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ULTRASTRUCTURAL STUDIES ON PLASTIDS OF GENERATIVE AND VEGETATIVE CELLS IN LILIACEAE. 6. PATTERNS OF PLASTID DISTRIBUTION DURING GENERATIVE CELL FORMATION IN ALOË SECUNDIFLORA AND A. JUCUNDA.

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SUMMARY

Electron microscopy was used to study the plastid distribution during pollen development in *Aloë secundiflora* and *A. jucunda*. During the first pollen mitosis the majority of plastids is located in the centre of the microspore. Only exceptionally, single plastids are present in the region of the later generative cell. Normally the generative cell does not contain plastids. The semiquantitative analysis of the plastid content of generative cells at different developmental stages indicates that plastid transmission into the generative cell is an exceptional case in *Aloë*. This behaviour is considered to be typical for species of the *Lycopersicon* type (HAGEMANN 1983).

1. INTRODUCTION

In continuing our studies on the plastid behaviour during the pollen development in Liliaceae (*cf.* SCHRÖDER 1984, 1985a, b, 1986), the present paper describes the plastid distribution during the formation and maturation of generative cells in two species of the genus *Aloë*. The cytological mechanisms underlying the male plastid inheritance are discussed and compared with the semi-quantitative data about the plastid content of generative cells in *Aloë*.

2. MATERIALS AND METHODS

Plants of *Aloë secundiflora* and *A. jucunda* are growing in the Botanical Garden of the Martin-Luther-University (Halle/S.). Anthers have been collected at various times until anthesis. The cytological stage of the pollen grains was determined by aceto-carmine staining of one anther per flower; the remaining five anthers of this flower were fixed with 3% glutaraldehyde in 0.1M cacodylate buffer (pH 6.8) for 4 hours at 20 °C. The materials were postfixed with 2% buffered osmium tetroxide (pH 7.0), carefully rinsed with tap water, dehydrated through a gradual acetone series followed by propylene oxide, and embedded in low-viscosity epoxy resin (SPURR 1969). Ultrathin sections were made using

an OMU 3-ultrotome (Reichert, Austria), stained with aqueous uranyl acetate followed by lead citrate, and examined using a TESLA BS 500 electron microscope. Semithin sections for light microscopy were stained with toluidine blue.

3. RESULTS

In order to study the plastid behaviour during the formation and maturation of the generative cell in *Aloë secundiflora* and *A. jucunda* the pollen development of these species was examined from the first pollen mitosis until anthesis. The pollen development can be subdivided into four stages: 1. The first pollen mitosis (including cytokinesis). 2. The stage of the wall-attached generative cell. 3. The stage of the generative cell immediately after its detachment from the pollen wall. 4. The mature spindle-shaped generative cell at anthesis. No differences were observed in both species studied concerning the ultrastructural features and the patterns of plastid distribution.

3.1. The first pollen mitosis

The division of the microspore nucleus takes place near the wall of the microspore (*fig. 1* and *2*). At early cytokinesis the plastids and mitochondria are clearly distinguishable; the plastids contain small starch grains and are larger than the mitochondria. The majority of the plastids and mitochondria are located in the centre of the pollen grain near the vegetative nucleus (*fig. 1*). Single plastids and single mitochondria are present near the generative nucleus at early cytokinesis (*fig. 1*) as well as at late cytokinesis (*fig. 2*).

3.2. The stage of the two-cellular pollen grain

Just after the first pollen mitosis the generative cell is wall-attached (*fig. 3* and *7*). It contains mitochondria, ribosomes, vacuoles and lipid droplets. Plastids could only be observed exceptionally (*fig. 8*). These plastids have the same ultrastructure as the plastids of the vegetative cell. They contain starch and do not show signs of degeneration (*fig. 8*). During further pollen development the generative cell becomes detached from the pollen wall, and moves towards the centre of the pollen grain (*fig. 4, 5* and *9*). The starch of plastids exceptionally transmitted into the generative cell disappears (*fig. 4*). These plastids have a simple structure, and are obviously intact organelles. Normally the generative cell does not contain plastids (*fig. 5* and *9*). The mature generative cell is spindle-shaped at anthesis (*fig. 6*) and does not regularly contain plastids either. The results of semi-quantitative analysis of the plastid content of generative cells at different developmental stages are presented in *table 1*.

4. DISCUSSION

The first review about cytological mechanisms underlying the purely maternal inheritance of plastids in angiosperms was given by HAGEMANN (1976). At that time only few data about generative cells without plastids were available. In

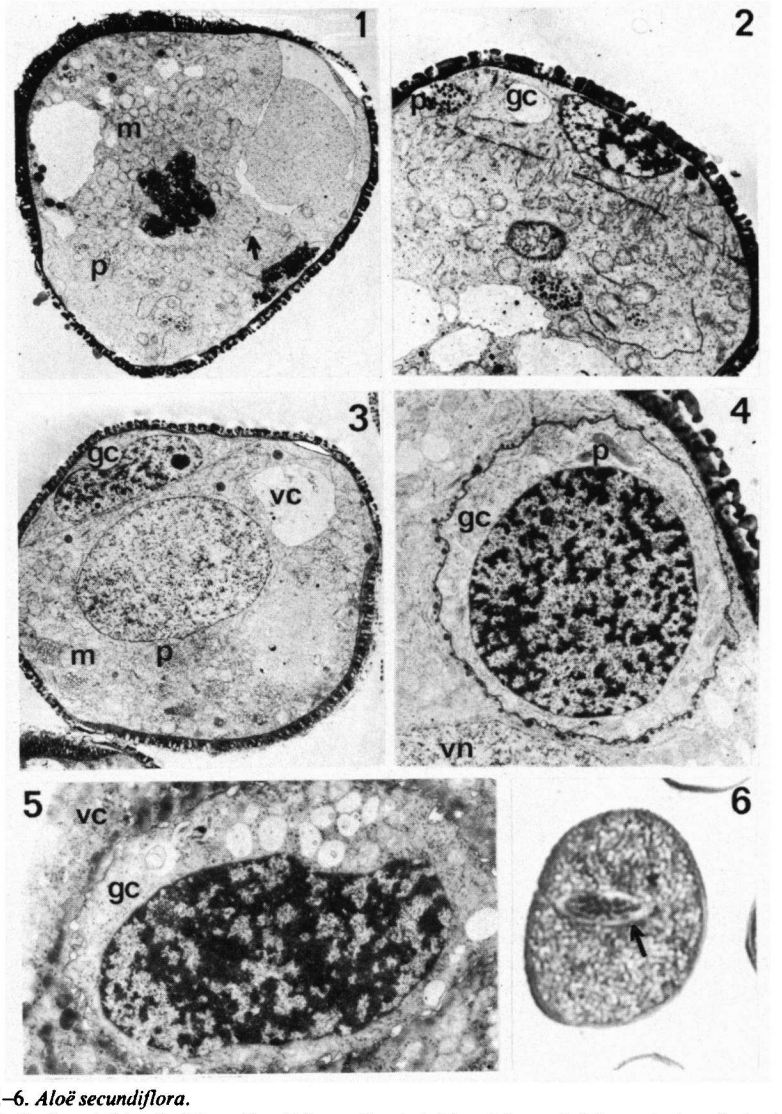

Fig. 1–6. *Aloë secundiflora.*

Fig. 1. Early cytokinesis. The cell wall formation is initiated (arrow). The majority of plastids (p) and mitochondria (m) is located in the centre of the microspore. 2.275 ×

Fig. 2. Late cytokinesis. The cell wall formation is nearly finished. Plastids (p) are also present in the generative cell (gc). 4.200 ×

Fig. 3. Stage of the wall-attached generative cell (gc). Plastids (p) are only present in the vegetative cell (vc). Mitochondria (m). 2.000 ×

Fig. 4. Generative cell (gc) immediately after its detachment from the pollen wall. Plastids (p) do not contain starch, have a simple structure, and do not show signs of degeneration. Vegetative nucleus (vn). 4.500 ×

Fig. 5. Cross section of a mature generative cell (gc), at anthesis. The generative cell is coated by a sheath of lipid droplets and lacks plastids regularly. Vegetative cell (vc). 8.400 ×

Fig. 6. Light micrograph of a mature pollen grain containing a spindle-shaped generative cell (arrow). 450 ×

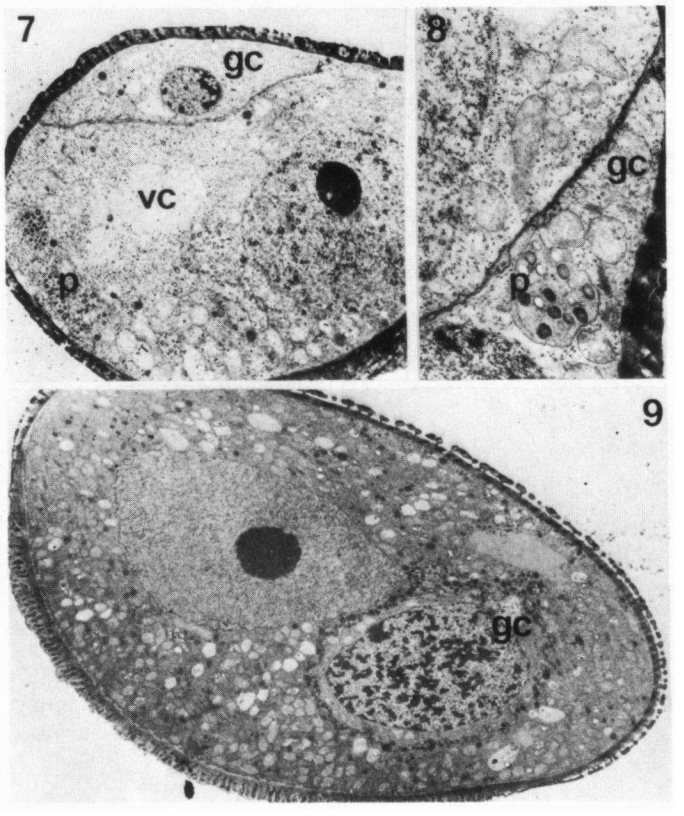

Fig. 7–9. *Aloë jucunda.*
Fig. 7. Stage of the wall-attached generative cell (gc). The generative cell does not regularly contain plastids. The vegetative cell (vc) contains numerous plastids (p) and mitochondria (m). 2.000 ×
Fig. 8. Generative cell (gc) containing a starch-filled plastid (p) at the same stage as shown in *fig.* 7. Mitochondria (m). Vegetative cell (vc). 8.400 ×
Fig. 9. Maturing generative cell (gc) without plastids. 2.800 ×

the meantime, during recent years more data were collected, and now two different cytological mechanisms are known to cause the lack of plastids in mature generative cells: 1. Formation of generative cells without plastids (*Lycopersicon* type, HAGEMANN 1983), 2. Degeneration of plastids during the maturation of the generative cell (*Solanum* type, HAGEMANN 1983).

The formation of generative cells without plastids is caused by the polar distribution of plastids within the microspore during first pollen mitosis (cf. VAN WENT 1984, SCHRÖDER 1985a). This plastid polarization is probably mediated by the cytoskeleton (VAN WENT 1984) and controlled by intracellular gradients (SCHRÖDER 1985a). However, as typical for living organisms, the plastid polarization does not work with a 100% efficiency (cf. HAGEMANN 1981). Therefore, single plastids can be irregularly transmitted into the generative cell as a normal

Table 1. Plastid content of generative cells during the pollen development in *A. secundiflora* and *A. jucunda*.

species	GC I		GC II		GC III	
	a	b	a	b	a	b
A. secundiflora	170	7	100	0	100	1
A. jucunda	150	2	120	1	80	0

GC I: stage of the wall-attached generative cell just after first pollen mitosis. GC II: stage of the generative cell immediately after its detachment from the pollen wall.
GC III: mature generative cell at anthesis. a: number of analysed generative cells (one section per generative cell). b: total number of plastids per studied sections.

event in species of the *Lycopersicon* type. This could be demonstrated by semi-quantitative analyses of the plastid content of generative cells in two species of *Aloë* (data presented in *table 1*). The same behaviour of plastids could be observed in two species of the genus *Gasteria* and in *Haworthia* (unpubl. data). An exceptional plastid content of generative cells in species of the *Lycopersicon* type was also demonstrated by genetical analyses (*Antirrhinum*, DIERS 1971; *Nicotiana*, MEDGYESY, pers. comm.).

An exceptional transfer of plastids into the generative cell during the first pollen mitosis can be caused by specific disturbances, too. The plastid distribution may be influenced either by drastic changes of the microspore environment (e.g. anther culture, REYNOLDS 1984) or by changes of the microspore metabolism (e.g. caused by virus infection, SCHRÖDER, in prep.). A delayed pollen development also causes an irregular plastid distribution in species of the *Lycopersicon* type (cf. SCHRÖDER 1985a).

We cannot recognize a biological sense of the inexact action of the cytological mechanisms controlling the plastid distribution during the first pollen mitosis. Probably, the components of the cytoskeleton are responsible for the intracellular arrangement of plastids (cf. MENZEL 1985); the plastids have to be connected with microtubules or microfilaments. We expect that the plastid population of a cell also contains single plastids which lost their ability to bind to the cytoskeleton in that the binding sites of microtubules or microfilaments on the outer plastid envelope are altered or missing. These plastids show a normal metabolic function (e.g. in photosynthesis), but they are randomly arranged within the cytoplasm and randomly distributed during cell divisions.

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REFERENCES

DIERS, L. (1971): Übertragung von Plastiden durch die Pollen bei Antirrhinum majus. II. *Molec.*

Gen. Genetics **113**: 150–153.

HAGEMANN, R. (1976): Plastid distribution and plastid competition and the induction of plastom mutations by nitroso urea-compounds. In: BÜCHER et al. (eds.): *Genetics and biogenesis of chloroplasts and mitochondria* North Holland, Amsterdam, pp. 331–338.

— (1981): Unequal plastid distribution during the development of the male gametophyte of angiosperms. *Acta Soc. Bot. Pol.* **50**: 321–327.

— (1983): The formation of generative and sperm cells without plastids in angiosperms and the underlying mechanisms. In: ERDELSKA, O. (ed.) *Fertilization and Embryogenesis in Ovulated Plants* Bratislava, pp. 97–99.

MENZEL, D. (1985): Fine structure on the association of the caulerpalean plastid with microtubule bundles in the siphonalean alga Chlorodesmis fastigiata Ducker (Udoteaceae). *Protoplasma* **125** 103–110.

REYNOLDS, T. (1984): An ultrastructural and stereological analysis of pollen grains of Hyoscyamus niger during normal ontogeny and induced embryogenic development. *Amer. J. Bot.* **71**: 490–504.

SCHRÖDER, M. -B. (1984): Ultrastructural studies on plastids of generative and vegetative cells in the family Liliaceae. 1. Lilium martagon L. *Biol.Zbl.* **103**: 547–555.

— (1985a): Ultrastructural studies on plastids of generative and vegetative cells in Liliaceae. 3. Plastid distribution during the pollen development in Gasteria verrucosa (Mill.) Duval. *Protoplasma* **124**: 123–129.

— (1985b): Ultrastructural studies on plastids of generative and vegetative cells in the family Liliaceae. 2. Fritillaria imperialis and F. meleagris. *Biol. Zbl.* **104**: 21–27.

— (1986): Ultrastructural studies on plastids of generative and vegetative cells in Liliaceae. 4. Plastid degeneration during generative cell maturation in Convallaria majalis L. *Biol. Zbl.* **105**, in press.

SPURR, A. R. (1969): A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**: 31–43.

WENT, J. L. VAN (1984): Unequal distribution of plastids during generative cell formation in Impatiens. *Theor. Appl. Genet.* **68**: 305–309.