sperm cells there are no longer lipid bodies. They are dispersed within the whole vegetative cell cytoplasm. (Fig. 11).

DISCUSSION

Cytoplasm differentiation of the vegetative and generative cell of pollen grain was described in several angiosperm species (Dickinson 1982; Pacini and Juniper 1984, Schroder 1984; Hause 1986; Bednarska 1988; Polowick and Sawhney 1993). The changes in the generative cell of *Arabidopsis thaliana* are similar to those observed in other angiosperm species. The generative cell of *Arabidopsis thaliana* had all usual organelles with the exception of plastids, although in *Brassica napus* degenerated plastids were present at the early stage of generative cell development (McConchi et al 1987).

In the vegetative cell of *A. thaliana* the two storage reserves, starch and lipid, were present during pollen maturation. Similarly to *Brassica napus*, the plastids of *A. thaliana* accumulate starch only as transient storage materials. The starch was observed at the bicellular and early tricellular pollen stages. The starch was utilized during the maturation process, just before anthesis. A similar change was observed eg. in *Hyacynthus* (Bednarska 1988) and *Lycopersicon* (Pacini and Juniper 1984; Polowick and Sawhney 1993).

The most interesting feature in the differentiation of vegetative cell of *A. thaliana* is the formation of a very regular sheath of the lipid bodies, around the generative cell and next, around the sperm cells. So far, there has been no information about the occurence of these specific lipid bodies around sperm cells. In tricellular pollen grain of *Brassica napus* (Charzynska et al. 1989a/, the lipid bodies did not surround the sperm cells, but with mitochondria, microbodies and endoplasmic reticulum they formed a specific band in the vegetative cell cytoplasm. In bicellular pollen grain, lipid bodies around the generative cell were described only in single species of the family of *Liliaceae* (Gimenez – Martin et al 1969, Dexheim er 1970, Sanger and Jackson 1971), and *Magnolia* x *soulangeuna* (Dinis and Mesquita 1994). In *Hyacynthus orientalis* these structures were named as lysosome – like structures (Bednarska 1988).

In the case of *Arabidopsis thaliana* and in other species, for example *Nicotiana* (Cresti et al. 1985), *Lycopersicon* (Polowick and Sawhney 1993) and *Magnolia* (Dinis and Mesquita 1994), lipid bodies are surrounded by rough ER. The intimate association of these organelles suggests the possible role of rough ER in lipid accumulation and mobilization (Jensen et al. 1974; Pacini and Juniper 1984; Cresti et al. 1985; Polowick and Sawhney 1993).

Lipid bodies associated with cisternae endoplasmic reticulum and mitochondia are specific for *Arabidopsis thaliana*. In seeds before germination and in algal cells most lipid granules are surrounded by ER and they are always in contact with mitochondria (Trelease et al. 1971; Vigel 1970, Ueda et al. 1985). Ueda et al. (1985) speculated that ER might transfer its contents of decomposed lipids to the dictiosome. Oil bodies in cell of mature seeds are very stable and do not aggregate or coalcasce, because their surface is shieldedby layer of uniqe protein termed oleosine (Huang 1996). The oil bodies in pollen grain are an important energy reserve. They are morphologically similar to those which are present in seed. Their surface seems to be covered by oleosins, which prevent the oil bodies from coalescing (Huang 1996).

LITERATURE CITED

BEDNARSKA E. 1988. Ultrastructural transformation in the cytoplasm of differentiatie *Hyacinthus orientalis* L. pollen cells. Acta Soc. Bot. Pol. . 97: 235-245

BRONNER R. 1975. Simultaneous demonstration of lipid and starch in plant tissue. Stain Technol. Baltimore, 50/1/: 1-4.

CHARZYNSKA M. 1984. Various pathways of sister cell differentiation vegetative and generative in the pollen grain *Tradescantia*. Post. Biol. Kom. 2: 589-592.

CHARZYNSKA M., MURGIA M., CRESTI M. 1989a. Ultrastructure of the vegetative cell of *Brassica napus* pollen with particular reference to microbodies. Protoplasma 152: 22- 28.

CHARZYNSKA M., MURGIA M., MILANESI C., CRESTI M. 1989 b. Origin of sperm cell association in the "male germ unit" of *Brassica* pollen. Protoplasma 149: 1-4.

CRESTI M., CIAMPOLINI F., MULCAHY D. L., MULCAHY G. 1985. Ultrastructure of *Nicotiana alata* pollen, its germination and early tube formation. Amer. J. Bot. 72: 719-727

CRESTI M., LANCELLE S. A., HEPLER P. K. 1988. Structure of the generative cell wall complex after freeze substitution in the pollen tubes of *Nicotiana* and *Impatiens*. J. Cell Sci. 88: 373-378.

CRESTI M., MILANESI C., SALVATICI P., VAN AELST A. C. 1990. Ultrastructural observation of *Papaver rhoeas* mature pollen grain. Botanica Acta 103: 349-354.

CRESTI M., BLACKMORE S., VAN WENT JL. 1992. Atlas of sexual reproduction in flowering plants. Springer Verlag, Berlin.

DAWSON EILSONZ. A., AARTS M. G. M., BRAITHWAITE A. F., BRIARTY L. G., M. B. J. 1993. Microspore and pollen development in the six male sterile mutant of *Arabidopsis thaliana*. Can. J. Bot. 71: 629-638.

DEXHEIMER L. 1970. Recherches cytophysiologiques sur les grains de pollen. Rev. Cytol. Biol. Veg. 33: 169-234

DICKINSON H. G. 1982. The development of pollen. Rev. Cytol. Biol. Veget. Bot. 5: 5-19.

DINIS A. M., MESQUITA J. F. 1994. Ultrastructural and Cytochemical Evidence for the Presence of Peroxisomes in the Generative Cell of *Magnolia x soulangeana* Pollen Grain. Ann. Bot. 73: 83-90.

GIMENEZ-MARTIN G., RISUENO N. C., LOPEZ-SAEZ J. F. 1969. Generative cell envelope in pollen grain as a secretion system a postulate. Protoplasma 67: 223-235.

HAUSE G. 1986. Organelle distribution during development of pollen grains. Grana 10: 21-30

HESS M. W. 1993. Membrane coating on the generative cell surface of freeze – substituted monocotyledones pollen. Protoplasma 176: 84-87.

HUANG A. H. C. 1996. Oleosin and Oil Bodies in Seed and Other Organs. Plant Physiol. 110: 1055-1061.

JENSEN W. A, . ASHTON M., HECKARD L. R. 1974. Ultrastructural studies of the pollen of subtribe, *Castileine* family *Scrophulariaceae*. Bot. Gaz. 135: 110-118.

McCONCHIE C. A., RUSSEL S. D., DUMAS C. TOUHY M., KNOX R. B. 1987. Quantative cytology of the sperm cells of *Brassica campestris* and *Brassica oleracea*. Planta 170: 446-452.

MISRA C. 1962. Contribution to the embriology of *Arabidopsis thalianum* (Gay and Monn.) Agra Univ. J. Res. 11: 191-199.

MURGIA M., DETCHEPARE S., VAN WENT J. L., CRESTI M. 1991. *Brassica napus* pollen development during generative cell and sperm cell formation. Sex. Plant Reprod. 4: 176-181.

NOGUCHI E. H., UEDA K . 1990. Structure of pollen grain of *Tradescantia*. Struct. Funct. 15: 379-384.

OWEN H. A., MAKAROFF C. A. 1995. Ultrastructure of microsporogenesis and microgametogenesis *Arabidopsis thaliana* (L.) Heynh. ecotype Wasilewskaja (*Brassicaceae*). Protoplasma 185: 7-21.

PACINI E., JUNIPER B. 1984. The ultrastructure of pollen grain development in *Lycopersicum peruvianum*. Caryologia 37: 21-50.

POLOWICK P. L., SAWHNEY V. K. 1993. An ultrastrucrural study of pollen development in tomato (*Lycopersicon esculentum*/, II. Pollen maturation. Can. J. Bot. 71: 1048-1055.

POSTEK M., TUCKER S. C. 1976. A new short chemical dehydration method for light microscopy preparation of plant material. Can. J. Bot. 54: 872-889

REGAN S. M., MOFFAT B. A. 1990. Cytochemical analysis of pollen development in wild – type *Arabidopsis* and male – sterile mutant. Plant Cell. 2: 877-889.

SANGER J. M. JACKSON W. T. 1971. Fine structure study of pollen development in *Haemanthus katharineae* Baker. III. Changes in organelles during development of vegetative cell. J. Cell Sci. 8: 317-329.

SCHRODER M. B. 1984. Ultrastructural studies on plastid of generative and vegetative cells on the family *Liliaceae*. Biol. Zbl. 103: 547-555.

SOUTHWORTH D. D. 1992. Freeze fracture of male reproductive cells. Int. Rev. Cytol. 140: 187-204.

TRELEASE R. N., BECKER W. M., GRUBER P. J., NEWCOMB E. H. 1971. Microbodies (glyoxysomes and peroxysomes) in cucumber cotyledons. Correlative biochemical and ultrastructural study in light and dark – grown seedling. Plant Physiol. 48: 461-475.

UEDA K., HATANO K., NOGUCHI T. 1985. The formation and cosumption of lipid droplets in the zygote and germinated cells of *Closterium ehrenbergii*. Bot. Mag. Tokyo 98: 263-269.

VAN AELST A. C., VAN WENT J, L. 1991. The ultrastructure of mature *Papaver dubium* L. pollen grains. Acta Bot. Neerl. 40: 319-328.

VIGEL E. L. 1970. Cytochemical and developmental changes in microbodies (glycoxysomes) and related of castor bean endosperm. J. Cell. Biol. 46: 435-453

ULTRASTRUKTURALNE BADANIA DOJRZEWAJĄCYCH ZIAREN PYŁKU *ARABIDOPSIS THALIANA* (L) HEYNH. (*BRASSICACEAE*)

STRESZCZENIE

W niniejszej pracy przedstawiono ultrastrukturalną dokumentację rozwoju ziaren pyłku *Arabidopsis thaliana* od późnego stadium mikrospory do dojrzałego pyłku. Po oderwaniu od intyny, sferycznego kształtu komórka generatywna, otoczona komórka wegetatywną zawierała wszystkie organelle z wyjątkiem plastydów. W komórce wegetatywnej obserwowano zwiekszenie liczby mitochondriów oraz zmiany w akumulacji skrobi i ciał lipidowych. Plastydy zawierały skrobię w dwukomórkowym pyłku oraz we wczesnym stadium trójkomórkowym. W czasie dojrzewania ziarna pyłku skrobia ulegała degradacji. Ciała lipidowe w komórce wegetatywnej tworzyły bardzo regularne otoczki wokół komórki generatywnej, a następnie wokół komórek plemnikowych. Tuż przed wypyleniem, w dojrzałych ziarnach pyłku ciała lipidowe były rozproszone w cytoplazmie komórki wegetatywnej.

SŁOWA KLUCZOWE: *Arabidopsis thaliana* komórka wegetatywna, komórka genetyczna, ciała lipidowe, skrobia, pyłek.