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INVESTIGATION OF EXINE AND ORBICULE FORMATION IN THE LILIUM ANTHER BY SCANNING ELECTRON MICROSCOPY

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SUMMARY

The features of the exine and orbicules in the *Lilium* anther were studied by scanning electron microscopy. The changes in the dimensions and shape of the orbicules and the elements of the exine during development are shown to indicate the course of sporopollenin deposition.

In the discussion the data obtained are compared with the results of transmission electron microscopy.

1. INTRODUCTION

Scanning electron microscopy has seldom been applied to investigate the sporopollenin-containing structures formed in the anthers of higher plants. This method is used mainly for illustrating the morphology of the mature pollen.

Studying pollen wall development of *Lilium longiflorum* (cf. HESLOP-HARRISON 1968) by scanning electron microscopy the investigation of the surface of the exine and orbicules can contribute to the understanding of the rôle of sporopollenin deposition in the development of these structures.

The results are compared with the data obtained by transmission electron microscopy (REZNICKOVA & WILLEMSE 1980).

2. MATERIALS AND METHODS

Complete anthers of greenhouse-grown plants of *Lilium* hybrid "Enchantment" at different stages of microsporogenesis and gametogenesis were fixed in a 96% ethanol-acetic mixture (3:1) for 6 hours. After washing in 70% ethanol the anthers were cut into pieces of 3–4 mm and placed in polyethylene tubes covered with gauze at both ends. The material was dehydrated in an ethanol series and transferred to amylacetate. After critical point drying in carbondioxide with the Balser's Union critical point dryer, the anther fragments were stuck to the holder with double sticky tape and covered with a gold layer in the Polaron sputter apparatus (SEM coating unit E5100). The samples were investigated with a scanning microscope Jeol U3 operated at 25 kV. The same classification of the different developmental stages is used as is described in REZNICKOVA & WILLEMSE (1980).

3. RESULTS

3.1. Morphology of the exine of the post-tetrad stages of microsporogenesis and gametogenesis

3.1.1. The sexine

In this study only the surface view on the sexine and nexine is considered. The pattern of the sexine is represented by muri, forming a reticulum. The sculpturing of the sexine consists of bacula with enlarged heads which can be considered as the equivalent of a reduced tectum.

At the stage of the young microspore the heads of the bacula form a relatively regular hexagon (*fig. 1*). The larger heads, about 2.2–2.8 μm, are situated at the corners of the hexagon. Every side of the hexagon includes either one or two smaller heads, about 0.7–1.1 μm in diameter. Sometimes these smaller intermediate heads are absent. Thus the structure of the sexine is not strictly regular. The intermediate heads develop differently but they are always smaller than the corner heads. The corner heads are nearly spherical and sometimes slightly lobed. The shape of the intermediate heads varies.

At the beginning of the stage of the vacuolated microspore, both the corner and intermediate heads become more spherical and some are lobed. The intermediate heads seem to enlarge (*fig. 2*). By the end of the stage of the vacuolated microspore all the heads are spherical and very close together (*fig. 3*). The appearance of the sexine at the stage of the young pollen grain changes significantly and points to the increase in cell volume. The distance between the heads increase (*fig. 4*). The distance between the heads remains very small till the stage of the pollen grain with a lens-like generative cell (*fig. 5*). One day before anthesis the distance between the heads is minimal (*fig. 6*).

3.1.2. The nexine

The area enclosured by the bacula corresponds to the outer surface of the nexine. The outer surface of the nexine is relatively homogeneous both at the stage of the young microspore and at the beginning of the stage of the vacuolated microspore. Single globules are dispersed over it (*figs. 7, 8*). More globules and lamellae are present on the outer surface of the nexine at the stage of the vacuolated microspore (*fig. 7*). The number and size of the globules increase at the postmitotic stages of the pollen grain development. By the stage of the pollen grain with a lens-like generative cell they have become abundant and they look more spherical (*fig. 5*).

A reticulate structure becomes noticeable on the outer surface of the nexine starting from the late microspore interphase. It looks more conspicuous at the stage of the pollen grain with a lens-like generative cell (*fig. 8*). During these stages holes are visible, indicating the channels present in the nexine.

3.2. Morphology of orbicules

At the stage of the young microspore the orbicules look like small round bodies

about 1.0 μm in diameter. At this stage the diameter of the orbicules is much less than that of the corner heads of the sexine (*figs. 9, 1*). Apart from the orbicules rod-like bodies with a diameter of about 0.23 μm are present on the locular surface of the tapetum at this stage (*fig. 9*).

The diameter of the orbicules has nearly doubled by the beginning of the stage of the vacuolate microspore and approaches that of the corner baculum heads. The orbicules seem to increase further during the stage of the vacuolated microspore. Thus, their diameter is about 2.7 μm by the end of this stage and is approximately equal to that of the corner heads of the bacula. The orbicules look like spherical bodies with flattened poles and consist of plates (*fig. 10*). At the post-mitotic stages the globules that make up the orbicules become more spherical. The shape of the orbicule is more complicated because of small globules adhering to the outer surface.

The orbicules seem to decrease in size at the stage of the pollen grain with the generative cell near the wall. Apart from the large orbicules with a diameter of about 2.0–2.2 μm, smaller orbicules with a diameter of about 1.2–1.3 μm are present (*fig. 11*).

At anthesis the inner locular face is covered with orbicules of different size (*fig. 12*). Fibrils appear at the stage of the late microspore interphase and become more conspicuous at the post-mitotic stages. The orbicules are linked with these fibrils (*figs. 11, 12*).

4. DISCUSSION

The changes in dimensions and character of the surface of the microspore and pollen grains as well as orbicules in the anthers of *Lilium* hybrid "Enchantment" can be compared with the mode of the cell growth and the process of sporopollenin deposition described in WILLEMSE & REZNICKOVA (1980) and REZNICKOVA & WILLEMSE (1980).

Many authors have demonstrated the deposition of the major part of the sporopollenin on the exine just after microspore release from the callose wall (BANERJEE et al. 1965; HESLOP-HARRISON & DICKINSON 1969; HESLOP-HARRISON 1971; CHRISTENSEN et al. 1972). This investigation also demonstrated a massive sporopollenin deposition during this interval. The heads of the bacula enlarge and become more spherical. How sporopollenin is deposited in the *Lilium* anther at the interval mentioned above becomes much more understandable from the comparison of the orbicule dimensions. The diameter of the orbicules doubles during this interval.

Most authors considered that sporopollenin deposition on the exine is complete when the tapetal tissue disintegrates during microspore mitosis in the *Lilium* anther (HESLOP-HARRISON & DICKINSON 1969; DICKINSON 1976). Meanwhile, the measurements of *Lilium* microspores and pollen grains revealed a significant increase in cell volume at the post-mitotic stages of pollen development (WILLEMSE & REZNICKOVA 1980); this suggests a change in the exine.

It was shown in the present investigation that the sexine structure changes in

the course of pollen development. The distance between the heads of the bacula may either increase or decrease. The size of the heads, at least of the intermediate ones, seems to increase slightly. The shape of the heads changes too. Thus, by the end of the stage of the vacuolated microspore the sexine looks compact. The distance between the heads is minimal and the heads enlarge and come in contact. This character of the sexine is quite different at the stage of the young pollen grain, where the distance between the heads increases. During this interval sporopollenin deposition probably has taken place. These changes coincide with the increase in pollen grain volume (WILLEMSE & REZNICKOVA 1980).

BANERJEE et al. (1965) suggested that the exine possesses elasticity which allows it to enlarge its surface according to the change in cell volume. This property of the exine may be attributed mainly to the nexine. The nexine differs from the major part of the sexine in its composition and staining characteristics and shows channels in periods of increase of cell volume (WILLEMSE & REZNICKOVA 1980). Deposition of sporopollenin on the sexine continues from the stage of the young pollen grain to the stage of the pollen grain with a lens-like generative cell during the constant increase in cell volume (WILLEMSE & REZNICKOVA 1980).

Consolidation of the exine structure may also be due to accretion of sporopollenin on the outer surface of the nexine. Sporopollenin is deposited here in the form of globules and membrane-like lamellae, their number being increased constantly. During the post-mitotic stages a reticulate structure develops. it is clearly seen at the stage of the pollen grain with a lens-like generative cell, by the time cell growth is complete.

The data obtained in the present investigation showed that the size of the orbicules increases from the stage of the young microspore to the stage of the late vacuolated microspore. The size of the orbicules differs at each stage investigated. This variability increases at the post-mitotic stages of pollen development. Numerous small orbicules arise at these stages. Their diameter is 2.0–2.5 times less than that of the larger orbicules. These data correspond to the results of direct measurements (REZNICKOVA & WILLEMSE 1980).

In the scanning electron micrographs the orbicules look like compound round bodies. The character of their surface is different at the different stages of microsporogenesis and gametogenesis. At the stage of the young microspore the surface is composed of spheres. The spheres change into plates at the stage of the vacuolated microspore and become spheres again at the post-mitotic stages but of greater size. These changes in the appearance of the spheres may be related to the mode of sporopollenin deposition on the orbicules (REZNICKOVA & WILLEMSE 1980). Thus, the data obtained by scanning electron microscopy prove the existence of the post-mitotic phase in sporopollenin deposition in the exine of the *Lilium* pollen.

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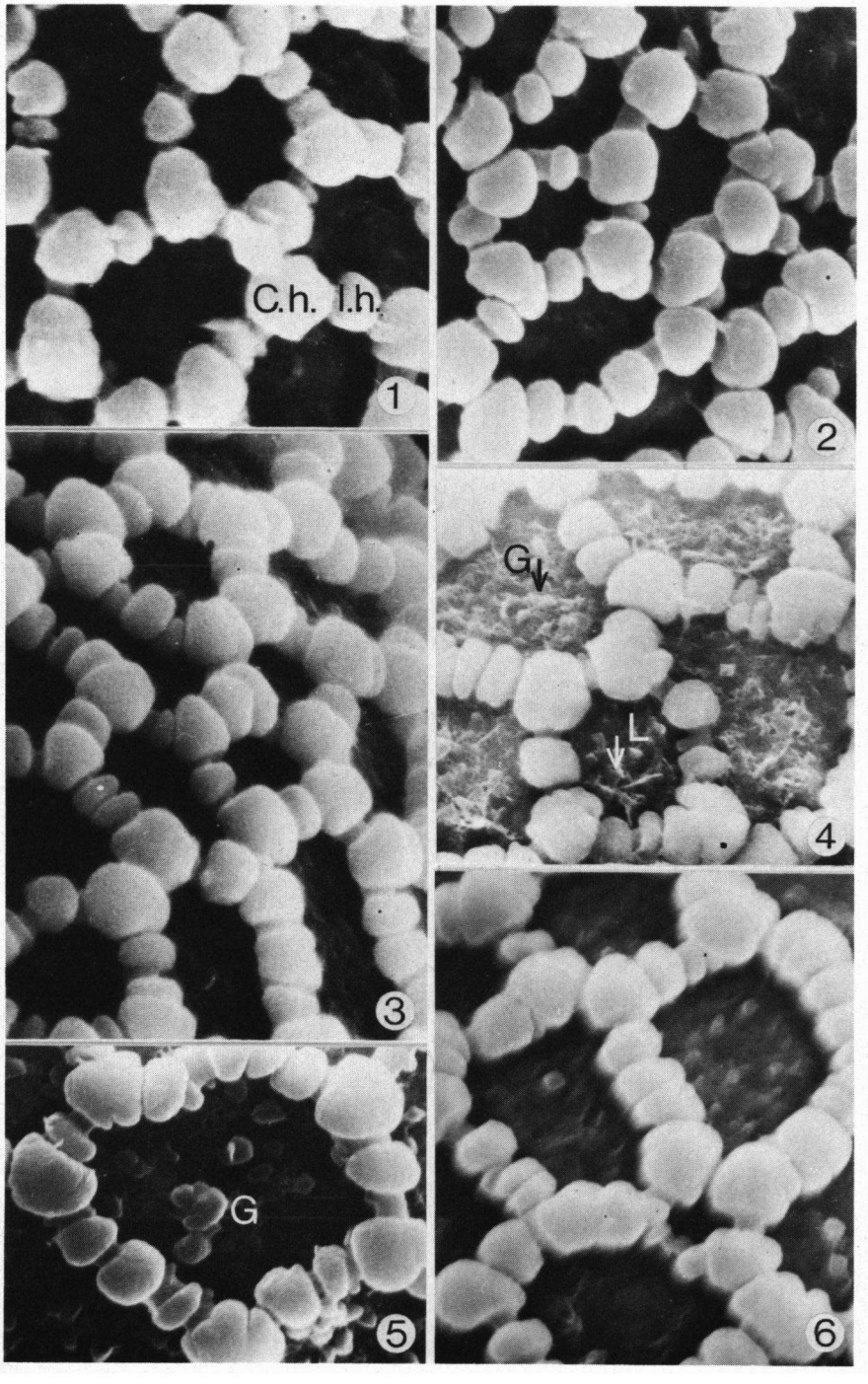
LEGENDS TO THE FIGURES

1. On the outer surface of the young microspore the distance between the heads is clear. C.h. – corner head, I.h. – intermediate head. × 5400.
2. At the vacuolated microspore stage the distance between the heads is shortened and the whole sexine looks more spherical. × 5400.
3. At the end of the stage of the vacuolated microspore the distance between the heads is minimal. × 5400.
4. Outer surface of the young pollen grain. The distance between the heads is increased due to a larger cell volume. The membrane-like lamellae (L) covered with sporopollenin and a first sign of globules (G) are seen on the surface of the enclosures. × 5400.
5. Pollen grain with a lens-like generative cell, sporopollenin globules are present on the surface of the enclosures. The sexine looks more spherical. × 5400.
6. Outer surface of the pollen grain two days before anthesis. All the heads are in contact with each other. × 5400.

Plate II.

7. The stage of the vacuolated microspore, membrane-like lamellae (L) are present on the surface of the enclosures. × 5400.
8. At the stage of the pollen grain with a lens-like generative cell the reticulate formation (R.f.) is clearly visible on the surface of the enclosures. × 10.800.
9. At the stage of the young microspore the orbicules (0) and rod-like bodies (arrow) are present on the locular face of the tapetal cell. × 10.800.
10. At the stage of the late vacuolate microspores the structure of the orbicules is more flattened. × 10.800.
11. Stage of the pollen grain with the generative cell near the wall the structure of the orbicules is more round. Small orbicules (arrows) are present. The reticulate formation (R.f.) is seen at the base of the orbicules. × 10.800.
12. The locular surface of the anther two days before anthesis, the orbicules are linked by fibrils. × 10.800.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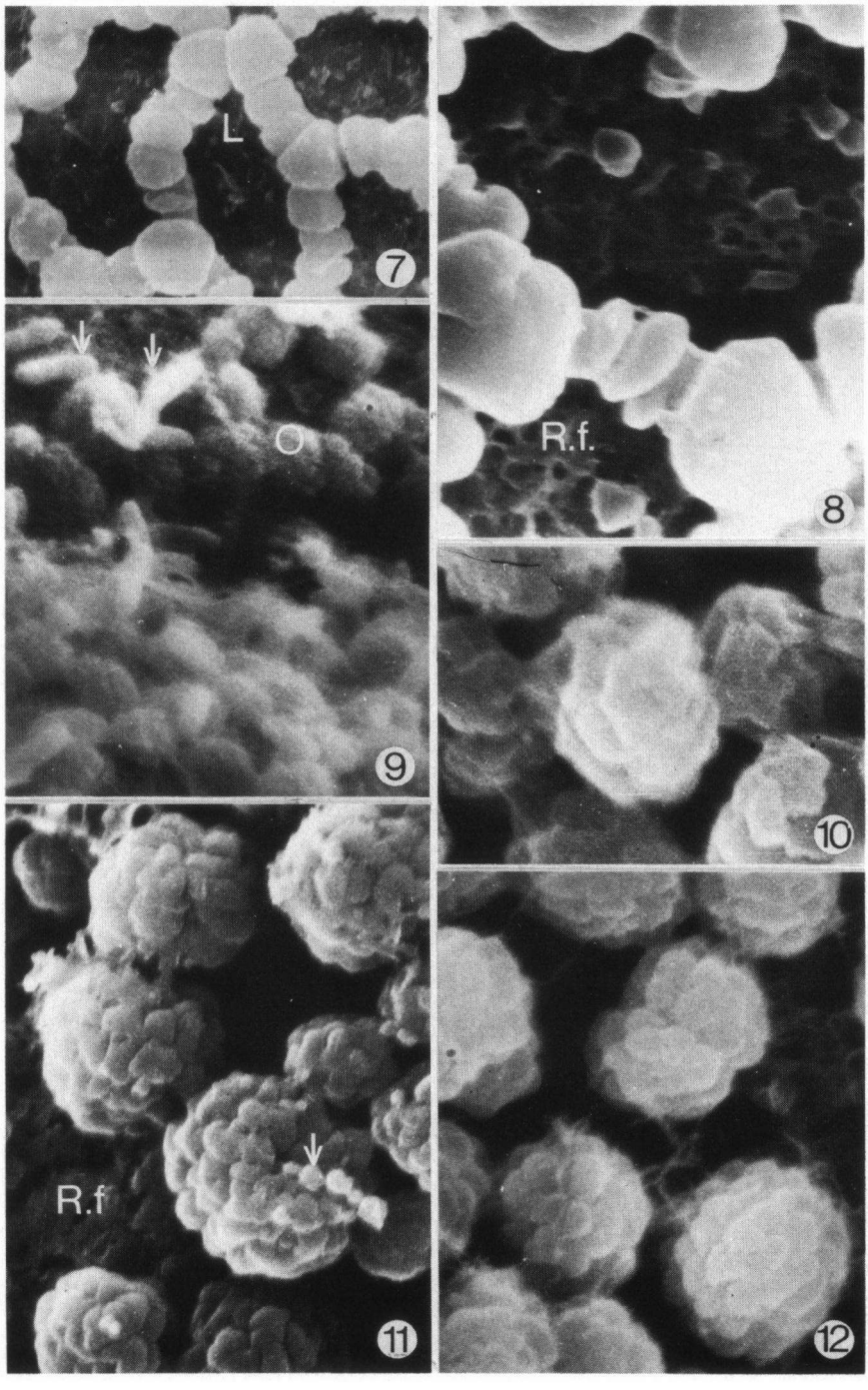
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