Ultrastructural transformations in the cytoplasm of differentiating *Hyacinthus orientalis* L. pollen cells

ELŻBIETA BEDNARSKA

Department of Plant Cytology and Genetics, Institute of Biology, Copernicus University, Gagarina
9, 87-100 Toruń, Poland

(Received: October 17, 1987. Accepted: November 27, 1987)

Abstract

The sequence of ultrastructural changes in the cytoplasm during the successive stages of pollen grain development in *Hyacinthus orientalis* pollen cells was studied. The cytoplasmic transformations of the generative cell included the elimination of plastids, increase in the number of mitochondria, assumption of a spindle shape with the aid of microtubules and the characteristic development of the vacuole system with the formation of so-called colored bodies. The cytoplasmic transformations of the generative cell encompassed changes in the plastids, which began to accumulate starch soon after the cell was formed, then released it shortly before anthesis, an increase in the number of mitochondria and an increase in the number of highly active dictyosomes just before anthesis. Changes in the structure of the border region between the differentiating pollen cells were associated mainly with the periodical appearance of a callose wall and the presence of lysosome-like bodies in the cytoplasm of the vegetative cell surrounding the generative cell. They arose soon after the disappearance of the callose wall and disappeared shortly before anthesis.

Key words: *Hyacinthus orientalis*, ultrastructural differentiation, pollen cell cytoplasm

INTRODUCTION

The development of pollen grains in *Angiospermae* presents a model situation for the study of cell differentiation. This derives from the fact that the maturation of pollen is a long series of metabolic and structural changes of two pollen cells which takes place in an almost closed system, such as is created by the thick spermoderm layer.

The ultrastructural and metabolic differentiation of pollen cells have been

the subject of study by several authors (among others Sanger and Jackson 1971a, b, c, Mascarenhas 1975 (a review), Dickinson 1982, Pacini and Juniper 1984). However, in spite of numerous studies, the data on the differentiation of the vegetative and generative cells is still often fragmentary. The studies either deal with only the metabolic aspects of the pollen grain (see Mascarenhas 1975, Tupy et al. 1983), or, in the case of ultrastructural studies, only with selected developmental stages or organelles, e.g. plastids (Schröder 1984, Hause 1986) or microtubules (Cresti et al. 1984, Van Lammeren et al. 1985). In addition there are no studies encompassing simultaneously the metabolism and ultrastructure of pollen cells in comparable stages of pollen development of the same species.

To date, studies on the differentiation of *Hyacinthus orientalis* pollen grain have included their morphology, some metabolical aspects (Bednarska 1981, 1984) and the ultrastructure of the nuclei of sister pollen cells (Bednarska and Górska-Brylass 1987). The results of these studies showed that both pollen cells of *Hyacinthus orientalis* undergo full interphase, albeit S phase does not occur simultaneously in them (Bednarska 1981). Differences in the level of RNA and protein synthesis in the generative and vegetative cells were also found (Bednarska 1984). The period of histone synthesis as well as the type of histones synthesized were determined in both cell types (Bednarska 1981). The ultrastructural changes in the nuclei of sister pollen cells, as analysed in the context of the course of their interphase and levels of RNA and protein synthesis, confirmed the metabolic differentiation of the generative and vegetative cells (Bednarska and Górska-Brylass 1987).

In this paper, which is a continuation of the studies on the differentiation of *Hyacinthus orientalis* pollen grain cells, the ultrastructural changes in the cytoplasm of the generative and vegetative cells during the successive stages of development are presented.

MATERIAL AND METHODS

The study was conducted on the pollen grains of *Hyacinthus orientalis* L., the diploid variety, Pink Pearl. The ultrastructure of the cytoplasm of the pollen cells was studied during seven morphologically determined stages, presented schematically on Fig. 1. A previously described, standard method (Bednarska and Górska-Brylass 1987) was used for the ultrastructural study. The number of mitochondria was determined by counting them on the electronmicrographs in randomly selected squares of $1 \mu\text{m}^2$ and $100 \mu\text{m}^2$. The percent of the area of the cytoplasm occupied by these organelles was calculated by a weight method:

$$P_{mit} = \frac{B}{A} 100,$$

where: A — weight of the cytoplasm, B — weight of the mitochondria, P — percent of area.

The occurrence of callose was determined by a fluorescence method according to Eschrich and Currier (1964). Acid phosphatase was identified by the Gomori method (1952).

Fig. 1. The studied morphological developmental stages of *Hyacinthus orientalis* L. pollen grain. Stage I — lens-shaped generative cell surrounded by a callose wall, Stage II — lens-shaped generative cell devoid of callose wall, Stage III — balloon-shaped generative cell, Stage IV — spherical generative cell, Stage V — lemon-shaped generative cell, Stage VI — spindle-shaped generative cell surrounded by lysosome-like structures, Stage VII — spindle-shaped generative cell without lysosome-like bodies

RESULTS

The cytoplasm of pollen cells was already differentiated the moment they were formed. During maturation of the pollen grain, further deepening of the differentiation of the cytoplasm of the sister pollen cells occurred, involving the different directions of development of elements of the vacuole system, plastids and mitochondria, as well as the border region between the sister cells.

THE VACUOLE SYSTEM

During the initial stage of development, the generative cell was relatively poor in elements of the vacuole system. Single vacuoles were found (Fig. 14), along with short cisterns of rough endoplasmic reticulum, lysosome-like bodies and dictyosomes surrounded by vesicles containing electron-dense inclusions (Fig. 3). A distinct increase in the number of elements of the vacuole system was seen in the spherical generative cell. In addition to dictyosomes, long ER channels (Fig. 4a), rolls of membranes (Fig. 4b), as well as vacuoles surrounding double-membraned organelles showing symptoms of degeneration (Fig. 9) were found. During the elongation of the generative cell, a sharp rise in the amount of smooth ER was seen. Short, distended channels and numerous vacuoles were also seen (Fig. 5). In the spindle-shaped generative cell, almost the entire cytoplasm was filled with vacuoles with diameters of 0.2-1.0 μm (Fig. 6), which correspond to the so-called colored bodies seen in the light microscope (named by Kostriukova 1939). The occurrence of the colored bodies in the cytoplasm of a mature generative cell is characteristic for *Hyacinthus orientalis*.

During the development of the vegetative cell, the degree of vacuolization decreased and the amount of long ER cisterns was reduced (compare Figs. 2 and 12). Just after the formation of the vegetative cell, large vacuoles containing electron-dense bodies or electron-dense granular material, were found in the cytoplasm (Fig. 2). During the further developmental stages (stages III-IV), few small vacuoles (about 1 μm in diameter) occurred in the cytoplasm. Shortly before anthesis, small vacuoles (0.3-0.8 μm diameter) were present in the vegetative cell. The sometimes contained spherical or ring-shaped electron-dense structures and often occurred in the vicinity of highly osmophilic bodies (Fig. 12) and rolls of membranes.

The number and activity of dictyosomes in the cytoplasm of the vegetative cell was different during the different stages of development of this cell. Relatively numerous, active dictyosomes occurred in the initial stages of development of the vegetative cell. They were surrounded by vesicles containing electron-dense inclusions (Fig. 2). During further stages of development, the number of dictyosomes on the cross sections of the cytoplasm of the vegetative cell decreased. A very noticeable increase in the number of very active dictyosomes was noted just before anthesis (Fig. 12). Golgi vesicles, which occurred very numerous in the entire cytoplasm of the vegetative cell at this stage of development, were either entirely or peripherally filled with an electron-dense content.

PLASTIDS

The presence of plastids was noted in the generative cell only during the first four stages of development, that is, until and including the stage of the spherical generative cell (Figs. 7, 8). Initially (stages I-II), they occurred as proplastids and sporadically as small amyloplasts (Fig. 7). No types of plastids at all were found in mature generative cells. In the vegetative cell, plastids were present throughout the entire period of pollen grain development. They underwent three basic stages of changes. Stage I — just after the division of the microspore — was characterized by accumulation of starch. In addition to numerous proplastids with an electron-transparent matrix, amyloplasts also occurred, often surrounded by ER channels (Fig. 10). Soon, all of the plastids were in the form of large (2-3 μm diameter) amyloplasts. The second stage of plastid change (developmental stages IV-V) was characterized by the process of partial lysis of starch. Plastids containing various numbers of starch grains as well as having no grains were observed on the electronmicrographs (Fig. 11). The third stage of changes in plastids which occurred just before anthesis was characterized by very intense, complete disintegration of starch. This was accompanied by a decrease in plastid size and concentration of their matrix which became electron-dense. During anthesis, all of the plastids were in the form of proplastids (Fig. 12).

Figs. 2 and 3. The cytoplasm of pollen cells shortly after they are formed. Fig. 2 — Present in the vegetative cell are large vacuoles (v), long ER channels (er), dictyosomes (d) surrounded by vesicles containing electron-dense inclusions (inset 35 000x) and mitochondria (m). $\times 25\ 000$. Fig. 3 — present in the generative cell are short ER channels, dictyosomes (d) surrounded by vesicles of a similar morphology as in the vegetative cell (arrow), lysosomes (l) and spherical and elongated mitochondria (m). $\times 39\ 750$

Fig. 4. The cytoplasm of a spherical generative cell rich in elements of the vacuole system. $\times 25\ 000$.
 Fig. 4a — long ER channels, dictyosomes and mitochondria are visible. Fig. 4b — coils of membranes and irregular vesicles are present in the cytoplasm (n — nucleus, d — dictyosome, l — lysosome, m — mitochondrium, mb — membranes)

Plate III

Fig. 5. The cytoplasm of a lemon-shaped generative cell filled with parallel long and short distended ER channels, $\times 46\,000$. Fig. 6. Colored bodies are present in the cytoplasm of a mature generative cell. $\times 11\,250$ (gc — generative cell, vc — vegetative cell, n — nucleus, m — mitochondrium)

Figs. 7 and 8. The occurrence of plastids in the generative cell. Fig. 7 — Callose stage — small amyloplasts (a) are visible in the cytoplasm, $\times 11\,250$. Fig. 8 — proplastid (p) in a spherical generative cell, $\times 25\,000$ (gc — generative cell, vc — vegetative cell, m — mitochondrion). Fig. 9. A vacuole surrounding a double membraned organelle showing symptoms of degeneration is visible in a spherical generative cell. $\times 32\,000$

Fig. 10. The cytoplasm of a vegetative cell shortly after its formation. Visible are proplastids (p) and amyloplasts (a) surrounded by ER channels (arrow). Mitochondria (m) are in spherical, elongated or basket form, $\times 25\ 000$. Fig. 11. The cytoplasm of a vegetative cell during the elongation of the vegetative cell. Visible are plastids lacking starch (p), amyloplasts (a) and numerous spherical mitochondria (m). $\times 11\ 250$

Fig. 12. The cytoplasm of a vegetative cell during anthesis. All plastids (p) containing electron-dense material are devoid of starch. Visible are spherical mitochondria (m) and numerous dictyosomes (d) and Golgi vesicles (arrow) as well as small vacuoles in the vicinity of highly osmophilic bodies. $\times 25\ 000$. Fig. 13. A spindle-shaped generative cell during anthesis. Microtubules (mt) arranged along the long axis of the cell are visible in the cytoplasm. $\times 25\ 000$ (gc — generative cell, n — nucleus)

Fig. 14a. Electron-dense lysosome-like bodies (arrow) are present in the cytoplasm of a vegetative cell surrounding a generative cell. $\times 25\,000$. Fig. 14b. The occurrence of acid phosphatase in the structures surrounding the generative cell, $\times 1200$. Fig. 15. Shortly before anthesis the lysosome-like bodies surrounding the generative cell become electron-transparent. $\times 13\,700$. Fig. 16. Lysosome-like bodies are lacking around the mature generative cell. A mitochondrion and cross-section of microtubules are visible in the cytoplasm of the generative cell. $\times 25\,000$ (gc — generative cell, N — nucleus, m — mitochondrion, v — vacuole, mt — microtubules)

MITOCHONDRIA

In both pollen cell types, mitochondria were present during the entire period of differentiation.

In the generative cell from the I to II stage of development, elongated and spherical mitochondria were seen, the latter being probably cross-sections of elongated ones (Fig. 3). In the spindle-shaped generative cell, only spherical mitochondria were found (Fig. 6). The change from elongated to spherical shape was accompanied by an increase in the number of mitochondria per cell. The number of mitochondria per unit of cytoplasm area rose on average from 0.1 per $1 \mu\text{m}^2$ at the stage of the parietally situated generative cell to about 0.2 per $1 \mu\text{m}^2$ at the spherical stage (Table 1). The surface of the cross section of the cytoplasm occupied by these organelles rose at this time from about 2% to about 3.2%. During the period of elongation of the generative cell the number of mitochondria per unit area of the cytoplasm decreased to about 0.15 per $1 \mu\text{m}^2$. The area of the cytoplasm occupied by these organelles also decreased to about 3%. However, taking into account the rise in the volume of the cytoplasm at this time, resulting from the decrease in the volume of the nucleus (Bednarska and Górska-Bryl 1987), the observed process should be interpreted as rather the dispersion of mitochondria than their elimination.

Just after the division of the microspore in the vegetative cell, mitochondria of elongated, spherical and basket shapes were observed (Fig. 10). Only spherical mitochondria (Fig. 12) were found in mature pollen grains. Similarly as in the generative cell, the passing from the elongated to spherical shapes was accompanied by a rise in the number of mitochondria (Table 1). The number of these organelles per unit of area rose from about 0.3 per $1 \mu\text{m}^2$ just after the division of the microspore, to about 0.6 per $1 \mu\text{m}^2$ during stage IV. The area of cytoplasm occupied at this time by these organelles rose from about 13% to about 22%. Taking into account the increase in cytoplasm volume at this time

Table 1

Mitochondrion size, number and the percentage of the cross-section area of the cytoplasm of pollen cells taken up by mitochondria in successive stages of development of pollen grain

Stage	Generative cells			Vegetative cells		
	size, μm^{-2}	number μm^{-2}	% cytoplasmic area	size, μm	number μm^{-2}	% cytoplasmic area
I	0.56-1.42	0.09	1.83	0.67-2.06	0.31	12.7
II	0.52-1.31	0.12	2.36	0.71-1.94	0.32	13.3
III	0.45-1.52	0.17	2.48	0.63-0.97	0.43	17.8
IV	0.52-1.90	0.22	3.26	0.76	0.61	22.6
V	0.50	0.19	3.29	0.71	0.57	23.8
VI	0.48	0.16	3.04	0.65	0.59	22.4
VII	0.45	0.15	2.98	0.63	0.58	21.7

(Bednarska 1984), the increase in the number of mitochondria should be taken as significant. The number of mitochondria did not undergo further significant changes during development.

MICROTUBULES

During the stages preceding the elongation of the generative cell, only few, single microtubules were seen in its cytoplasm. Their number increased when the process of elongation began. Bundles of microtubules arranged parallel to the long axis of the cells were found in the spindle-shaped generative cell (Fig. 13).

Ordered systems of microtubules were never seen in the vegetative cell.

BORDER REGION

As the result of differentiating mitosis of the microspore and of cytokinesis, the pollen cells were separated by a callose wall. Single-membraned structures called lomasomes appeared in the space between the plasma membranes of the two cells. Lomasomes occurred until the stage of the spherical generative cell, inclusively (Fig. 9). During the following stages of development, until anthesis, the space between the pollen cells was filled with a granular and fibrillar material.

After the disintegration of the callose cell, electron-dense, spherical structures (Fig. 14a) giving a positive acid phosphatase reaction (Fig. 14b) appeared in the cytoplasm of the vegetative cell adjoining the generative cell. These lysosome-like structures surrounded the generative cell during the entire period of its differentiation. Just preceding anthesis, they became electron-transparent (Fig. 15) and completely disappeared in the mature pollen grain (Fig. 16).

DISCUSSION

Similarly as in other angiospermous species (Sanger and Jackson 1971a), the cytoplasm of *Hyacinthus orientalis* pollen cells is already differentiated at the moment the generative and vegetative cells are formed. This differentiation concerns mostly the number of organelles. In the newly formed vegetative cell, the number of mitochondria, plastids and elements of the vacuole system is decidedly higher than in the generative cell. During maturation of the pollen grain, the ultrastructural differences between these cells become more pronounced, leading to functional differences between them.

The cytoplasmic transformations of the generative cell encompass two main processes: the preparation of the cytoplasmic genetic material for the future male gametes and the adaptation of the cell's shape for transportation in the

cytoplasm of the pollen tube. In *Hyacinthus orientalis* these processes are accompanied by the characteristic development of the vacuole system with the formation of colored bodies. Both mitochondria and plastids are present in the generative cell in the initial period of its development, whereas in the mature cell, only mitochondria were found to be present. The number of mitochondria increases during maturation of the generative cell. This is indicated by the increase in the number of these organelles per unit of cross section area of the cytoplasm and the passing of the mitochondria from elongated to spherical in shape. It may be that this process is connected with the transferring of these organelles through the paternal line.

The elimination of plastids during the maturation of the generative cell is a frequent process and has been described in many species of plants as the so-called *Solanum* type (see Hegemann 1983). In *Hyacinthus orientalis* the elimination of plastids probably occurs through intracellular digestion at the spherical stage of the generative cell (Fig. 9).

The adaptation of the shape of the generative cell for transportation in the cytoplasm stream of the pollen tube calls for the assumption of a spindle-like shape. This process is carried out with the aid of microtubules, similarly as has been described in *Haemanthus* (Sanger and Jackson 1971b) and *Petunia*, *Malus* and *Olea* (Cresti et al. 1984).

A characteristic process in the differentiation of the cytoplasm of *Hyacinthus orientalis* generative cells is the formation of specialized forms of vacuoles called colored bodies. Their genesis is connected with the development and then fragmentation and distention of cisternae of smooth ER during the period of elongation of the generative cell (Fig. 5). Colored bodies are not a common structural element of the cytoplasm of generative cells. They have been described on the light microscope level among others in the generative cell of *Lilium mártagon* (Nawaszin 1910 cited by Kostriukova 1939), *Haemanthus katharinae* (Wójcicki 1926) and *Iris* sp. (Krupko 1959). A survey of over 60 species has shown that they are characteristic of the generative cells in the families *Amaryllidaceae* and *Iridaceae* and for some species from the family *Liliaceae* (Górska-Brylasek and Bednarska — unpublished data). In spite of the fact that several of the species having colored bodies in their generative cells, e.g. *Lilium* (Bopp-Hassenkamp 1960), *Narcissus* (Dexheimer 1970) or *Haemanthus* (Sanger and Jackson 1971b) have been the object of ultrastructural studies, these bodies have not yet been analyzed in the electron microscope. This is probably caused by their high sensitivity to osmotic factors during fixing, due to which they are not always visible on the electron micrographs. The function of the colored bodies is unknown.

The cytoplasmic transformations of the vegetative cell aimed at preparing it in terms of substrates and energy for the germination and growth of the pollen tube, are reflected in the direction of the changes in its plastids, mitochondria and dictyosomes.

The plastids go through 3 basic types of changes: in the initial phase the process of amylogenesis takes place, during which similarly as in *Lycopersicon peruvianum* (Pacini and Juniper 1984), the amyloplasts are surrounded by ER. After the period of partial starch lysis, just before anthesis, abrupt complete lysis of starch takes place and all of the plastids become proplastids. The development of plastids seen in the vegetative cell of *Hyacinthus orientalis* is therefore similar to that which occurs in many plant species which accumulate starch in their pollen grain as a storage material.

The direction of changes in mitochondria in the vegetative cell is connected mainly with the increase in the number of these organelles. This is expressed mainly by the increase in the number of mitochondria per unit of cytoplasmic area and the transition from elongated to spherical form. The increase in the number of mitochondria during maturation of the vegetative cell seems to be a universal process. It has been described in numerous species, among others in *Linum usitatissimum* (Vazart 1969), *Tradescantia bracteata* (Mephram and Lane 1970) and *Haemanthus katherinae* (Sanger and Jackson 1971c).

Two peaks of activity of dictyosomes were observed in the vegetative cell – immediately after the division of the microspore and shortly before anthesis. During the initial stage of development when the dictyosomes produce vesicles containing electron-dense inclusions, the high activity of these structures is probably connected with the increase of the surface of the grain, which at that time very intensively increases its volume (Bednarska 1984). Vesicles with a similar morphology are also produced by the dictyosomes of the generative cell when it adheres to the wall of the pollen grain. Similar Golgi vesicles have been described by Willemse and Reznickova (1980) in the microspore of *Lilium*, suggesting that they participate in the building of the sporoderm. A significant increase in the number of very active dictyosomes producing a different type of vesicle than in the early period (compare Figs. 3 and 12) occurs shortly before anthesis. This increase has been observed in the vegetative cell of all of the plant species studied hitherto (among others Sanger and Jackson 1971c, van Went 1974, Russel and Cass 1981). The common opinion is that the production and accumulation of Golgi vesicles during the final period of maturation of the pollen grain is a way of storing compounds and membranes for the building of the pollen tube wall.

The changes in the border region between the pollen cells of *Hyacinthus orientalis* mirror the process of the regulated interaction between the generative and vegetative cells. The temporary presence of the callose wall between the cells is a universal phenomenon and is an important factor leading to their differentiation (Górska-Bryllass 1970). Inhibition of the formation of the callose wall leads to the development of two undifferentiated pollen nuclei (Charzyńska and Pannenko 1976). The separation of the two cells with callose must surely create a barrier which highly limits exchange between these

two cells early in their development. This takes place during phase G_1 of both cells, after which the interphases of the cells lose their synchrony (Bednarska 1981). After the callose wall disappears and the pollen cells are separated only by a pair of membranes, an additional structure composed of lysosome-like bodies appears on the border between the generative and vegetative cells. These bodies do not disappear until anthesis, that is, until the cells have completed their differentiation. The occurrence of similar structures around the generative cell has only been described in a few species of plants from the *Liliaceae* family (Gimenez-Martin et al. 1969, Dexheimer 1970, Sanger and Jackson 1971c). The function of these structures in the process of differentiation of the pollen cells is unknown.

The presented cytoplasmic transformations of the generative and vegetative cells reflect the successive stages leading to their functional distinctness. Nevertheless, the changes occurring in the cytoplasm of the pollen cells, which is the medium in which the sister pollen nuclei differentiate, are only one of the elements of this process in the pollen grain of *Hyacinthus orientalis*. This process also includes the deepening of metabolic differentiation of the pollen cells (Bednarska 1981, 1984), which is reflected in the diverse patterns of ultrastructural changes in the nuclei (Bednarska and Górska-Brylarska 1987) and cytoplasm of these cells. Following both the ultrastructural and metabolic changes in the pollen cells at the same time throughout the entire period of their differentiation makes it possible to reach a fuller interpretation of the phenomena occurring in the maturing pollen grain.

Acknowledgments

The author wishes to thank doc. dr hab. Alicja Górska-Brylarska for guidance in the preparation of this paper.

This study was conducted under program MR II/2.3. This program is coordinated by the Botanical Garden of the Polish Academy of Science.

REFERENCES

- Bednarska E., 1981. Autoradiographic studies of DNA and histone synthesis in successive differentiation stages of pollen grain in *Hyacinthus orientalis*. Acta Soc. Bot. Pol. 50: 367-380.
- Bednarska E., 1984. Ultrastructural and metabolic transformations of differentiating *Hyacinthus orientalis* pollen grain cells. I. RNA and protein synthesis. Acta Soc. Bot. Pol. 53: 145-158.
- Bednarska E., Górska-Brylarska A., 1987. Ultrastructural transformations of nuclei in differentiating *Hyacinthus orientalis* L. pollen grain cells. Acta Soc. Bot. Pol. 4: 667-685.
- Bopp-Hassenkamp G. 1960. Elektronenmikroskopische Untersuchungen an Pollenschläuchen zweier Liliaceen. Z. Naturforsch. 15b: 91-94.
- Charzyńska M., Pannenko I., 1976. Inhibition of cytokinesis in the microspores of *Tradescantia bracteata* by caffeine. Acta Soc. Bot. Pol. 45: 469-470.
- Cresti M., Ciampollini F., Kapil R. N., 1984. Generative cells of some angiosperms with particular emphasis on their microtubules. J. Submicroscop. Cytol. 16: 317-326.

- Dexheimer I., 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.
- Eschrich W., Currier H. B., 1964. Identification of callose by its diachrome and fluorochrome reaction. Stain Technol. 39: 303-307.
- Gimenez-Martin G., Risueno N. C., Lopez-Saez J. F., 1969. Generative cell envelope in pollen grains as a secretion system a postulate. Protoplasma 67: 223-235.
- Gomori G., 1952. Microscopic histochemistry principles and practice. Univ. Chicago Press, Chicago.
- Górska-Brylasek A., 1970. The "callose stage" of the generative cell in pollen grains. Grana 10: 21-30.
- Hause G., 1986. Organelle distribution during pollen development of *Pisum sativum* L. Biol. Zentralbl. 105: 283-288.
- Hegemann R., 1983. The formation of generative and sperm cells without plastids in angiosperms and the underlying mechanisms. Embryogen. Ovul Plants (Erdelska, O. et al. eds.) Račkova Dolina. pp. 97-99.
- Kostriukova K. J., 1939. Observations *in vivo* on the formation of the male sex cells *Lilium martagon*. L. Compt. Rend. Acad. Sci. USSR 22: 444.
- Krupko S., 1959. Występowanie oksyflawonów i flawonów w generatywnej komórce irysa. Acta Soc. Bot. Pol. 28: 169-234.
- Mascarenhas J. P., 1975. The biochemistry of angiosperm pollen development. Bot. Rev. 41: 259-314.
- Mephum R. H., Lane G. R., 1970. Observation on the fine structure of developing microspores of *Tradescantia bracteata*. Protoplasma 70: 1-20.
- Pacini E., Juniper B., 1984. The ultrastructure of pollen grain development in *Lycopersicon peruvianum*. Caryologia 37: 21-50.
- Russell S. D., Cass P., 1981. Ultrastructure of the sperm of *Plumbago zeylanica*. I. Cytology and association with the vegetative nucleus. Protoplasma 107: 85-107.
- Sanger J. M., Jackson W. T., 1971a. Fine structure study of pollen development in *Haemanthus katharinae* Baker. I. Formation of vegetative and generative cells. J. Cell Sci. 8: 289-301.
- Sanger J. M., Jackson W. T., 1971b. Fine structure study of pollen development in *Haemanthus katharinae* Baker. II. Microtubules and elongation of the generative cells. J. Cell Sci. 8: 303-315.
- Sanger J. M., Jackson W. T., 1971c. Fine structure study of pollen development in *Haemanthus katharinae* Baker. III. Changes in organelles during development of vegetative cell. J. Cell Sci. 8: 317-329.
- Schröder M. B., 1984. Ultrastructural studies on plastids of generative and vegetative cells in the family *Liliaceae*. Biol. Zbl. 103: 547-555.
- Tupy J., Süß J., Hrabětová E., Rihová L., 1983. Developmental changes in gene expression during pollen differentiation and maturation in *Nicotiana tabacum* L. Biol. Plant. 25: 231-237.
- Van Lammeren A. A. M., Keijzer C. J., Willemse M. T. M., Kieft H., 1985. Structure and function of the microtubular cytoskeleton during pollen development in *Gasteria verrucosa* (Mill.) H., Duval. Planta 165: 1-11.
- Vazart J., 1969. Structure et évolution de la cellule générative du lin, *Linum usitatissimum* L., au cours premiers stades de la maturation du pollen. Rev. Cytol. Biol. Veg. 32: 101-114.
- van Went J. L., 1974. The ultrastructure of *Impatiens* pollen. In: Fertilization in higher plants. Linskens H. F. (ed.). North-Holland Publ. Co., Amsterdam. pp. 81-88.
- Willemse M. T. M., Reznickova S. A., 1980. Formation of pollen in the anther of *Lilium*. Development of pollen wall. Acta Bot. Neerl. 29: 127-140.
- Wójcicki Z., 1926. Grains de pollen tubes polliniques et spermatogénèse chez *Haemanthus katharinae* Bak. II Bul. Acad. Pol. Sci. Letters-B., Sci Natur. pp. 535-557.

Ultrastrukturalne transformacje cytoplazmy różnicujących się komórek pyłkowych Hyacinthus orientalis L.

Streszczenie

Badano sekwencję przemian ultrastrukturalnych cytoplazmy komórek pyłkowych *Hyacinthus orientalis* podczas kolejnych etapów dojrzwania ziarna pyłkowego. Przemiany cytoplazmatyczne komórki generatywnej obejmują: eliminację plastydów i wzrost liczby mitochondriów, przyjęcie wrzecionowatego kształtu przy udziale mikrotubul oraz charakterystyczny rozwój systemu wakuolarnego, związany z wytworzeniem tzw. ciał barwnych. Przemiany cytoplazmatyczne komórki wegetatywnej obejmują: zmiany plastydów, które wkrótce po powstaniu komórki gromadzą skrobię uwalniając ją na krótko przed antezą, wzrost liczby mitochondriów oraz wzrost liczby bardzo aktywnych diktiosomów tuż przed antezą. Zmiany struktury strefy granicznej między różnicującymi się komórkami pyłkowymi związane są przede wszystkim z okresowym występowaniem ściany kalozowej oraz z obecnością, w cytoplazmie komórki wegetatywnej otaczającej komórkę generatywną, ciał lizosomopodobnych. Powstają one po zaniku ściany kalozowej i zanikają na krótko przed antezą.