TAPETUM DEVELOPMENT IN TRANSGENIC TOBACCO (*NICOTIANA TABACUM* L.) PLANTS WITH MODIFIED LEVEL OF HISTONE H1 VARIANTS

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ABSTRACT

The phenomenon of male sterility has often been observed in investigations on the role of histone H1 in regulation of morphogenetic and cytological processes in transgenic tobacco plants. These changes were accumulated by disturbances in flower development, consisting in lengthening of the pistil style in relation to stamen heads. This prevented pollination and production of seeds. As similar abnormalities occurred also in the present investigations (depending on combination, the sterility% was 84.4 to 19.9, at only 8.1 in the control), the main problem of our investigations was an attempt to explain their reasons. It is commonly known that one of the conditions for formation of fertile pollen is the properly functioning tapetum. Here, we carried out observations of ultrastructure of anther tapetum control cells in respect of abnormalities which occurred during microsporogenesis of transgenic plants with inactivated expression of two major (A, B) and two minor (C, D) histone H1 variants. The investigations were carried out on the following groups of plants: (1) control group with a full set of histone variants (K), (2) with inactivated A and B variants (–AB); (3) with inactivated A, B, C and D variants (–ABCD), (4) with inactivated C and D variants (–CD).

It was found that tapetal development was normal in all the investigated groups of plants, and the sequence of changes was similar as in the control. However, certain ultrastructural differences appeared when tapetum functioned as secretory tissue, and in the degeneration phase. In tapetal cell cytoplasm, with participation of rER, lipid bodies were formed, which, having penetrated to the cell surface and to locules, took part in formation of pollen grain sporoderm. Both in the control and in the remaining combination, excluding –ABCD, these bodies looked similar: they were grey, homogenous and surrounded by black jagged deposits. In –ABCD plants, these bodies were more translucent, slightly rarefied, and not surrounded by the deposits. Moreover, in –CD plants, large lipid deposits were frequently observed between remainders of degraded tapetal cells. They did not occur in the control and the remaining combinations.

KEY WORDS: *Nicotiana tabacum* L., histone H1 variants, tapetum, sterile pollen grains, Übisch bodies.

INTRODUCTION

The histone H1 is one of the main structural proteins of chromatin, occurring in nucleus of almost all eukaryotic organisms (fungi, plants and animals) (Kasinsky et al. 2001; Jerzmanowski 2002). In plant and animal cells it occurs in the form of numerous non-allelic variants, exhibiting slight differences in their aminoacid sequence (Kasinsky et al. 2001; Khochbin 2001). Tobacco (*Nicotiana tabacum* L.) has six variants of histone H1: two major ones (H1A and H1B), making some 90% of all H1, and four minor ones (H1C, H1D, H1E and H1F), occurring in trace quantities.

Investigations of the histone H1 role in the regulation of morfogenetic and cytological processes have confirmed the presumption that it is not a general repressor of transcrip-

tion and it participates in specific regulation of the expression of particular genes (Prymakowska-Bosak et al. 1996, 1999). Experimental reduction of the level of the main H1 variants in tobacco combined with elevated levels of its small variants, has caused various phenotypic changes (Prymakowska-Bosak et al. 1999; Ślusarczyk et al. 2001b). No influence on vegetative development and differentiation of plants has been observed; however, there have been disturbances of flower development consisting in lengthening of the pistil's style, in comparison with the level of anther's tip, which made pollination and seed production impossible. Analysis of microsporogenesis in those plants revealed asynchronous course of meiosis, appearance of chromosomal aberrations (chromosomes "lost" during the process of division, ana- and telophase chromosome "bridges") and finally, formation of sterile pollen grains (Ślusarczyk et al. 2001a, b). Similar changes occurred also in our investigations on plants with modified proportion of histon H1 variants (Przewłoka et al. 2002). Disorders of microsporogenesis process and the percentage of sterile pollen grains were different in particular combinations (e.g. the percentage of sterility oscillated between 19.9 to 84.4 at the level 8.1 in control plants) (Przewłoka et al. 2002; Ślusarczyk et al. 2003).

Therefore in the present paper we have made an attempt to explain the reason of the observed disorders, especially in terms of developmental correlation between tapetum and male gametophyte, because one of the crucial conditions for formation of fertile pollen is the presence of properly functioning tapetum. Disturbances in development of this layer of cells lead to formation of, among others, sterile pollen grain (Bino 1985; Mariani et al. 1990; Chaudhury 1993; Zhang et al. 1994; Matsuda et al. 1996).

Anther tapetum of angiosperms is a layer of specialized cells, surrounding the sporogenous tissue in the developing anther. Tapetum is important for nourishing of developing pollen grains (Shivanna and Johri 1985; Chapman 1987; Pacini 1990), supplying them with different enzymes and exine (a layer covering pollen grains) precursors (Pacini 1985; Murgia et al. 1991; Leśniewska 1996; Clement et al. 1998; Piffanelli et al. 1998; Leśniewska and Charzyńska 2000). This indicates the importance of interaction between cells of tapetum, which is a part of sporophyte, and cells of germ line. Tapetum cells transmit all impulses and nutrients destined for developing male gametophyte. Passing through tapetum, these compounds are metabolized and transferred to microsporocytes and microspores as precursors or final products.

In our investigations, we carried out observations of anther-tapetum-cell ultrastructure in connection with previously described changes, which occurred in microsporogenesis process in transgenic tobacco plants with inactivated expression of two main (A, B) and two lateral (C, D) histone H1 variants (Ślusarczyk et al. 2003). In the investigations, we analysed four groups of transgenic tobacco plants with different level of histone H1 variants:

K – control with the full set of histone variants,
– AB – with inactivated A and B variants,
– ABCD – with inactivated A, B, C and D variants, and
– CD – with inactivated C and D variants.

MATERIAL AND METHODS

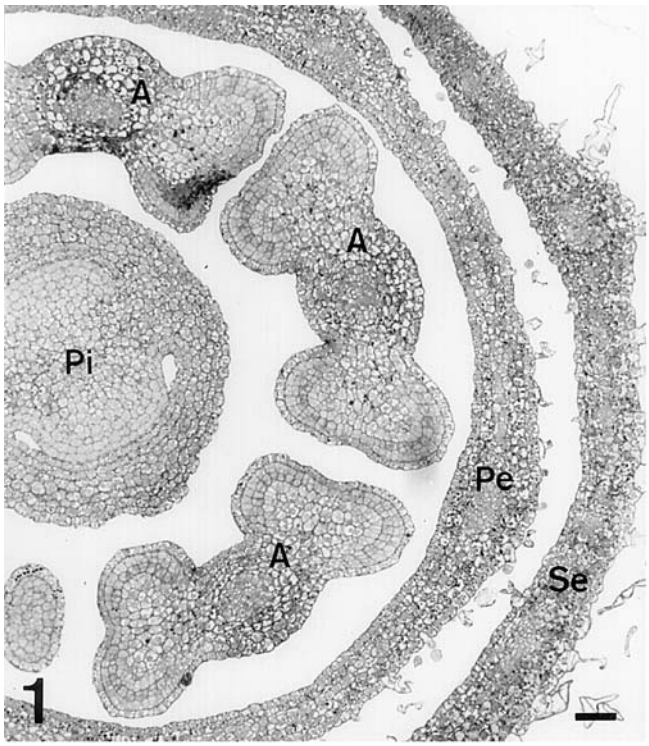
The methods for obtaining and cultivation of plants and their morphological and molecular characteristics were presented by Przewłoka et al. (2002). Cytological disturbances in the process of microsporogenesis and formation of male gametophyte were described by Ślusarczyk et al. (2003). The latter work, basing on changes in ultrastructural organization of cells in subsequent stages of sporogenous tissue and pollen grains of control plants, distinguishes nine developmental stages of the tapetum layer. These stages, correlated with the length of flower buds, were distinguished in preparations squeezed in acetoorcein (the detailed description of making the preparations is given in the work of Ślusarczyk et al. (2001)). Basing on these criteria, the following stages were distinguished: early archesporial (5 mm-long buds), archesporial (6 mm-long buds), early meiotic (7 mm-long buds), older meiotic (8 mm-long buds), tetrad (9 mm-long buds), microspore (10 mm-long buds), vacuolated microspores (14 mm-long buds), immature pollen grains (16 mm-long buds), mature pollen grains (fully-blown flowers, 45 mm-long).

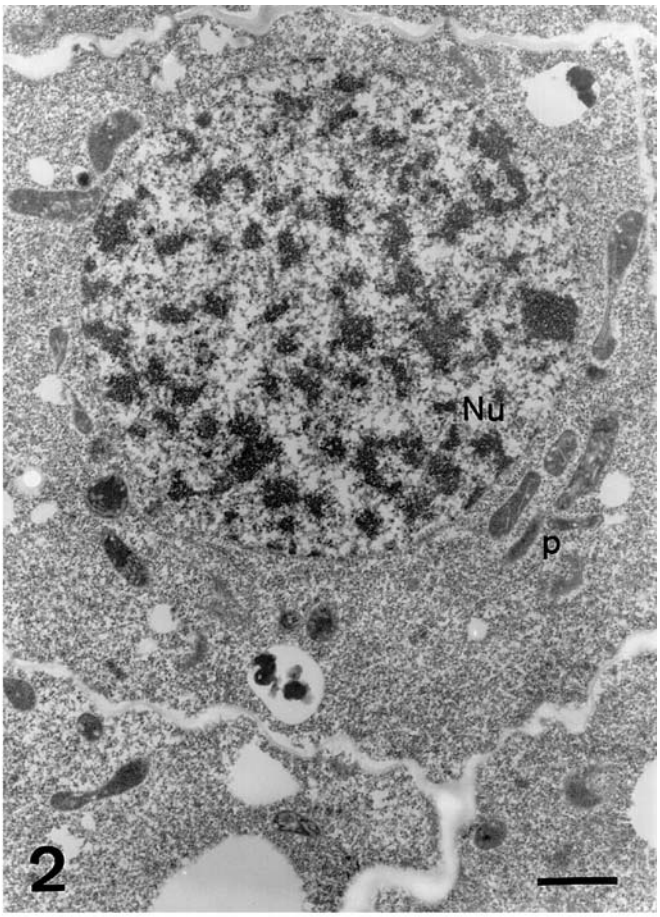
Ultrastructural changes in anther tapetum cells of the investigated groups of plants were analysed in transmission electron microscope (TEM). Anthers, dissected from the flower buds in particular developmental stages, were fixed for 2 hours in 2% glutaraldehyde at pH 7.2 (0.1 M cacodylate buffer), then infiltrated in vacuum thermostat at 0.6 atm vacuum for 1/2 hour. The material was then postfixed for 2 hours in 2% OsO_4, dehydrated in ethanol, and, through propylene oxide, embedded in the Epon/Spurr mixture of epoxy resins. Cross ultrathin sections (±80 nm) were cut with LKB ultramicrotome (Sweden), contrasted in saturated water solution of uranyl acetate (30 min.) and lead citrate (30 min.), according to Reynolds (1963). The preparations were observed in JEOL JEM – 1200 EX transmission electron microscope at the voltage of 90 kV. Electronograms were made according to the classical photographic method, using Kodak negative films.

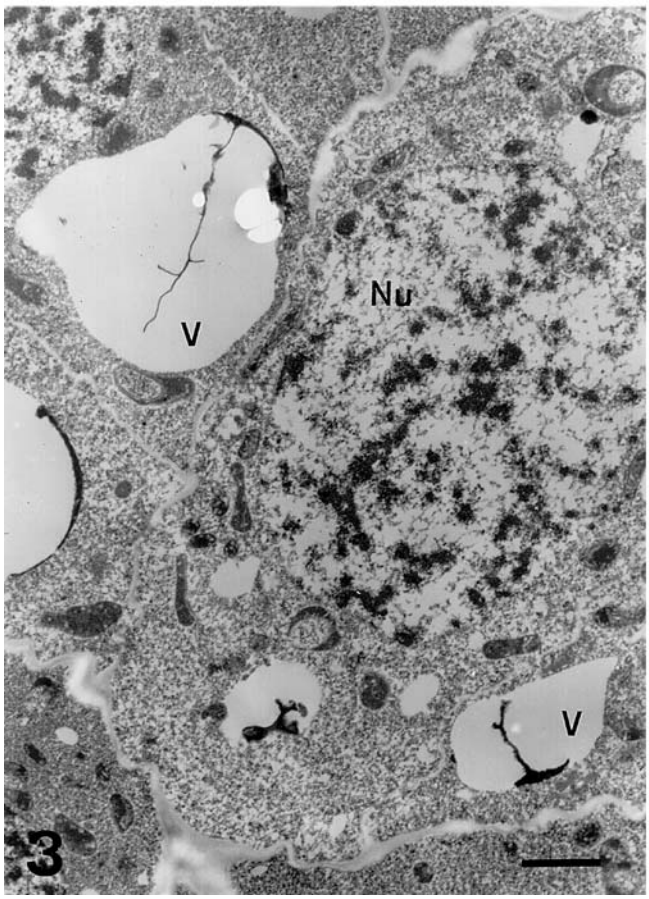
RESULTS

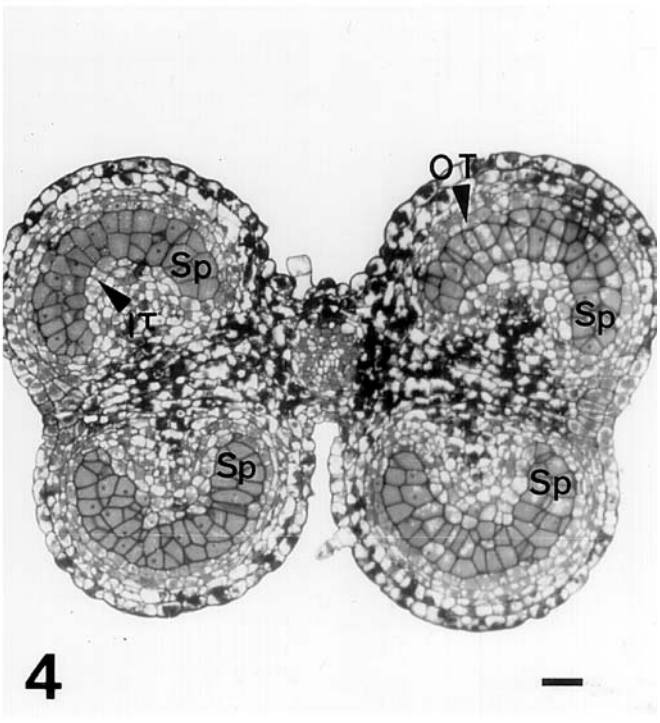
Early archesporial stage (5 mm-long bud)

In cross sections of anthers from 5 mm-long flower buds, in all groups of investigated plants, four future pollen sacs were distinguishable as distinct protuberances. Each of them contained the epidermis and less differentiated subepidermal layers of meristematic type. Sporogenous tissue cells were located in the center, tightly surrounded with a layer of tapetum cells (Fig. 1). Tobacco tapetum is heteromorphic, i.e., composed of outer and inner tapetum. Inner tapetum cells are derived from connective tissues, while outer tapetum cells are differentiated from anther wall layers (Periasamy and Swamy 1966). This results in certain differences in ultrastructure of those cells. The cytoplasm of outer tapetum cells was dense as it contained a considerable number of free ribosomes, and few cell organelles (Fig. 2). They were plastids of various shapes, with dense matrix, and oblong mitochondria located around the cell nucleus. Dictyosomes (weakly active, without vesicles) and endoplasmic reticulum were rarely met. The outer ta-









Figs 1-4. Development of tapetum of *Nicotiana tabacum* L. at archesporial stage in control plant.
Fig. 1. The cross section of the floral bud part (length 5 mm) in the control plants. Visible sepals, petals, anthers and part of the pistil. Four future pollen sacs separated in a form of distinct protuberances. Bar = 5 µm
Fig. 2. A cell of the outer tapetum in control plants. In centre nucleus and some organelles. Bar = 1 µm
Fig. 3. A more vacuolated inner tapetal cell in control plant. In cytoplasm visible evenly distributed organelles. Bar = 1 µm
Fig. 4. The cross section of the anther (6 mm-long-bud). The sporogenous tissue separated in form of croissant surrounded tapetum layer. Bar = 10 µm
A – anther; IT – inner tapetum; OT – outer tapetum; Nu – nucleus; Pe – petals; Pi – pistil; Se – sepals; Sp – sporogenous tissue; V – vacuole; p – plastid.

petum cell nuclei contained distinctly delimited chromosomal territories and pale karyolymph.

Inner tapetum cells contained a rarer cytoplasm and were more vacuolated, their organelles were distributed similarly as in the outer tapetum cells (Fig. 3). Chromatin of inner tapetum cell nuclei was more dispersed, in comparison with the outer tapetum (Figs 2 and 3).

In –AB, –ABCD and –CD combinations, ultrastructural differentiation of tapetum layer cells was at this stage similar as in the control.

Archesporial stage (6 mm-long bud)

At this stage, both in the control and in plants with different levels of histone H1 variants, the sporogenous tissue the took form of croissant-shaped bands (Fig. 4). In comparison with the previous stage, tapetum cells showed slight ultrastructural differences. In cytoplasm of the outer tapetum cells, single, long cisterns of rough ER appeared. Oval or oblong plastids with starch grains and mitochondria were met as well. Single lipid bodies were also seen within the cytoplasm. Cell nuclei, with distinct envelope and very rare karyolymph contained scarce, small chromocenters (Fig. 5). In the inner tapetum cells, the cytoplasm was rarefied, with numerous vacuoles. Organelles within it looked normally. At this stage, like at the previous one, no ultrastructural damages were observed in tapetal cells of control plants and plants with different level of histone H1 variants.

Early meiotic stage (7 mm-long bud)

The pollen sac in control plants and the remaining combinations was filled with meiocytes surrounded with callose. The difference in ultrastructure of the inner and outer tapetum cells was still visible in all plants. Cytoplasm of the outer tapetum cells, in comparison with the previous stage, became denser and richer in organelles (Fig. 6). The quantity of rough ER increased markedly, and its cisterns were large and swollen. Numerous mitochondria contained lucent matrix. Their cristae, although short, were very numerous, filling almost the whole mitochondrial matrix. Plastids were rarely met. They had a dense homogenous matrix and very scarce thylakoids. In comparison with the previous stage, the cytoplasm contained more dictyosomes and vesicles of dictyosomal origin. The outer tapetum began to turn into a very active tissue, although the structure of nuclei and nucleoli did not indicate this. Nuclear chromatin was thickened, and nucleus compact (Fig. 6).

The inner tapetum cells, in all the investigated combinations, had dense cytoplasm, but they were much more vacuolated. The number of organelles within the cytoplasm was also higher, in comparison to the previous stage. There were many mitochondria with very lucent matrix, numerous ER cisterns and vesicles of dictyosomal origin, like in the outer tapetum cells. Karyolymph of the inner tapetum nuclei was also dense, with very thick heterochromatin areas. Within the cytoplasm of both types of tapetal cells, only single lipid bodies were seen (Figs 6 and 7).

No differences were still observed at that stage between tapetal ultrastructure of control plants and plants with modified level of histone H1 variants.

Older meiotic stage (8 mm-long bud)

In the cross section of pollen sac of a control plant, meiocytes surrounded with callose occurred. They were usually at metaphase of meiosis I. Meiosis in plants with modified level of histone H1 variants, especially in –AB and –ABCD combinations, was asynchronous, with numerous chromosomal aberrations (Ślusarczyk et al. 2003).

Binuclear cells, both of the outer and inner tapetum, contained many vesicles and vacuoles of different size (Fig. 8), in which distinct black deposits occurred. Cytoplasm of outer tapetum cells contained still a larger amount of rough ER, dictyosomes, ribosomes, plastids and myelin structures, as well as mitochondria of different shapes with swollen cristae (Fig. 8). Inner tapetum cells were slightly more vacuolated, although their ultrastructural organization was similar to the ones of the outer tapetum.

In spite of distinct differences in the process of the meiotic division, as compared to the control, tapetal differentiation in –AB, –ABCD and –CD combinations progressed similarly as in the control plants.

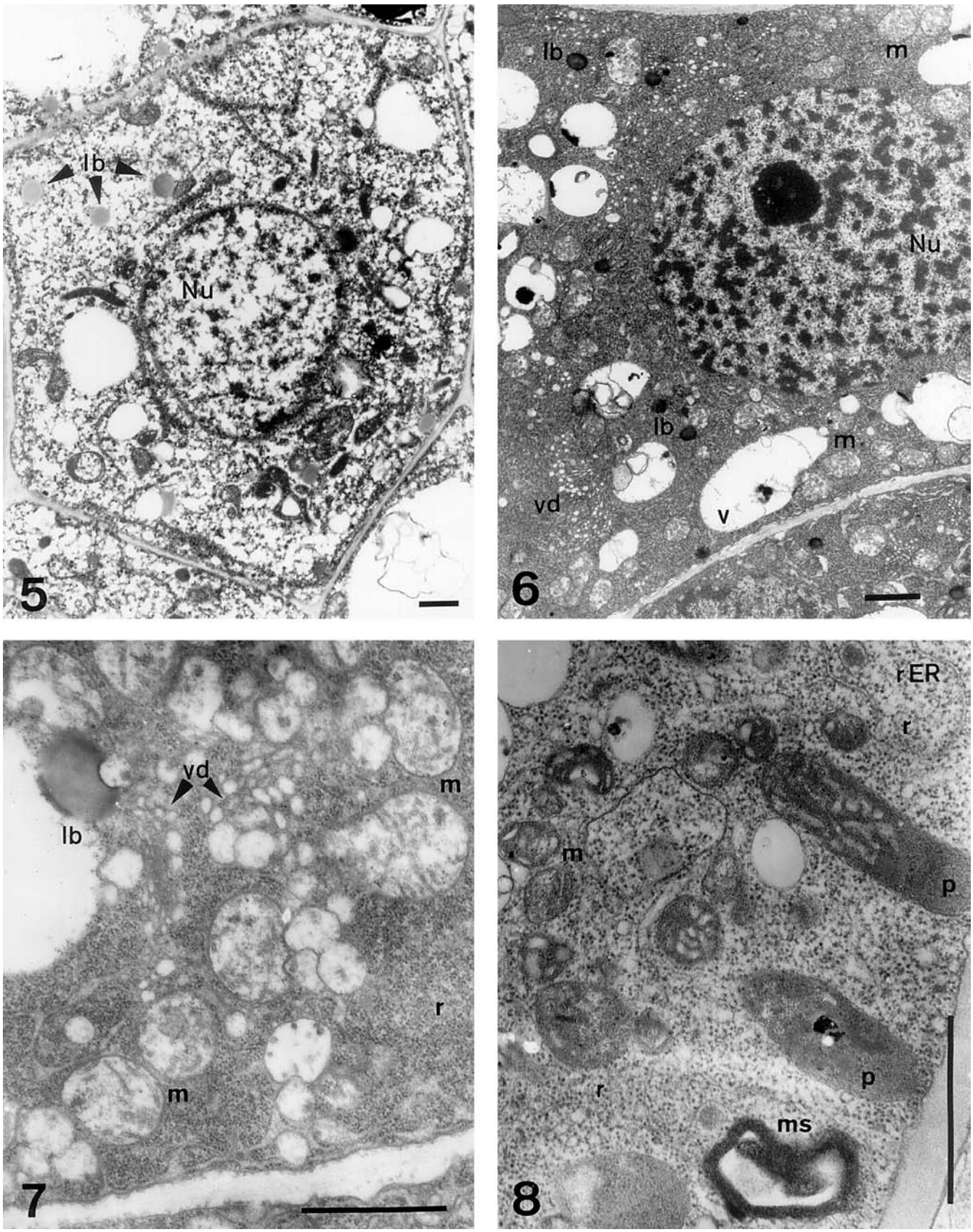
Tetrad stage (9 mm-long bud)

At that stage, microspore tetrads, surrounded with callose, occurred in cross section of the pollen sac of control plants and the ones with modified histone H1 variant levels. The cytoplasm of outer tapetum cells was very dense, lipid bodies still not very numerous. Dark lipid deposits were still observed in vacuoles. In cytoplasm there were numerous rER stacks often accompanied by lipid bodies (Fig. 9). There were still many small vacuoles and oblong plastids, sometimes containing starch grains. The nuclei contained many heterochromatin concentrations (Fig. 9), more than at the previous stage. Plasmodesmata between adjacent tapetum cells still existed, although cell wall hydrolysis had already begun. The inner tapetum cell ultrastructure was similar, except for groups of small vesicles at their surface, evidencing the onset of secretion.

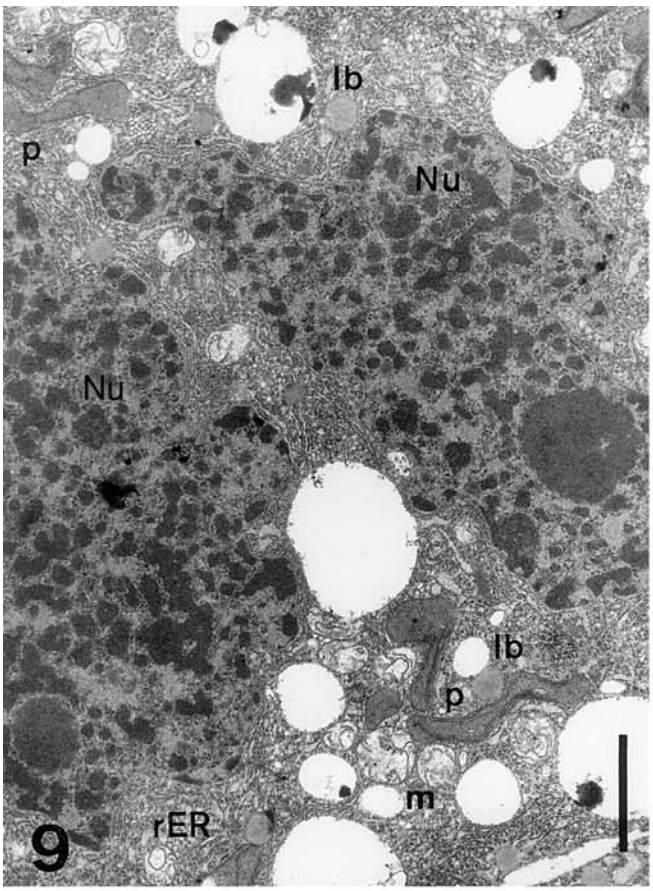
Ultrastructural organization of the outer tapetum cells in plants with modified levels of histone H1 variants was similar. Their cytoplasm was also dense, with numerous larger and smaller vacuoles, distributed evenly within the cell. Dark lipid deposits occurred in many vacuoles. Mitochondria and plastids with dark matrix and swollen thylakoids, sometimes containing starch grains, were less frequent. Cellulose/pectin cell walls were also hydrolysed, although plasmodesmata between neighboring cells still existed. In the cytoplasm near the walls, many mitochondria, single dictyosomes and rather numerous vesicles of dictyosomal origin were visible. A very large number of concentric systems of rough ER were observed within the cytoplasm. In the cytoplasm of –AB and –CD plants, single electron-dense lipid bodies were observed in the vicinity of the rER cisterns (Fig. 10), which did not occur in the control. In tapetal cells of –ABCD plants, lipid bodies were occasional at this stage. Ultrastructural organization of the inner tapetum cells was similar in all investigated groups of plants and it indicated the preparation to the secretion phase.

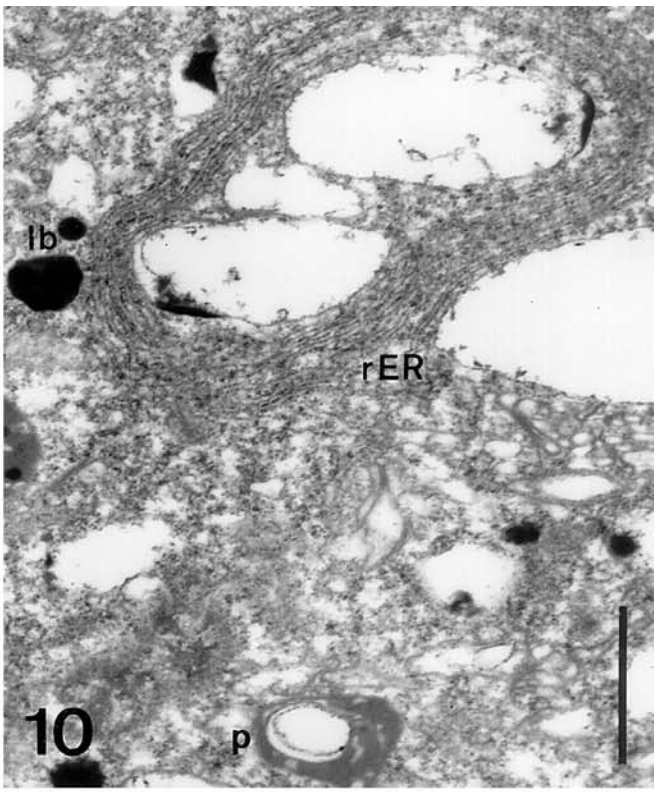
Microspore stage (10 mm-long bud)

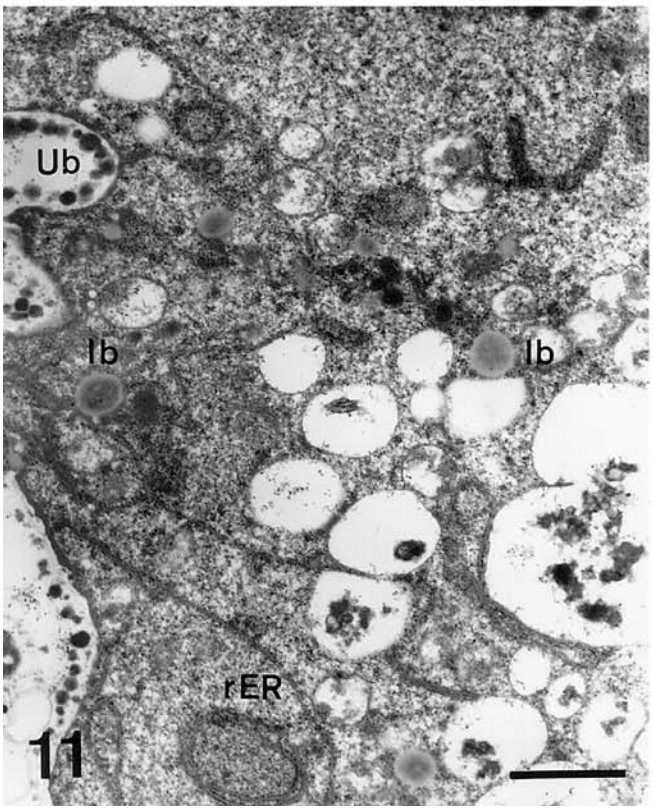
Pollen sacs, both in control and in the plants with different levels of histone H1 variants, contained numerous microspores surrounded with forming sporoderm. As compared to the previous stage, the outer tapetum cells still had dense cytoplasm with very numerous organelles; lipid bodies were more frequent (Fig. 11). Tapetal cell walls underwent further hydrolysis and plasmodesmata between the

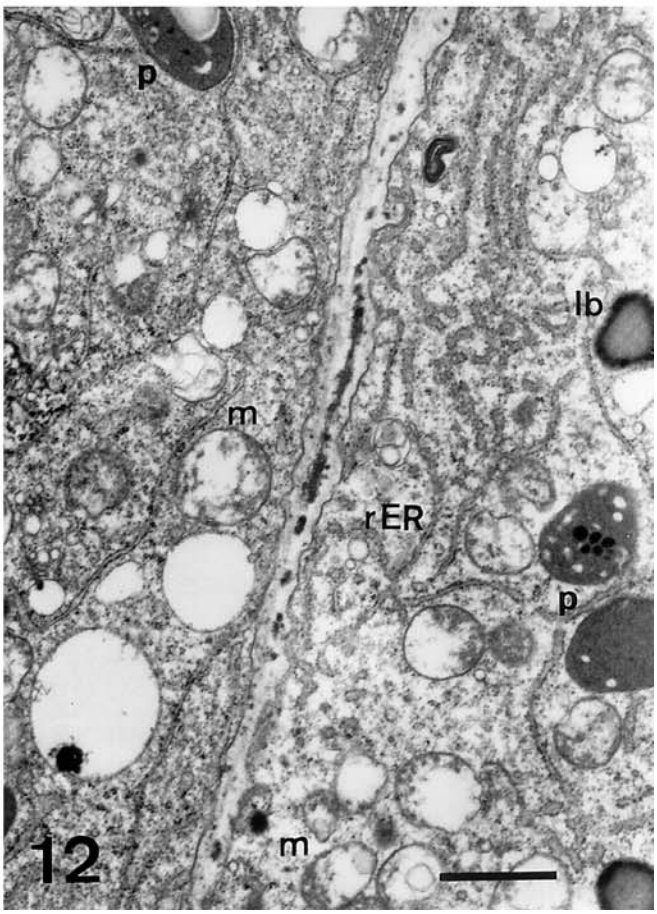


Figs 5-8. Differentiation of tapetum cells in control plant – continuation. Bars = 1 µm.
Fig. 5. A cell from the outer tapetum (6 mm-long-bud), in cytoplasm visible single lipid bodies.
Fig. 6. A part of cell from the outer tapetum (7 mm-long-bud) with dense cytoplasm, rich in organelles.
Fig. 7. Mitochondria and vesicles of dictyosomal origin in a cell from the inner tapetum.
Fig. 8. Numerous ribosomes and plastids in the cell of outer tapetum (8 mm-long-bud).
Nu – nucleus; rER – rough endoplasmic reticulum; lb – lipid body; m – mitochondrion; ms – myelin structure; p – plastid; r – ribosome; vd – vesicles of dictyosomal.









Figs 9-12. Differentiation of tapetum cells in control plant contrary to –CD and –ABCD plants. Bars = 1 µm.

Fig. 9. A binuclear cell of the control plant of outer tapetum.

Fig. 10. Rough ER cisterns in cell of the outer tapetum in –CD plant. On the left visible electron-dense lipid bodies.

Fig. 11. A part of the outer tapetum cell in control plant in the secretory phase. In surface of the cells on the locule-facing side present numerous orbicular vesicles.

Fig. 12. A part of two outer tapetal cells in –AB plant near their radial surfaces. In cytoplasm numerous organelles and lipid bodies in characteristics envelope.

Nu – nucleus; rER – rough endoplasmic reticulum; Ub – Übisch body; lb – lipid body; m – mitochondrion; p – plastid.

cells were disappearing. Small orbicular vesicles (Übisch bodies) were already present on the locule-facing surface of pleated plasmalemma, and in the spaces between neighboring cells.

Inner tapetum cells had similar ultrastructural organization as the outer tapetum cells; they showed, however, lower secretory activity and were slightly more flattened. Single orbicules were still visible at their surface. Plastids contained sometimes dark deposits and starch grains.

In plants with modified level of histone H1 variants, the density of cytoplasm of inner and outer tapetum cells was raised as compared to the previous stage. Cytoplasm of these cells, similarly as in the control, contained numerous organelles. Lipid bodies were more numerous there than at the previous stage, and they were surrounded with black "shreds", small in –AB, –CD and control plants (Fig. 12), and more homogenous in –ABCD plants (Fig. 13). Small vacuoles within the cell contained sometimes dark deposits. Cell walls underwent further hydrolysis. Many ribosomes, mitochondria and vesicles of dictyosomal origin were located near the plasmalemma (Fig. 12), participating in cell wall hydrolysis or transporting lipid bodies (pro-orbicules) to the plasmalemma surface. Cell plasmalemma was plicate, with orbicules (Fig. 13).

Vacuolated microspore stage (14 mm-long bud)

In so large buds, pollen sacs of anthers in control plants contained mononuclear microspores at vacuolation stage. By that moment of microspore development, cellulose/pectin tapetal cell walls were wholly hydrolyzed. Their surface, at the loculus and at the radial wall side, was covered abundantly with orbicular vesicles (Fig. 14). They occurred as well in spaces between neighboring tapetal cells, and at the middle layer surface, but in smaller number. Outer tapetal cytoplasm was markedly rarefied as compared to the previous stage. It contained numerous vacuoles, plastids with starch grains, and lipid bodies, usually situated around rER cistern stacks. Inner tapetal cells showed similar organization.

Pollen sacs of all combinations of plants with modified level of histone H1 contained mononuclear vacuolated microspores as well. They had typical structure, like in control; however, many degraded ones were observed. Their description is given by Ślusarczyk et al. (2003). Outer tapetal cells had dense cytoplasm containing very numerous lipid (pro-orbicular) bodies. In all studied combinations, these bodies abundantly filled tapetal cells. In –AB and –CD plants, the lipid bodies were surrounded with black "jagged" structures (Fig. 15), which were absent in –ABCD plants, where lipid bodies were grey and homogenous (Fig. 16). Apart of that difference, tapetal cell ultrastructure in the studied combinations was very similar. The cell surface, at the locule-facing side and between neighboring cells, was lined with numerous orbicular (Übisch) bodies, like in the control. Cytoplasm contained numerous rER stacks, distributed all over the cell. They were very often accompanied by concentrations of lipid bodies (pro-orbicules) in their neighborhood. Numerous mitochondria were observed, especially close to the cell surface, at the sites of orbicule penetration or their synthesis at rER stacks (Fig. 15). Cell walls were hydrolyzed by that stage, and cell protoplasts got slightly flattened. Inner tapetal protoplasts, when compared to outer ones, were more flattened and smaller, slightly more degraded.

Immature pollen grain stage (16 mm-long bud)

In control plants, pollen sacs were filled with pollen grains with the vegetative cell and generative cell, located at the wall. Ultrastructure of outer and inner tapetum showed clear symptoms of degradation: flattening of cell protoplasts and marked rareness of their cytoplasm. It contained long, sometimes tangled rough ER cisterns, and many smaller or larger vacuoles. Outside the cell protoplast, abundant orbicular bodies were visible (Fig. 17).

In plants with modified histone H1 variants level, the inside of pollen sacs was filled with immature pollen grains, composed of the vegetative cell and the generative cell, located at the wall, like in the control. However, apart of normally formed pollen grains, also degraded ones occurred, at the percentage typical for a given combination (for detailed description see Ślusarczyk et al. 2003).

In all investigated groups of plants, anther tapetum cells showed marked symptoms of degradation as compared to the control. Markedly flattened cell protoplasts were observed, with numerous Übisch bodies located at their surface (Fig. 18). Cell cytoplasm had already become significantly degraded. It contained very numerous vacuoles and vesicles as well as remainders of organelles, hard to identify. In –AB and –CD plants, within such degraded cells, large lipid deposits were often observed (Fig. 18), which were also occasionally seen in the control. They were, however, absent in –ABCD plants (Fig. 19).

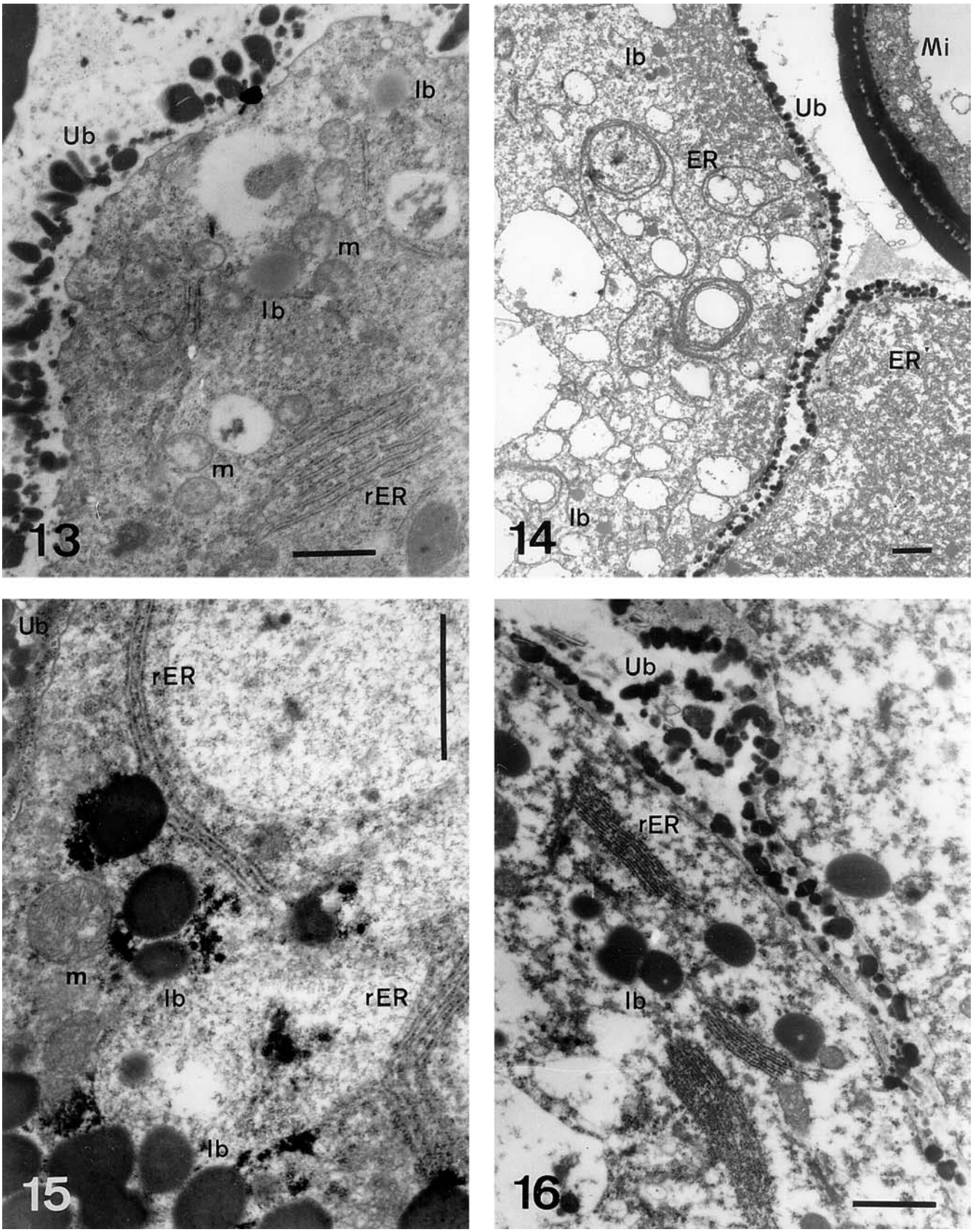
Mature pollen grain stage (developed flower, 45 mm)

Pollen sacs of the anthers were filled with mature pollen grains. They contained the vegetative cell with the generative cell inside. Tapetal cells of control plants and of the ones with different histone H1 variant levels were almost completely degenerated. Only their remainders were seen, strongly flattened, empty inside and with very numerous orbicular bodies at their surface (Fig. 20). In –CD plants degraded remainders of those cells still contained large lipid bodies (Fig. 21). In comparison with the previous stage, they disappeared from –AB plants.

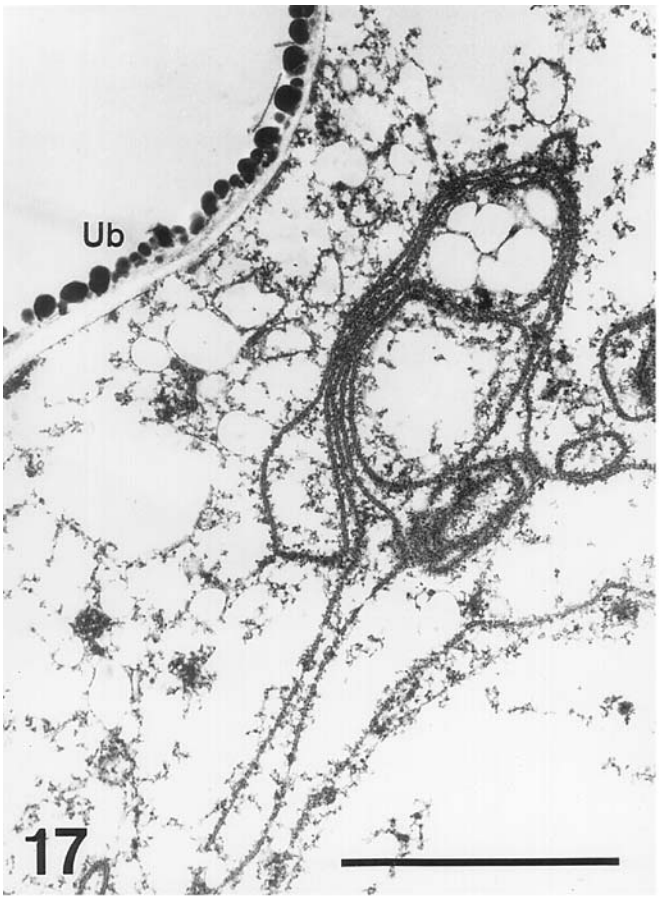
DISCUSSION

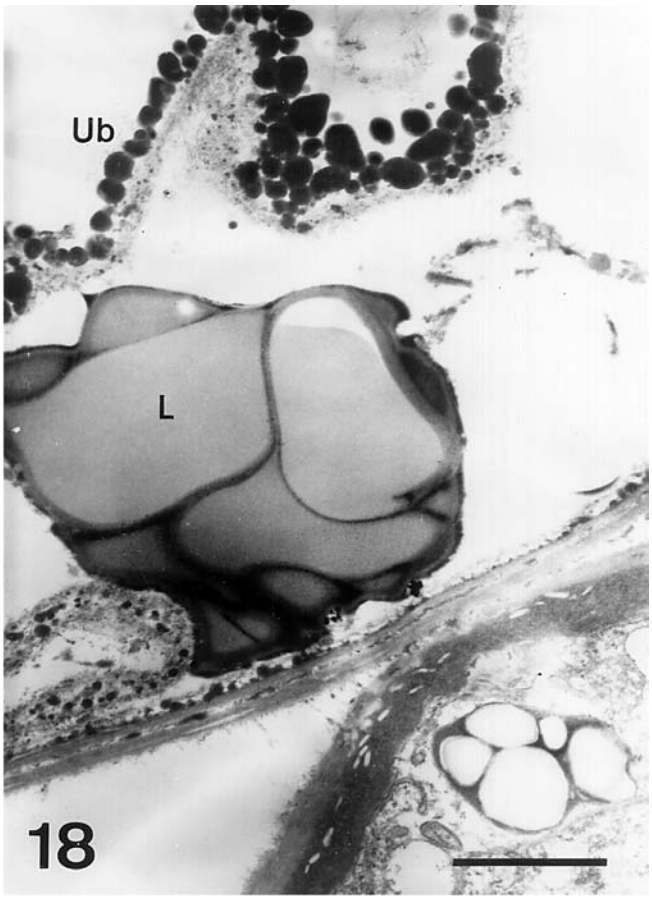
Previous investigations on the mechanism of action of histone H1 have shown that both increases of the level of its minor variants while reducing the major ones (Prymakowska-Bosak et al. 1999; Ślusarczyk et al. 2001a), and inactivation of particular histone H1 variants (Przewłoka et al. 2002; Ślusarczyk et al. 2003) lead to formation of distinct phenotypic changes consisting in disturbances of flower and male gametophyte development. Those changes resulted in development of male-sterile plants. We showed that their underlying basis involves defects in homologous chromosome pairing and their proper meiotic segregation (Prymakowska-Bosak et al. 1999; Ślusarczyk et al. 2001b).

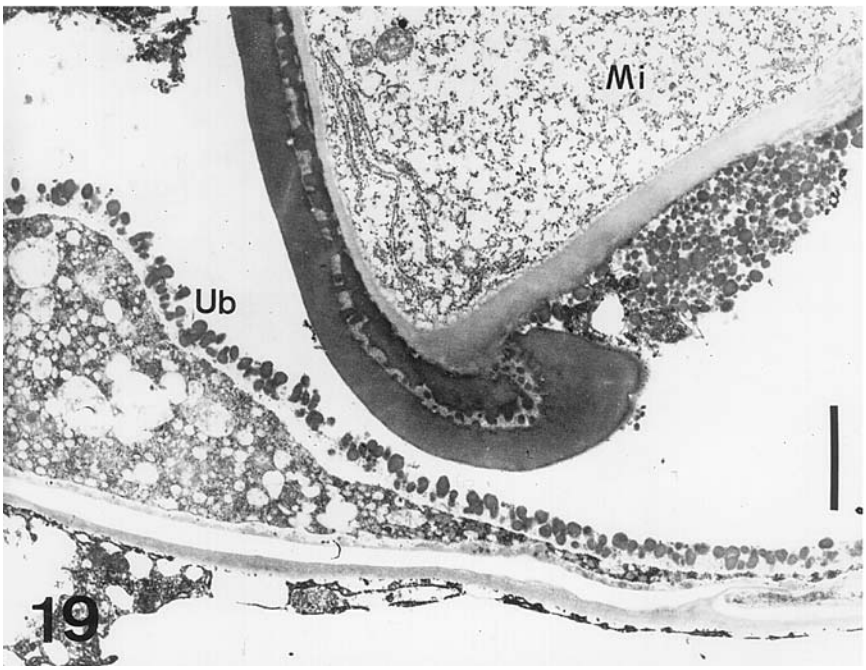
As a necessary condition for production of fertile pollen is the presence of properly functioning tapetum, we carried out ultrastructural studies of development of that tissue in plants with different levels of particular histone H1 va-



Figs 13-16. Development of tapetum in control plant and in plants with modified level of histon H1 variants – continuation. Bars = 1 µm.
Fig. 13. A part of the outer tapetum cell in –ABCD plant. In surface visible very numerous orbicules and in cytoplasm present characteristics homogenous lipid bodies.
Fig. 14. Rough ER cisterns in the tapetal cells of control plant in secretory phase.
Fig. 15. Lipid bodies in the outer tapetum cell in –CD plant surrounded with black "shreds".
Fig. 16. Homogenous lipid bodies in the outer tapetum cell in –ABCD plant.
rER – rough endoplasmic reticulum; Mi – microspore; Ub – Übisch body; lb – lipid body; m – mitochondrion.



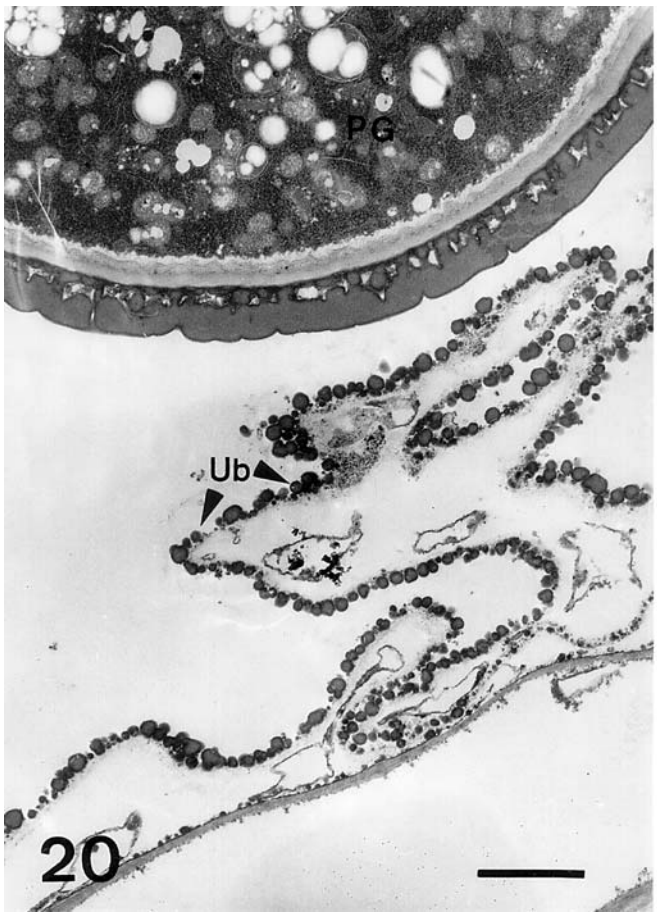


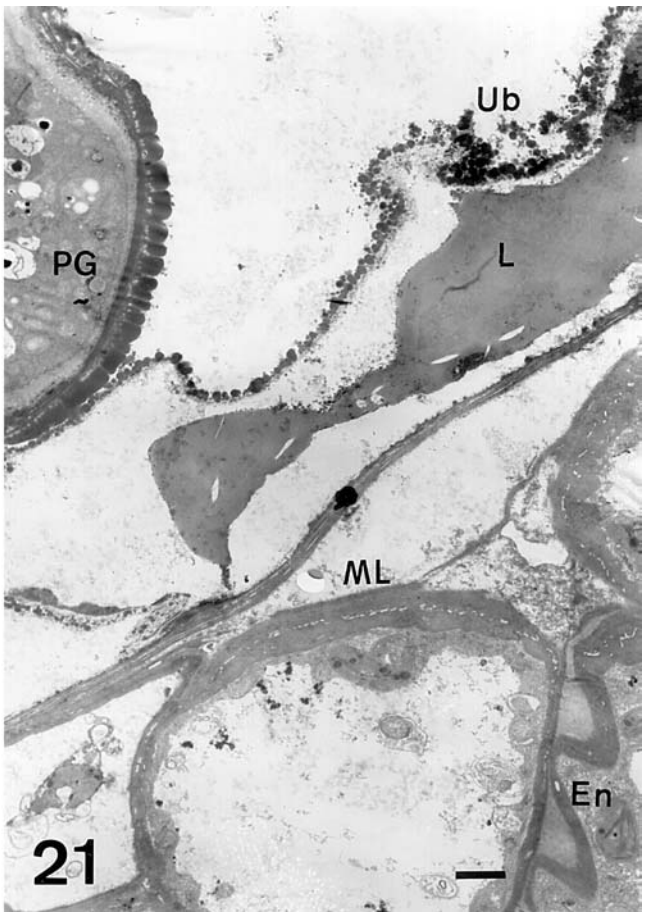


Figs 17-19. Tapetum of control plant and in plants with modified level of histon H1 variants in degradation phase. Bars = 2 µm.
Fig. 17. A part of outer tapetal cell in control plant during structural disintegration of organelles.
Fig. 18. A part of degraded outer tapetum cell in –CD plant with a characteristic large lipid deposit.
Fig. 19. A degraded tapetal cell of –ABCD plant without lipids deposits and a part of atypically microspore.
Mi – microspore; Ub – Übisch body; L – lipid deposit.

riants. Tapetum in tobacco, like in plants of Solanaceae family, is of secretory type (Periasamy and Swamy 1966; Pacini et al. 1985). A typical feature of its cells is the production of sporopollenin precursors and an element of pollen grain wall, in the form of orbicules (Übisch bodies).

Cell development in this layer of anther in three studied groups of plants (–AB, –ABCD and –CD) was generally very close to the control. Particular stages of development and differentiation of cells in this layer were synchronized with relevant developmental stages of sporogenous tissue. No distinct abnormalities were noted in functioning of tapetal cells in the investigated groups of plants, although they produced different amounts of sterile pollen grains (–AB – 84.4%, –ABCD – 81.4%, –CD – 19.9% – see Ślusarczyk et al. 2003). Cell organelles in tapetal cells of those plants were organized and distributed similarly as in the control. A certain difference was noted in structure of lipid bodies in –ABCD plants. They were light grey and seemed





Figs 20-21. Degradation of tapetal cells – continuation. Bars = 2 µm.
Fig. 20. A degraded tapetal cell and a part of pollen grain of the control plant.
Fig. 21. Degraded tapetum from lipids deposits of the –CD plant.
EN – endothecium; L – lipid deposit; ML – middle layers; PG – pollen grain; Ub – Übisch body.

to be less condensed in comparison with lipid bodies in the control and in –AB and –CD plants. They were not surrounded by black "jagged" structures. These bodies, after transformation, became orbicules, which participated in development of sporoderm, i.e., the wall covering pollen grains. However, in plants with modified level of histone H1 variants (–AB, –ABCD and –CD), sporoderm was sometimes abnormally formed (very thick and deformed, very thin, or sometimes absent) (Ślusarczyk et al. 2003). It referred however to all investigative combinations, not only to –ABCD one. Another observed difference was the long persistence of lipid remainders in degraded tapetal cells of –AB plants, and particularly long in –CD group, when pollen grain development had been finished. They were probably not completely used by developing male gametophytes, which were largely defective, particularly in –AB combination (84.4%). Thus histone H1 variants appear to be an important regulatory factor in microsporogenesis process in sporogenous tissue (especially A and B variants), but they do not significantly affect the functioning of tapetal tissue.

Formation of viable pollen is a complex process, depending on proper course of the developmental program of sporophyte and gametophyte tissues of anther (Koltunow et al. 1990; Murgia et al. 1991; Goldberg et al. 1993; Polowick and Sawhney 1993; Clement et al. 1998; Piffanelli et al. 1998). It takes place in the anther, in the pollen sac, which is surrounded by a layer of unicellular tissue, i.e. tapetum. Tapetal cells derive from the same precursor cells as developing male gametophytes and surround them completely during their development. Such a spatial dependence indicates that exchange of nutritive, structural or regulatory substances between sporophytic anther tissues and developing male gametophytes occurs through the tapetum. However, investigations carried out by us now show, that the difference of the proportion of the histone H1 variants does not cause significant disorders in the tapetum development and functioning, thou it touches on cells of germ line, what in most cases makes impossible to carry the correct development program. Considering chromosome location of H1 and its part in forming the higher level of chromatin structures, we can presume that the original reason of observed changes are disturbances in meiotic chromosome structure.

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