Acta Bot. Neerl. 40(4), December 1991, p. 319–328

The ultrastructure of mature *Papaver dubium* L. pollen grains

A. C. VAN AELST and J. L. VAN WENT

Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, 6703 BD Wageningen, The Netherlands

SUMMARY

The mature pollen of *Papaver dubium* is bicellular. The exine is composed of a nexine, bacula and a perforated tectum. The vegetative cell contains numerous mitochondria, small vesicles and ribosomes. Spherosomes, plastids and larger vesicles are present in relatively smaller numbers. Plastids do not contain starch. Dictyosomes are very low in number. RER is extensively present and has a stacked configuration, while single RER cisterns are located along and close to the surface of the plasma membrane. The generative cell is spindle shaped with tail-like extensions, and it shows an undulating outline in transverse sections. Bundles of microtubules are present in the outward bends of the cell. The generative cell contains a reduced number of mitochondria, ribosomes, ER and vesicles, and it does not contain plastids.

Key-words: generative cell, *Papaver dubium*, pollen grain, ultrastructure, vegetative cell.

INTRODUCTION

Pollen grains and pollen tubes function as carriers of generative cells or sperm cells. The vegetative cell of the pollen grain is regarded as a storage cell, which contains a high amount of reserve substances needed for extensive cell growth and wall synthesis during germination and pollen tube growth (Shivanna & Johri 1985). Among species there are considerable differences in length of the incubation period needed for the pollen to germinate (lag period). There are also prominent differences in the cytoplasmic composition of the vegetative cells among species, especially with respect to the number of dictyosome vesicles at the mature pollen stage. It is likely that these differences in the ultratructure of the cytoplasm among species are related to the specific physiology of the pollen grain and its performance during the lag period. The presence of a high amount of Golgi-vesicles seems to be related with a short lag time (Van Went 1974). In this paper, the ultrastructure of mature pollen grains of *Papaver dubium* L. is presented and discussed in view of its rapid germination which can take place within about 20 min (Hoekstra 1986).

MATERIALS AND METHODS

Mature pollen of field-grown *Papaver dubium* L. was dried for 24 h over silicagel at 20°C and subsequently stored for 4 months at −20°C. For chemical fixation and germination

tests the pollen was rehydrated at 20°C in moistured air (RH = 100%) for 1 h to avoid imbibitional damage (Hoekstra 1984). The vitality of the rehydrated pollen was controlled by determining the germination percentage on solid medium (according to Hoekstra & Van Roekel 1988). Only pollen of samples with a germination percentage of at least 85% were fixed.

For transmission electron microscopy, rehydrated pollen was fixed for 1·5 h at room temperature in 5% glutaraldehyde in 0·1 M phosphate buffer (pH 7·1) containing 0·44 M sucrose, and postfixed for 1 h in 1% osmium tetroxide in the same buffer and sucrose concentration. The 0·44 M sucrose was used during the fixation to create an isotonic condition. The samples were poststained with 1% uranyl acetate in 70% ethanol for 1 h during the dehydration procedure. Following dehydration in an ethanol series, the pollen was embedded in Epon 812. Thin sections were stained with a LKB ultrastainer, using standard solutions of uranyl acetate for 15 min at 35°C and lead citrate for 30 s at 20°C. For freeze fracturing, rehydrated pollen was mixed with 30% glycerol. Samples were frozen in liquid propane. The frozen samples were fractured and shadowed in a BAF 400 (Balzers).

For scanning electron microscopy, dried pollen grains were sputter-coated with gold palladium and observed directly.

For low-temperature scanning electron microscopy, rehydrated pollen grains were mounted with tissue TEK II oct., frozen, and sputter coated with gold/palladium and observed at −130°C (Van Aelst *et al.* 1989).

Organelle section surface measurements were done on seven different micrographs of sectioned pollen grains with a total surface area of 400 μm^2. These measurements were realized with a Contron MOP-30 image analyser (Zeiss).

RESULTS

The mature, dried pollen grain of *Papaver dubium* is ellipsoidal in shape and tricolpate (Fig. 1a). The germinal pores are clearly visible as infoldings of the surface. In the hydrated condition the pollen grain is spherical and the germinal pores can be identified only by the presence of larger surface particles (Fig. 1b). The exine consists of three sub-layers, the nexine, bacula and tectum (Fig. 1c). The mature pollen grain is bicellular, and the cytoplasm of the vegetative cell does not contain large vacuoles. The generative cell is located near the vegetative nucleus (Fig. 2a).

The vegetative cell

In transverse section the vegetative nucleus is sickle-shaped with a lobed surface (Fig. 2a). The nuclear envelope has a large number of randomly distributed nuclear pores (Fig. 2b). The nucleus is highly heterochromatic, and the heterochromatic regions consist of two different phases. There are areas with homogeneous electron density and areas with highly electron-dense small granules (Fig. 2c).

The cytosol of the vegetative cell is rather electron dense, which diminishes the visibility of the membranes. The plastids are spherical and have only few thylakoids and no starch. Mitochondria are numerous (about 8 per 10 μm^2) and evenly distributed. They are spherical and have well developed cristae (Fig. 3).

Two classes of vesicles are present in the vegetative cytoplasm. The first class (larger vesicles) comprises irregularly shaped vesicles with an average section surface of 0·097 μm^2 (SD 0·045μm^2) (350 nm diameter) (Fig. 3, l_1), and spherical vesicles with an

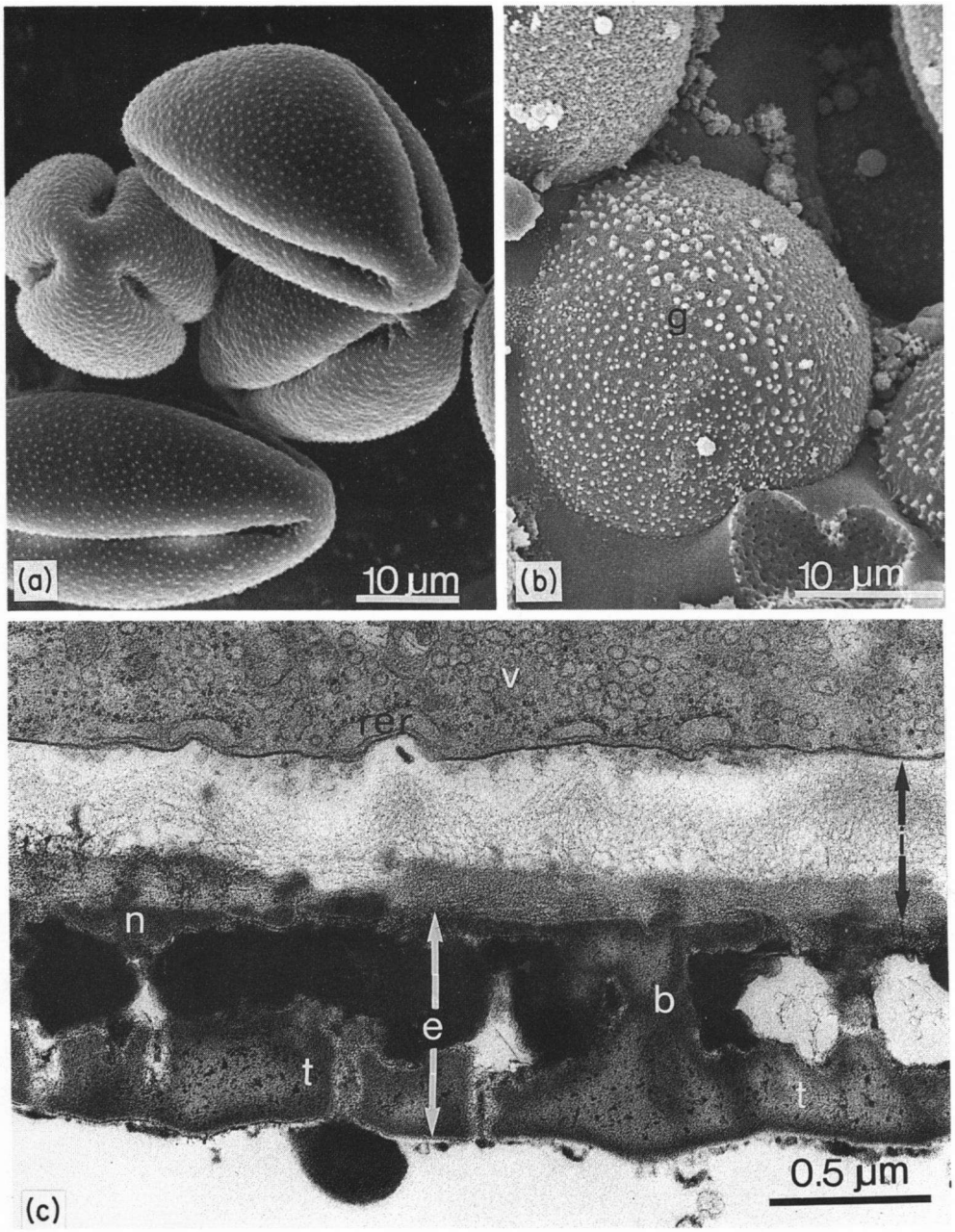

Fig. 1. (a) Scanning electron micrograph of dried mature pollen grains of *Papaver dubium*. × 1400. (b) Low temperature scanning electron micrograph of a fully hydrated pollen grain. g = germinal pore. × 2000. (c) Detail of the pollen wall and peripheric region of the vegetative cytoplasm. e = exine, n = nexine, b = bacula, t = tectum, i = intine, rer = rough endoplasmatic reticulum, v = small vesicle. × 45 000.

average section surface of 0·032 µm^2 (SD 0·016 µm^2) (200 nm diameter) (Fig. 4a, l_2). In both types of vesicles some fibrillar material can be observed. The second class is formed

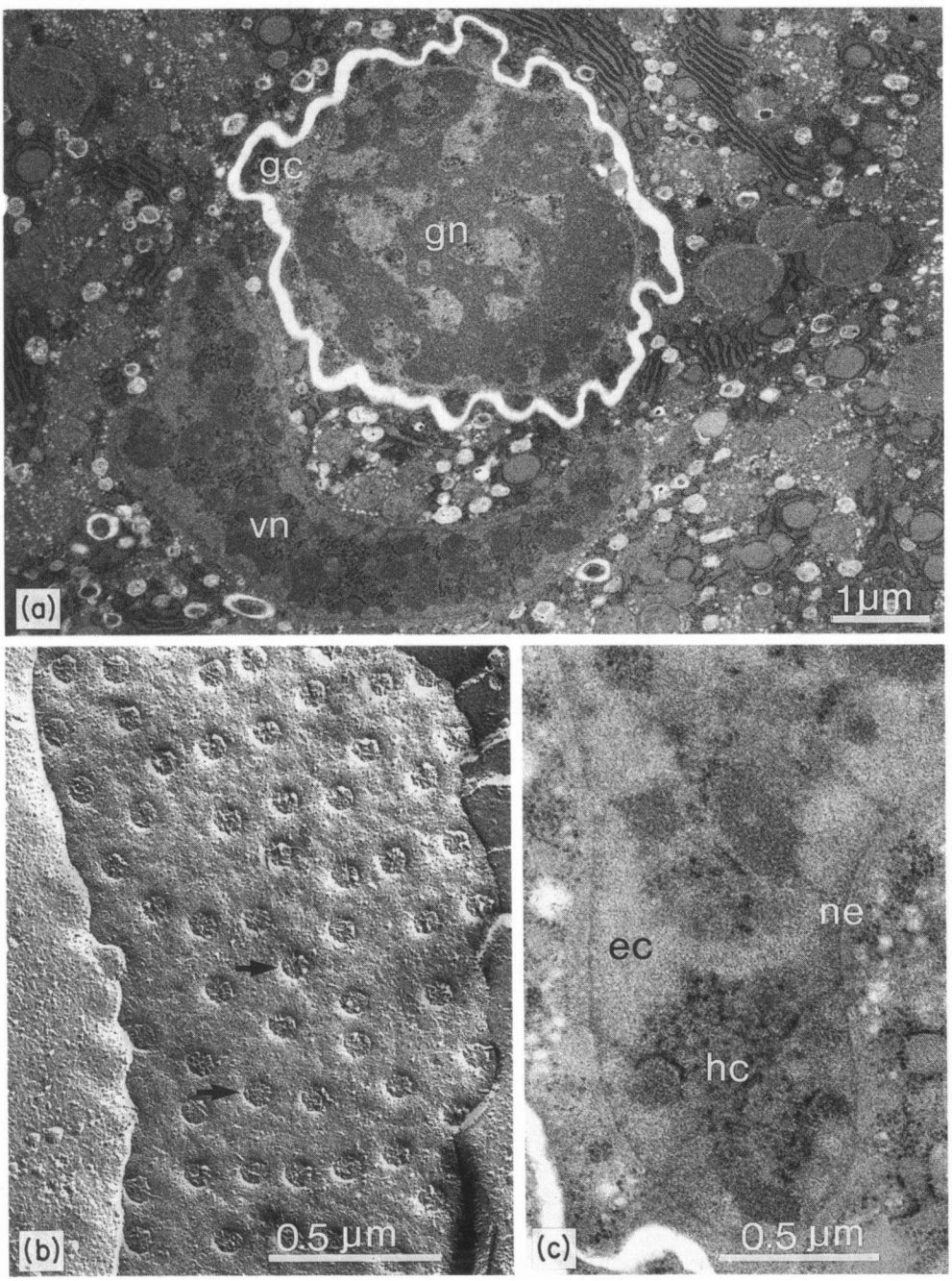

Fig. 2. (a) Detail of vegetative nucleus (vn), generative cell (gc) and generative nucleus (gn) in hydrated pollen grain of *Papaver dubium*. × 13 500.(b) Freeze-fracture image of the nuclear envelope of the vegetative nucleus. Arrows point to nuclear pores. × 46 000. (c) Detail of the vegetative nuclear matrix. Euchromatic region and two different heterochromatic phases are present. ne = nuclear envelope, hc = heterochromatin, ec = euchromatin × 43 000.

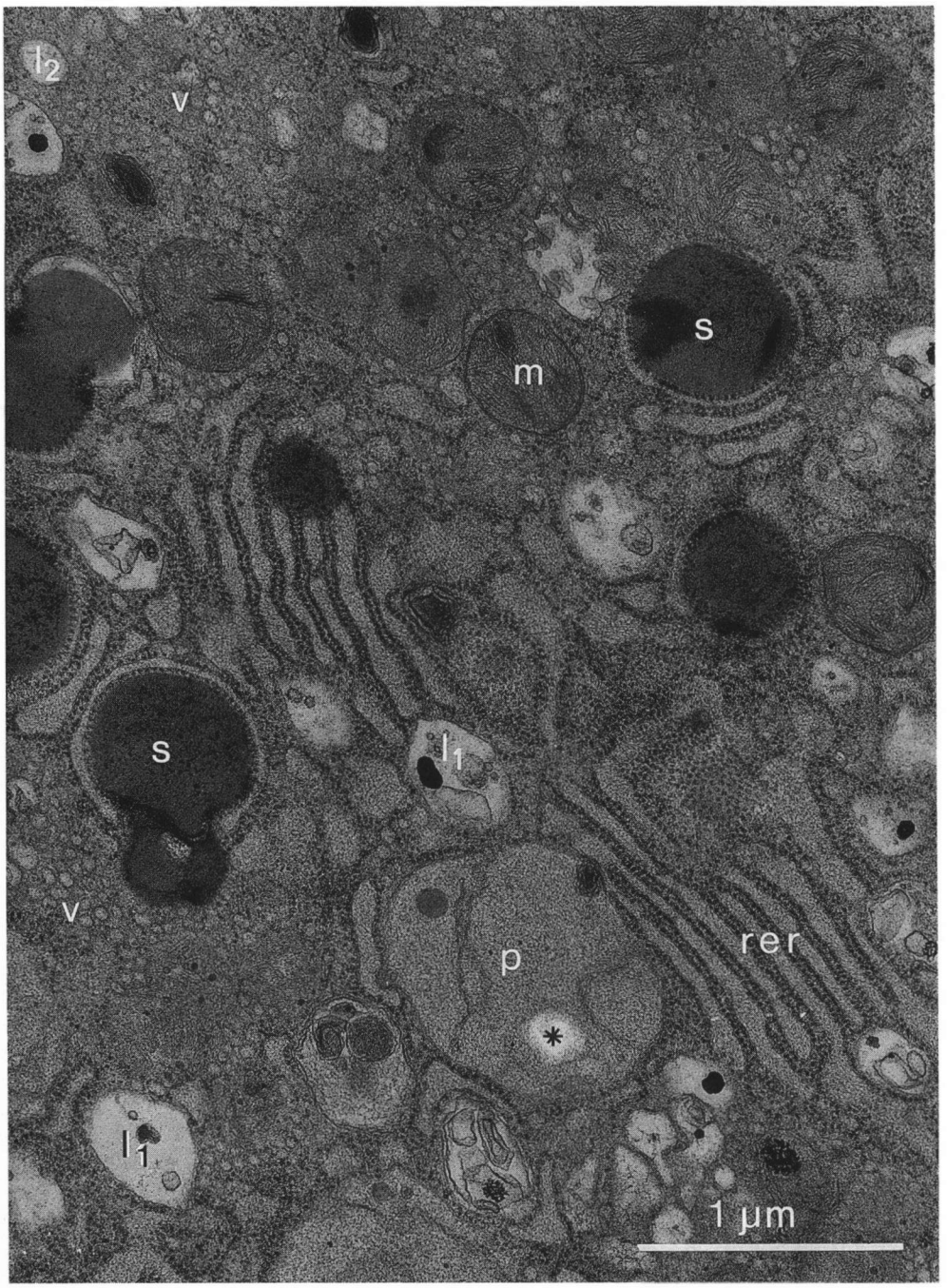

Fig. 3. Detail of the cytoplasm of the vegetative cell in hydrated pollen grain of *Papaver dubium*. p = plastid with a small starch grain (*), m = mitochondrion, l_1 = large irregular vesicle, l_2 = large spherical vesicle, v = small vesicle, s = spherosome, rer = rough endoplasmatic reticulum. × 35 000.

by much smaller vesicles (1921 nm^2/SD 480 nm^2) (50 nm diameter) which have an electron-translucent content. The number of these small vesicles is very high, 186 per

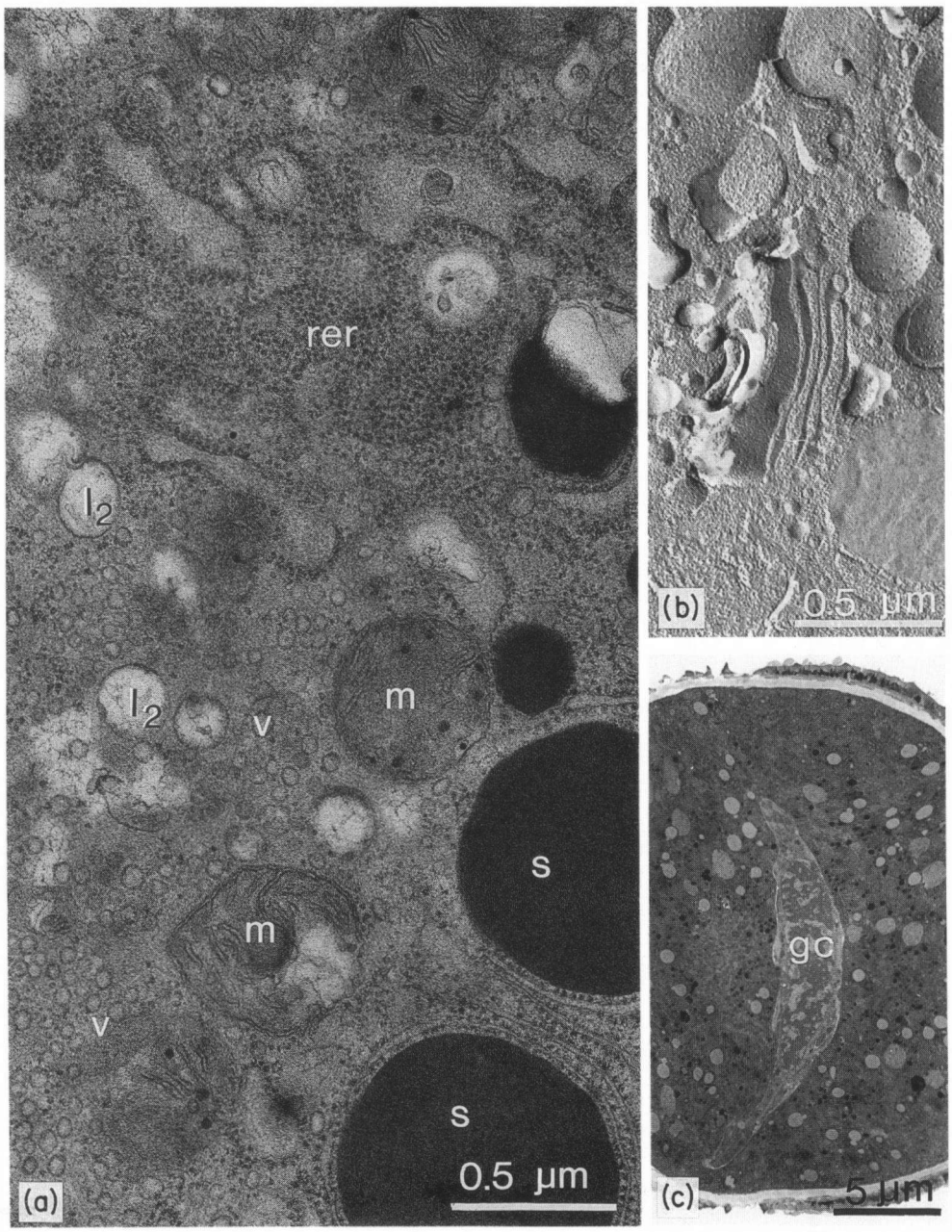

Fig. 4. (a) Detail of the cytoplasm of the vegetative cell in hydrated pollen grain of *Papaver dubium*. m = mitochondrion, l_2 = large spherical vesicle, v = small vesicle, s = spherosome, rer = rough endoplasmic reticulum. × 45 000. (b) Freeze-fracture image of a dictyosome in the vegetative cell. × 46 000. (c) Section showing the spindle shape of the generative cell (gn). × 3100.

10 µm^2 (SD 75) (Figs 3 and 4a). Dictyosomes are low in number, and they are small with only few cisterns (Fig. 4b). The cytoplasm contains spherosomes of uniform size and

round profile (Figs 3 and 4a). Rough endoplasmic reticulum (RER) is extensively present. Many of the cisterns are arranged in stacks. Others, however, are single or in pairs frequently associated with spherosomes and plastids. Remarkably is the presence of single RER cisterns along the major portion of the surface of the plasma membrane bordering the pollen wall. Of this RER cistern only the membrane facing the cytoplasm bears ribosomes (Fig. 1c). The cisterns are slightly swollen and contain a moderately electron-dense matrix (Figs 3 and 4a). The average section surface percentages of a number of cell organelles were measured and calculated: plastids 4·3% (SD 1·6%); spherosomes 9·81% (SD 5·7%); mitochondria 6·9% (SD 2·1%); RER 23·2% (SD 4·6%); large vesicles 5·4% (SD 1·5%); small vesicles 3·5% (SD 1·4%); (Fig. 6).

The cell constituents appear to be unevenly distributed. Especially the RER cisterns, spherosomes and plastids show a tendency to be arranged in clusters. These clusters themselves are randomly distributed in the vegetative cell (Fig. 3).

The generative cell

The generative cell is curved and spindle-shaped (Fig. 4c). In transverse sections, the central region of the generative cell has a round profile with an undulating outer surface (Figs 2a and 5a). The distance between the plasma membranes of the generative cell and the vegetative cell is rather uniform in size and about 60–100 nm. The space between the two plasma membranes is highly electron translucent. In freeze fracture replicas the distance between the two plasma membranes is found to be very small and regular (Fig. 5b). Cytoplasmic connections as pores or plasmodesmata between the vegetative and the generative cell were not found.

The cytoplasm of the generative cell contains mitochondria, microtubules, ribosomes, ER and vesicles. Plastids have not been observed. The generative nucleus contains large areas with heterochromatin and smaller areas with euchromatin. In the euchromatic areas some granular structures are found (Fig. 5a). Microtubules are abundantly present in the peripheral region of the cytoplasm and oriented parallel to the long axis of the cell. Most of the microtubules are arranged in bundles and seem to be predominantly located in the outward bends of the generative cell. Also the tail-like cell extensions contain bundles of microtubules, oriented parallel to the tail direction (Fig. 5c).

DISCUSSION

Our results show that mature *Papaver* pollen have a specific ultrastructure, characterized by the presence of extensive RER and numerous small vesicles. In these respects, *Papaver* pollen appears to be an intermediate pollen type, in comparison to pollen species as *Nicotiana* (Cresti *et al.* 1985) and *Linaria* (Cresti *et al.* 1988), which are marked by the presence of extensive RER and few small vesicles, and pollen of species as *Impatiens* (Van Went 1974) which contain an extremely high number of small vesicles and only a few RER cisterns. The small vesicles are supposed to be derived from the dictyosomes and to be involved in the future exocytosis of cell wall precursors. The vesicles in the mature pollen grain could be regarded as a stock of wall precursors used during pollen germination and early pollen tube growth. It was proposed earlier (Van Went 1978) that there might be a relation between the length of the activation period needed for pollen germination and the presence and number of small vesicles at the mature pollen stage. Pollen with few small vesicles at the mature stage are supposed to need a longer period, whereas pollen with abundant small vesicles may germinate very rapidly (within a few

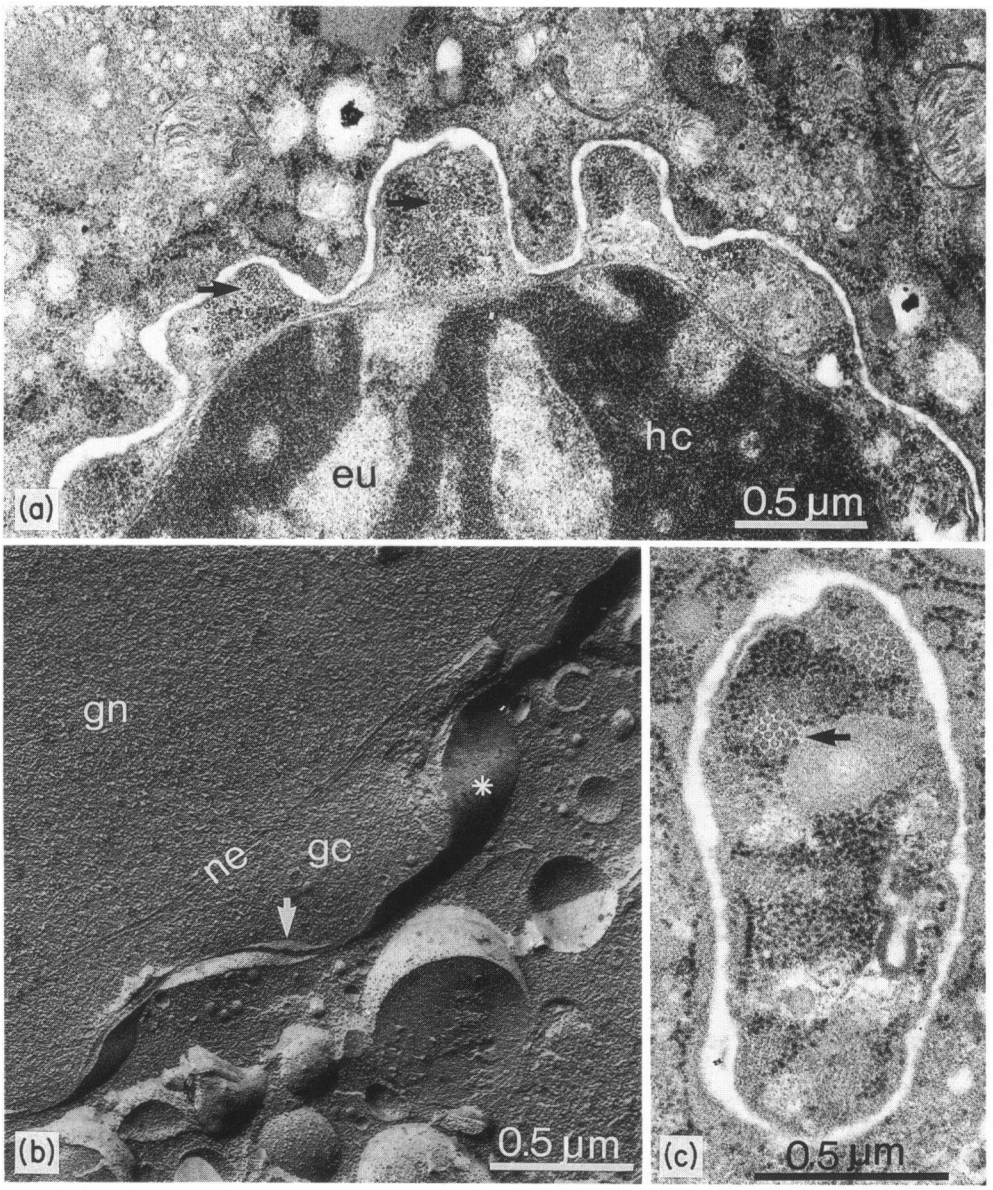

Fig. 5. (a) Detail of the generative cell and generative nucleus in hydrated pollen grain of *Papaver dubium*. In the generative nucleus euchromatin (eu) and heterochromatin (hc) is present. Microtubules (arrow) are arranged parallel to the long axis of the cell and located predominantly in the outward bends of the cell surface. × 35 000. (b) Freeze-fracture image of the generative cell. The plasma membranes of the generative cell (arrow) and vegetative cell (asterisk) are very close to each other. gc = generative cell, gn = generative nucleus, ne = nuclear envelope. × 38 000. (c) Transverse section through tail-like part of the generative cell showing bundles of microtubules (arrow). × 51 000.

minutes). *Papaver* pollen are reported to germinate after 20 minutes of incubation on a solid germination medium (Hoekstra 1986). The actual activation time might be longer, because of the foregoing rehydration period in moistured air. However, such an activation

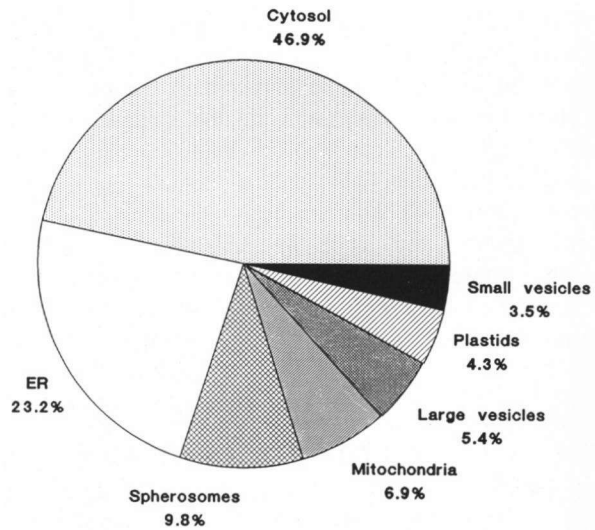

Fig. 6. Diagram of the mean section surface percentages of the cell constituents in the vegetative cell of the pollen grain of *Papaver dubium*.

time is still relatively short. The observed combination of rapid germination and presence of numerous Golgi-vesicles in the mature *Papaver* pollen support the fore-mentioned hypothesis.

Most of the extensive RER in the *Papaver* pollen is arranged in stacks. There are, however, also single RER cisterns, many of which are surrounding the spherosomes and plastids, or located along the plasma membrane of the vegetative cell. It has been proposed that the stacked RER configuration is related to an inactive state of the pollen grain. During activation of the pollen of *Nicotiana* the RER stacks disappear, and the cisterns become dispersed in the cytoplasm (Cresti *et al.* 1985). The presence of both stacked and single RER cisterns in the rehydrated *Papaver* pollen may indicate that during the rehydration period activation of the pollen has already started. The association of RER cisterns with spherosomes, plastids and the plasma membrane may be indicative for functional relationships. The RER–spherosomes–plastids association could be involved in the mobilizing and processing of the stored lipids. The RER–plasma membrane association may be involved in the local regulation of the Ca^{2+} metabolism in the cell periphery (Quader 1990). Besides the small vesicles in the rehydrated *Papaver* pollen also a second class of vesicles is present. These vesicles are larger and appear to be present in two types; irregularly shaped vesicles and spherical vesicles. Both types of vesicles appear to have the same content, and they are supposed to be closely related. In our opinion the irregularly shaped vesicles are fusion products of the spherical vesicles. Such fusion may be induced by rehydration, and reflect the formation of larger vacuoles during the start of pollen activation and germination.

The generative cell of *Papaver dubium* contains a rather small amount of cytoplasm, a relatively large portion of which contains only ribosomes and microtubules. Bundles of microtubules are common in generative cells (Cresti *et al.* 1984; Lancelle *et al.* 1987; Ciampolini *et al.* 1988). The position of these bundles of microtubules in the outward bends of the cell and in the tail-like extensions support the idea of microbules involvement in the shaping of the generative cell (Heslop-Harrison *et al.* 1988). Clearly, changes in

shape of isolated sperm cells from spindle-shape to spherical are associated with the disappearance of the microtubules (Tanaka 1988; Theunis & Van Went 1990).

In our chemically fixed material the space between the plasma membranes of the vegetative cell and the generative cell is quite wide and electron-translucent, as is usually observed after chemical fixation. However, observations with cryo-scanning electron microscopy shows a close contact between the two membranes (Van Aelst *et al.* 1989). Also freeze substitution methods confirm that the space between vegetative and generative plasma membrane is very small, and in addition that the space between the membranes is filled with material of which the nature is unknown (Cresti *et al.* 1987; Van Went & Gori 1989; Tanaka 1988). The few fibrillar inclusions that we sometimes found could be remnants of this material.

ACKNOWLEDGEMENTS

The authors wish to thank T. van Roekel and Dr F. A. Hoekstra for providing the dried freeze-stored pollen, and S. Massalt for making the prints. This research was carried out in the framework of contract no BAP 0202-NL of the Commission of the European Communities.

REFERENCES

Ciampolini, F., Moscatelli, A. & Cresti, M. (1988): Ultrastructural features of *Aloe ciliaris* pollen. I. Mature grain and its activation in vitro. *Plant Sex. Reprod.* **1:** 88–96.

Cresti, M., Ciampolini, F. & Kapil, R.N. (1984): Generative cells of some angiosperms with particular emphasis on their microtubles. *J. of Submicrosc. Cytol.* **16:** 317–326.

—, —, Mulcahy, D.L.M. & Mulcahy, G. (1985): Ultrastructure of *Nicotiana alata* pollen, its germination and early tube formation. *Amer. J. Bot.***72:** 719–727.

—, Lancelle, S.A. & Hepler, P.K. (1987): Structure of the generative cell wall complex after freeze substitution in pollen tubes of *Nicotiana* and *Impatiens*. *J. Cell Sci.*, **88:** 373–378.

—, Milanesi, C., Tiezzi, A., Ciampolini, F. & Moscatelli, A. (1988): Ultrastructure of *Linaria vulgaris* pollen grains. *Acta Bot. Neerl.* **37:** 379–386.

Heslop-Harrison, J., Heslop-Harrison, Y., Cresti, M., Tiezzi, A. & Moscatelli, A. (1988): Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. *J. Cell. Sci.* **91:** 49–60.

Hoekstra, F.A. (1984): Imbibitional chilling injury in pollen: involvement of the respiratory chain. *Plant Physiol.* **74:** 815–821.

— (1986): Water content in relation to stress in pollen. In: Leopold, A.C. (ed.): *Membranes, metabolism and dry organisms.* 102–122. Comstock, Ithaca, New York.

— & Van Roekel, T. (1988): Desiccation tolerance of *Papaver dubium* L. pollen during its development in anther. *Plant Physiol.* **88:** 626–632.

Lancelle, S.A., Cresti, M. & Hepler, P.K. (1987): Ultrastructure of the cytoskeleton in freeze-substituted pollen tubes of *Nicotiana alata*. *Protoplasma* **140:** 141–150.

Quader, H. (1990): Disintegration of cisternae of the endoplasmatic reticulum visualized by confocal laser scanning microscopy: evidence for the involvement of calcium and the cytoskeleton. *Protoplasma* **155:** 166–175.

Shivanna, K.R. & Johri, B.N. (1985): *The Pollen Grain. Structure and function.* Wiley Eastern, New Delhi.

Tanaka, J. (1988): Isolation of generative cells and their protoplasts from pollen of *Lilium longiflorum*. *Protoplasma* **142:** 68–73.

Theunis, C.H. & Van Went, J.L. (1990): Isolated sperm cells of *Spinacia oleracea* L. In: Barnabas, B. and Liszt, K. (eds): *Characterization of Male Transmission units in Higher plants.* 25–30. MTA Copy, Budapest.

Van Aelst, A.C., Mueller, T., Dueggelin, M. & Guggenheim, R., (1989): Three dimensional observations on freeze-fractured frozen hydrated *Papaver dubium* pollen with electron microscopy. *Acta Bot. Neerl.* **38:** 25–30.

Van Went, J.L. (1974): The ultrastructure of *Impatiens* pollen. In: Linskens, H.F. (ed.): *Fertilization in Higher Plants.* 81–88. North-Holland Publ. Company, Amsterdam.

— (1978): The ultrastructure of *Impatiens* pollen tubes. *Soc. Bot. Fr. Actualites Botaniques* **125:** 149–153.

— & Gori, P. (1989): The ultrastructure of *Capparis spinosa* pollen grains. *J. Submicrosc. Cytol. Pathol.* **21:** 149–156.