Acta Bot. Neerl. 32 (3), May 1983, p. 177–183.

ULTRASTRUCTURE OF S_3 S_4 GENOTYPE POLLEN GRAINS OF OENOTHERA ORGANENSIS*

M. CRESTI[1], F. CIAMPOLINI[1] and R. N. KAPIL[2]

[1] Department of Botany, University of Siena, Siena 53100, Italy
[2] Department of Botany, University of Delhi, Delhi 110007, India

SUMMARY

The exine of the S_3S_4 genotype pollen grains of *O. organensis* is furnished with beaded viscin threads as an efficient device for their transport by pollinating agents. The sexine structure is expedient for absorption of water and stigmatic exudate. The vegetative cell cytoplasm has the pertinent organelle system for the synthesis of starch, lipids, proteins and polysaccharides. The generative cell is, however, dormant but has a normal complement of subcellular bodies capable to resume their activity efficaciously after pollen hydration and activation; and does not transmit paternal plastome to the zygote.

1. INTRODUCTION

It is well recognized that in *Oenothera organensis* the pollen grains with a certain S-factor are unable to produce a functional tube in the stigma and style with the same allele, and the action of this factor is gametophytically controlled (see CLELAND 1972, DE NETTANCOURT 1977 and references therein). It is also known at the light microscopic level that in some heterogeneous races of *Oenothera* two distinct classes of pollen grains – active and nonactive – exist. The present research, first in the series of a larger programme, to correlate various pollen genotypes with their subcellular characteristics as well as with their fertility, was initiated for a better understanding of the incompatibility phenomenon in this plant. The ultrastructural features of pollen grains of the self-incompatible S_3S_4 genotype are described here in the first instance.

2. MATERIAL AND METHODS

The seedlings of *Oenothera organensis* (genotype S_3S_4) were supplied by K. SREE RAMULU (Euratom-Ital, Wageningen, The Netherlands). They were grown in the Botanical Garden of Siena University and pollen grains gathered from the mature anthers of these plants.

The scanning electron micrographs were made with the help of a Philips 501 B SEM, at 7.2 kV, from fresh pollen grains mounted on aluminium stubs with biadhesive tape, after sputter-coating with gold.

*Research supported by CNR Grant no. 81.00352/04 – Cytomorphology.

For transmission electron microscopy, the pollen grains were prefixed in 3% glutaraldehyde in 0.066M cacodylate buffer, pH 7.2, for 45 min., at room temperature. After rinsing in the same buffer, the material was post-fixed in 1% OsO_4. Following dehydration in ethanol and embedding in SPURR's ERL, sections were cut on LKB Ultrotome III using a diamond knife, and stained with uranyl acetate and lead citrate. The grids were examined with a Zeiss EM 9A electron microscope at 60 kV.

3. OBSERVATIONS

3.1. External morphology

According to the terminology of MOORE & WEBB (1978), the pollen grains of *O. organensis* are trizonoporate, with large vestibulate pores (*fig. 1*). The exine is granulate (*fig. 2*), thickest in the apoporium region, gradually becoming thin towards the poral margins and is almost undifferentiated in the poral region (*fig. 2*).

In lower magnifications, a large number of viscin threads appear to be arising away from the middle portion of the pollen grain, more in the vicinity of the zone bordering the poral area (*fig. 1*). They frequently appear entangled (*fig. 1*) and are sometimes seen to be having swollen ends. Individual threads have a diameter of approximately 0.35–0.40 μm, and when highly magnified, appear beaded, exhibiting remarkable morphological similarity with the exine surface. Often they occur singly but couple of them may sometimes be twisted together (*fig. 2*).

3.2. Internal morphology

3.2.1. Pollen wall

Ultrathin sections reveal three regions of the sexine – an outermost, electron-dense, smooth part of tectum at places draped by a thin tryphine-like layer (arrows); covering almost equally-spaced spherical, lobed or elongated subunits of sporopollenin connected with each other so as to produce a loose reticulum that sometimes touches the nexine forming spongy columellae of irregular shapes and sizes (*fig. 3*); and an electron translucent luminar region (L) which is probably filled with the stigmatic exudate at the time of the contact of the pollen grain with the stigma. The foot layer is indistinguishable (see also SKVARLA et al. 1976). The nexine (N) is thick, lightly stained, and fairly uniform (*fig. 3*). The intine is, however, thin but denser than the nexine, and granular in nature because of the presence of numerous membranous, fibrillar and vesicular bodies (*fig. 3, I*). It is in close contact with the electron dense plasma membrane.

3.2.2. Vegetative cell

The cytoplasm is characterized by abundant cell organelles and inclusions, and most prominent of these are the plentiful spindle-shaped, amyloplasts completely filled with starch of the same shape (*fig. 4*). A large number of lipidic bodies, of various sizes, are also very conspicuous (*fig. 4*) and are often seen in close

association with RER cisternae (*fig. 5*) which occasionally become enlarged and contain electron dense material. Accumulation of lipid droplets near the plasma membrane is quite common. Golgi bodies are composed of 5–7, flattened, dictyosomic cisternae, abundantly distributed in the cytoplasm (*Fig. 5*). They seem to be producing two types of vesicles (*fig. 6*) – the smaller (0.075–0.080 μm) with electron dense contents and the larger (0.40–0.50 μm) possessing fibrillar material – that are sometimes observed to fuse. Rough endoplasmic reticulum and ribosomes, often aggregated into polysomes, are many times situated near to the amyloplasts (*fig. 6*). Mitochondria are large and spherical; and innumerable small vacuoles gorge the cell cytoplasm (*fig. 4*).

The vegetative nucleus is large, and irregularly invaginated. It contains one small nucleolus and weakly electron dense chromatin.

3.2.3. Generative cell

This cell is highly lobed and located in the central part of the pollen grain. It is surrounded by two plasma membranes with an electron translucent space between them (*fig. 7*). The nucleus too is lobed and possesses one nucleolus. A normal complement of round mitochondria, dictyosomes producing vesicles, free ribosomes and rarely polysomes, but very few cisternae of RER, is visible (*fig. 7*). Plasmodesmata connections between the generative cell and the vegetative cell cytoplasm, plastids and microtubules were not encountered in any of the micrographs examined.

4. DISCUSSION

SKVARLA et al. (1976) have described and illustrated the viscin threads on the pollen grains of several species of *Oenothera* but *O. organensis* finds no mention. The threads in this taxon have a close morphological resemblance with those of *O. hookeri* but they are neither grouped together, like in the latter, nor do they arise from the main body of the pollen grain. According to these authors, beaded viscin threads constitute a particularly efficient system for passive adhesion of pollen to the bodies of pollinating agents, not concerned with it as a food source, so that S_3S_4 genotype pollen grains have the certainty of being appropriately transported because of the presence of this feature. Additionally, the 'spongy' nature of the sexine is advantageous too, for quick absorption of sufficient amount of water and stigmatic exudate for their hydration and activation. The role of the tryphine layer, which is considered to contain some proteins responsible for incompatibility reaction, and in *Raphanus* (a self-incompatible system) produces thick bands of callose around the stigmatic papillae (see KAPIL & TIWARI 1978), requires to be histochemically examined in *O. organensis*.

The cytoplasm of the S_3S_4 pollen grains of *O. organensis* presents the picture of an active lipid-, starch-, and protein-synthesizing machinery of a fertile genotype. While the RER is engaged in producing large amounts of lipids (as against SER in *Forsythia,* DUMAS et al. 1974); the amyloplasts are engendering the characteristic spindle-shaped starch grains (which in *O. muricata* are indicative of

pollen fertility, RENNER 1919); and the ribosomes, polysomes and RER are concerned with the formation of proteins. Besides, the abundance of dictyosomes, vigorously breeding two kinds of vesicles which subsequently fuse, is suggestive of the existence, in advance, of the metabolic events leading to the generation of materials needed in the formation of the tube after pollen germination (see also KOZAR 1979). Conversely, the pollen grains of *Impatiens* (VAN WENT 1974) have a comparatively small number of ribosomes and dictyosomes and are poorly equipped for augmenting large quantities of proteins and polysaccharides.

The generative cell cytoplasm of *O. organensis*, at this stage, is relatively metabolically dormant but is suitably provided with organelles likely to revert to bustle after pollen hydration and activation. Some of these changes after hydration have been noticed in this species by DICKINSON & LAWSON (1975) who came across a striking increase in the number of small single-membraned vesicles and fibrillar bodies, large areas of ER rich in ribosomes, appearance of small but transitory vacuoles as well as in enzymatic activity. The microtubules too would probably appear after pollen activation as observed by CRESTI et al. (1983) in the generative cells of some other species. With only plasma membrane contact with the tube cell cytoplasm and in the absence of plasmodesmata and large intercellular connections seen by KOZAR (1979) in *Coryphanta*, the generative cell is more or less isolated, prior to its division to form the sperms, from the penetration of large molecular materials.

Transmission and presence of plastids in the generative cells of *Oenothera*, and in some other plants, have been referred to by DIERS (1963) and by CLAUHS & GRUN (1977; see also CRESTI et al. 1983) but their absence in *O. organensis* needs special mention since this genus is a classical example in which the plastids exhibit "continuity from one generation to another, specificity of behaviour, and genetic activity" (CLELAND 1972). The present investigation documents an evidence of non-production of mixed cells having egg and sperm plastids side by side, even after successful fertilization, so that the uniparental plastome-genome interaction is likely to maintain the characteristics of this S_3S_4 genotype, unless the plastids of the egg mutate.

ACKNOWLEDGEMENT

We express our gratitude to Professor G. Sarfatti for his counsel in preparing this manuscript and thank Mrs. Ida Clemente for secretarial help. A research grant from the CNR (Italy) enabled R. N. Kapil to participate in this investigation.

REFERENCES

CLAUHS, R. P. & P. GRUN, (1977): Changes in plastid and mitochondrion content during maturation of generative cells of *Solanum* (Solanaceae). *Amer. J. Bot.* **64**: 377–383.

CLELAND, R. E. (1972): *Oenothera – Cytogenetics and Evolution*, pp. 370. Academic Press: London, New York.

CRESTI, M., F. CIAMPOLINI & R. N. KAPIL (1983): Generative cells of some angiosperms with particular emphasis on their microtubules. *Protoplasma* (in press).

DE NETTANCOURT, D. (1977): *Incompatibility in Angiosperms*, pp. 230. Springer-Verlag: Berlin, Heidelberg, New York.

DIERS, L. (1963): Elektronenmikroskopische Beobachtungen an der generativen Zelle von Oenothera hookeri Torr. et Gray. *Z. Naturforschg.* **18b**: 562–566.

DICKINSON, H. G. & J. LAWSON (1975): Pollen tube growth in the stigma of Oenothera organensis following compatible and incompatible intraspecific pollinations. *Proc. R. Soc. Lond.* B **188**: 327–344.

DUMAS, C., A. PERRIN, M. ROUGIER & P. ZANDONELLA (1974): Some ultrastructural aspects of different vegetal glandular tissues. *Portugal. Acta Biol.* **14**: 501–520.

KAPIL, R. N. & S. TIWARI (1978): Plant embryological investigations and fluorescence microscopy: an assessment of integration. *Internat. Rev. Cytol.* **53**: 291–331.

KOZAR, F. (1979): Ultrastructure of pollen of Coryphanta vivipara. *Pollen et Spores* **21**: 5–15.

MOORE, P. D. & J. A. WEBB (1978): *An Illustrated Guide to Pollen Analysis*. pp. 133. Hodder and Stoughton: London, Sydney, Auckland, Toronto.

RENNER, O. (1919): Zur Biologie und Morphologie der männlichen Haplonten einigen Oenotheren. *Zeitschr. Bot.* **11**: 305–380.

SKVARLA, J. J., P. H. RAVEN & J. PARGLOWSKI (1976): Ultrastructural survey of Onagraceae pollen. In *The Evolutionary Significance of the Exine*, eds. I. K. FERGUSON & J. MULLER, pp. 447–480. Academic Press: London.

WENT, J. L. VAN (1974): The ultrastructure of Impatiens pollen. In *Fertilization in Higher Plants*, ed. H. F. LINSKENS, pp. 81–88. North Holland Publishing Company: Amsterdam, Oxford.

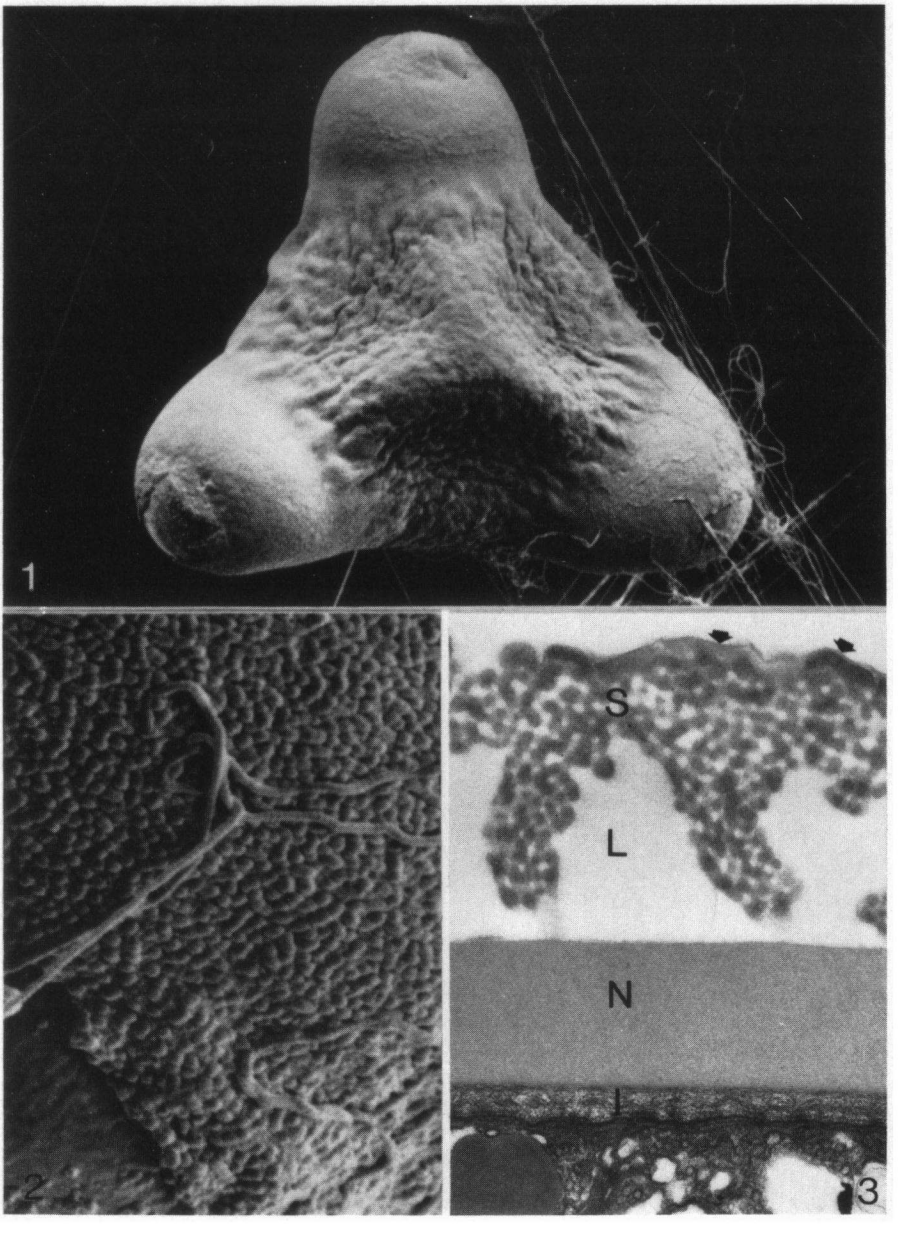

FIG. 1. *Oenothera organensis*, mature pollen with viscin threads. × 510.

Fig. 2. Magnification of exine. In the pore region exine is almost undifferentiated. Viscin threads are often single; two twisting threads are also seen. × 4,000.

Fig. 3. Pollen wall showing intine (I); lumina (L); nexine (N) and sexine (S). × 19,200.

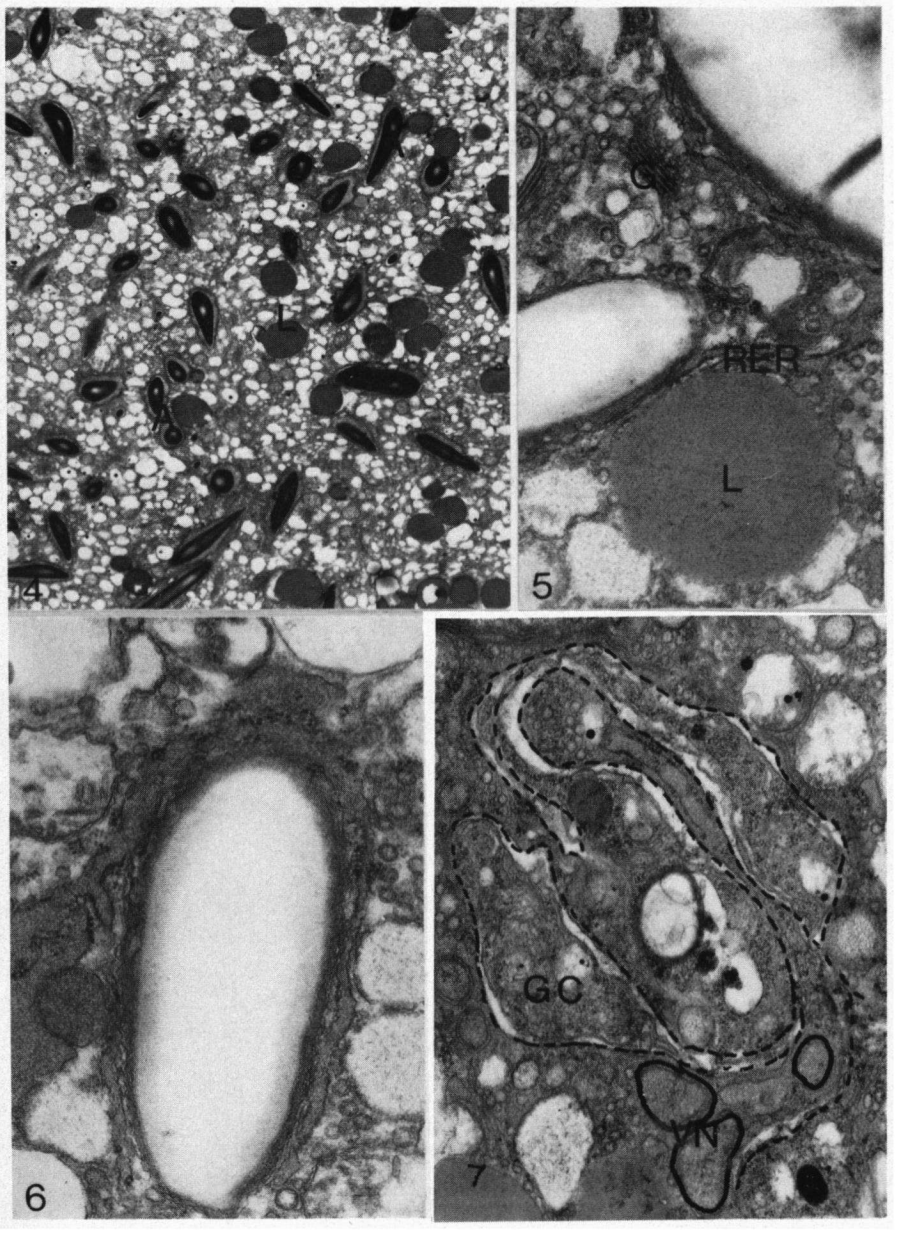

Fig. 4. General view of vegetative cytoplasm. (A) Amyloplast, (L) lipidic bodies. ×4,300.

Fig. 5. Golgi bodies (G) producing vesicles. Rough Endoplasmic Reticulum (RER) is associated with lipidic bodies in the vegetative cytoplasm. × 28,330.

Fig. 6. Amyloplast surrounded by ER; at places small vesicles seem to fuse with the larger ones (arrow). ×38,330.

Fig. 7. Portion of generative cell (GC - dotted line) and lobed nucleus of vegetative cell (VN - uninterrupted line). × 28,000.