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 process. 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 (pH 7, 4) at room temperature for 4 hrs. Then they were rinsed in three changes of buffer and postfixed in 1% OsO₄ 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 nucleus 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 μ m.

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 bicellular pollen stage, arrow – generative cell, tc – tapetal cell, bar – 10 μ m.

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., Inu – larger part of nucleolus, m – mitochondrion, 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 μ m.

Fig. 7. Portion of pollen at the same stage in Fig. 6. Spherulic lipid bodies deposit in the vegetative cell bordering with the generative cell, d – dictyosome, gn – generative nucleus, 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 μ m.

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 than generative cell nucleus. The vegetative cell nucleus contains two parts of nucleolus, differing in size. The bigger part of nucleolus is granular and vacuolate, while the smaller part is fibrillar (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 elliptical 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 dictyosomes 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 specific 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, just 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 *Hyacinthus* (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 occurrence 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, Dexheimer 1970, Sanger and Jackson 1971), and *Magnolia x soulangeana* (Dinis and Mesquita 1994). In *Hyacinthus 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 mitochondria 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 dictyosome. Oil bodies in cell of mature seeds are very stable and do not aggregate or coalesce, because their surface is shielded by layer of unique protein termed oleosin (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).

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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 komórką vegetatywną zawierała wszystkie organelle z wyjątkiem plastydów. W komórce vegetatywnej obserwowano zwiększenie liczby mitochondriów oraz zmiany w akumulacji skrobi i ciał lipidowych. Plastidy zawierały skrobię w dwukomórkowym pyłku oraz we wczesnym stadium trójkomórkowym. W czasie dojrzewania ziarna pyłku skrobia ulegała degradacji. Ciała lipidowe w komórce vegetatywnej 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 vegetatywnej.

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