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Ultrastructure of the egg apparatus of *Spinacia*

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Abstract

The egg apparatus of *Spinacia* was studied from the time the embryo sac reaches its maximal size to just before fertilization, i.e., until about 8-9 hours after pollination. At maturity each synergid has a large elongated nucleus and prominent chalazal vacuoles. Numerous mitochondria, plastids, dictyosomes, free ribosomes, rough endoplasmic reticulum (RER), and lipid bodies are present. The cell wall exists only around the micropylar half of the synergids and each cell has a distinct, striated filiform apparatus. In general, degeneration of one synergid starts after pollination. The egg cell has a spherical nucleus and nucleolus and a large micropylar vacuole. Numerous mitochondria, some plastids with starch grains, dictyosomes, free ribosomes, and RER are present. A continuous cell wall is absent around the chalazal end of the egg cell.

INTRODUCTION

The ovary has a single ovule, which is ortho-amphitropous, bitegmic and crassinucellate (Wilms, 1980). The fact that there is only one embryo sac per ovule means that pollen tube growth can be regulated after artificial pollination. When such an embryo sac is located below a multi-layered nucellar tissue, the exact moments of pollen tube discharge and fertilization can be localized rather easily.

In view of the limited information available on the process of fertilization in *Spinacia* the present study was undertaken to determine the ultrastructure of the egg apparatus prior to fertilization.

MATERIAL AND METHODS

Spinacia oleracea L., cv. Prévital was grown in the greenhouse of the Botanical Laboratory at Wageningen at ±25° C. The dissected ovaries were fixed in glutaraldehyde and osmium tetroxide (GA-OsO_4) and

embedded in Epon. A complete description of the precedure has been given earlier (Wilms, 1980). Observations were made on a Philips EM 301.

RESULTS

The two synergids and the egg cell are long, pear-shaped cells; the former partially surround the latter. The length of each cell is about 60 μm, whereas the width of the synergids is about 20 μm, and that of the egg cell is 10 μm at the micropylar part and about 25 μm at the chalazal part. Two-third of the egg apparatus at the chalazal pole is surrounded by the central cell. The boundaries of the egg apparatus vary considerably, from only two membranes at the chalazal side of the cells, to a specially thickened filiform apparatus (FA) at the micropylar pole of the synergids. All longitudinal walls of the cells of the egg apparatus have plasmodesmata near their micropylar region (Fig. 1).

The two synergids develop similarly until maturity. Each has chalazal vacuoles with mainly micropylar cytoplasm (Fig. 1). The nucleus is initially spherical and becomes elongated towards the FA. The total lenght of the nuclear envelope in median longitudinal sections increases approximately two-fold. During further development concentration of heterochromatin occurs but the nucleolus starts to disintegrate.

The synergid cytoplasm has a rich complement of organelles (Fig. 2) including plastids with few diminishing starch grains, some short thylakoids, mitochondria with well-developed cristae sometimes attached to ribosomes, dictyosomes with many vesicles and electron-dense contents, and RER as single cisternae or as short swollen lamellae. Free ribosomes are abundant but decrease later and form small clusters. The lipid bodies increase during maturation both in number and diameter. When degeneration of the synergid starts, generally after pollination, electron-dense material accumulates at the membranes of mitochondria and plastids. Subsequently the mitochondrial cristae collapse and disappear and the ER lamellae become disorganized. The nucleus diminishes in size and disintegrates, whereas lipid bodies develop and aggregate.

The egg cell has chalazal cytoplasm and large micropylar vacuoles. Its large nucleus, about 14 μm in diameter, is located in the chalazal cytoplasm and has a nucleolus with a diameter of approximately 6 μm. The karyoplasm often contains some myeline-like structures.

The chalazal cytoplasm contains large plastids (Fig. 3) with sometimes sacculate lamellae and accumulated starch as grains, numerous slightly elongated mitochondria with an average diameter of 0.5 μm having well-defined membranes and cristae like those of the synergids, rare dictyosomes, some lamellar RER and many ribosomes, single as well as clustered (Fig. 4).

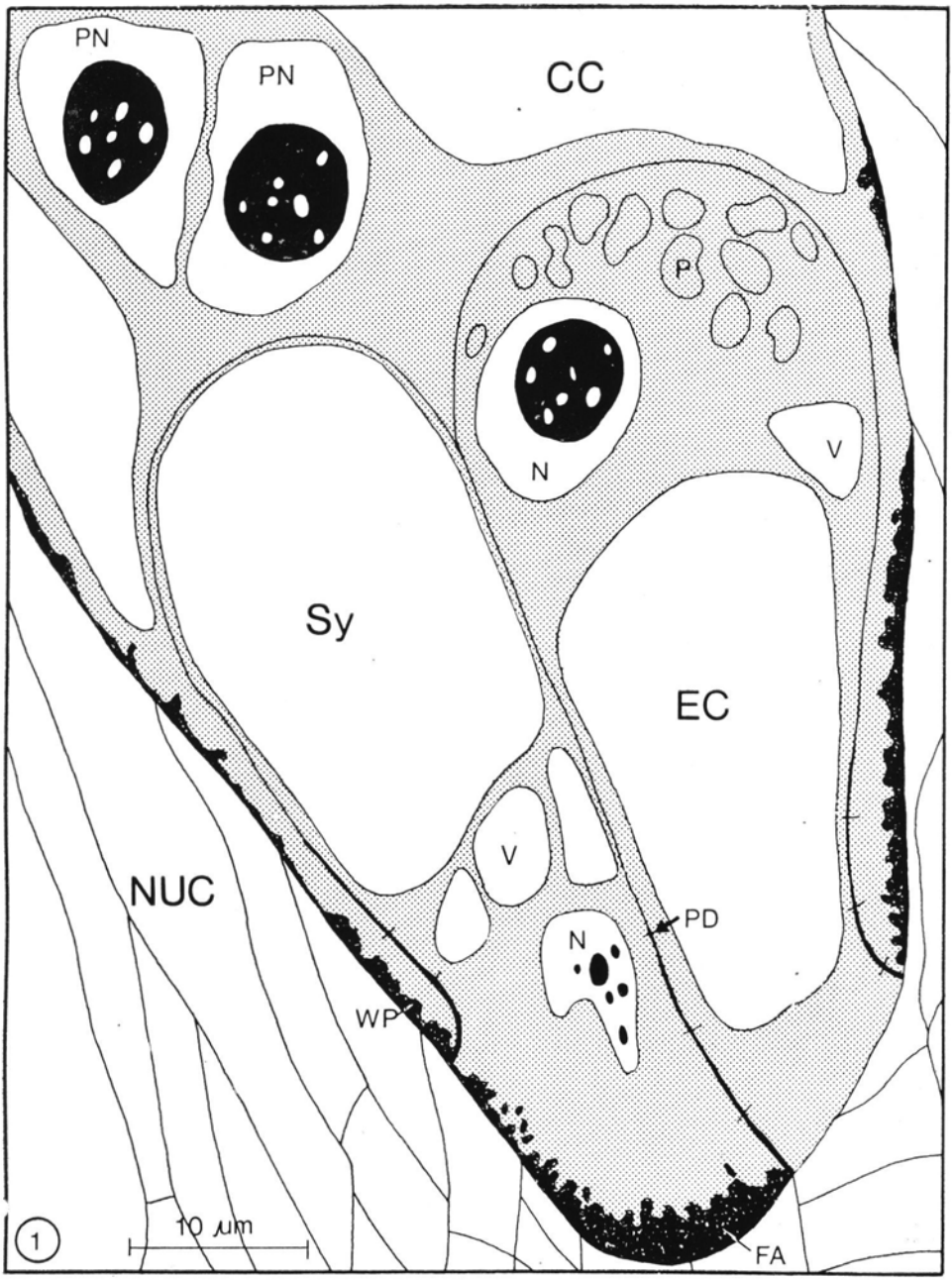

Fig. 1. Longitudinal diagrammatic representation of micropylar part of mature embryo sac of *Spinacia oleraceae*. Note opposite polarity of egg cell and synergid (2400 ×)

CC — central cell, EC — egg cell, FA — filiform apparatus, N — nucleus, NUC — nucelus, P — plastid, PN — polar nuclei, PD — plasmodesmata, Sy — synergid, V — vacuole, WP — wall projection.

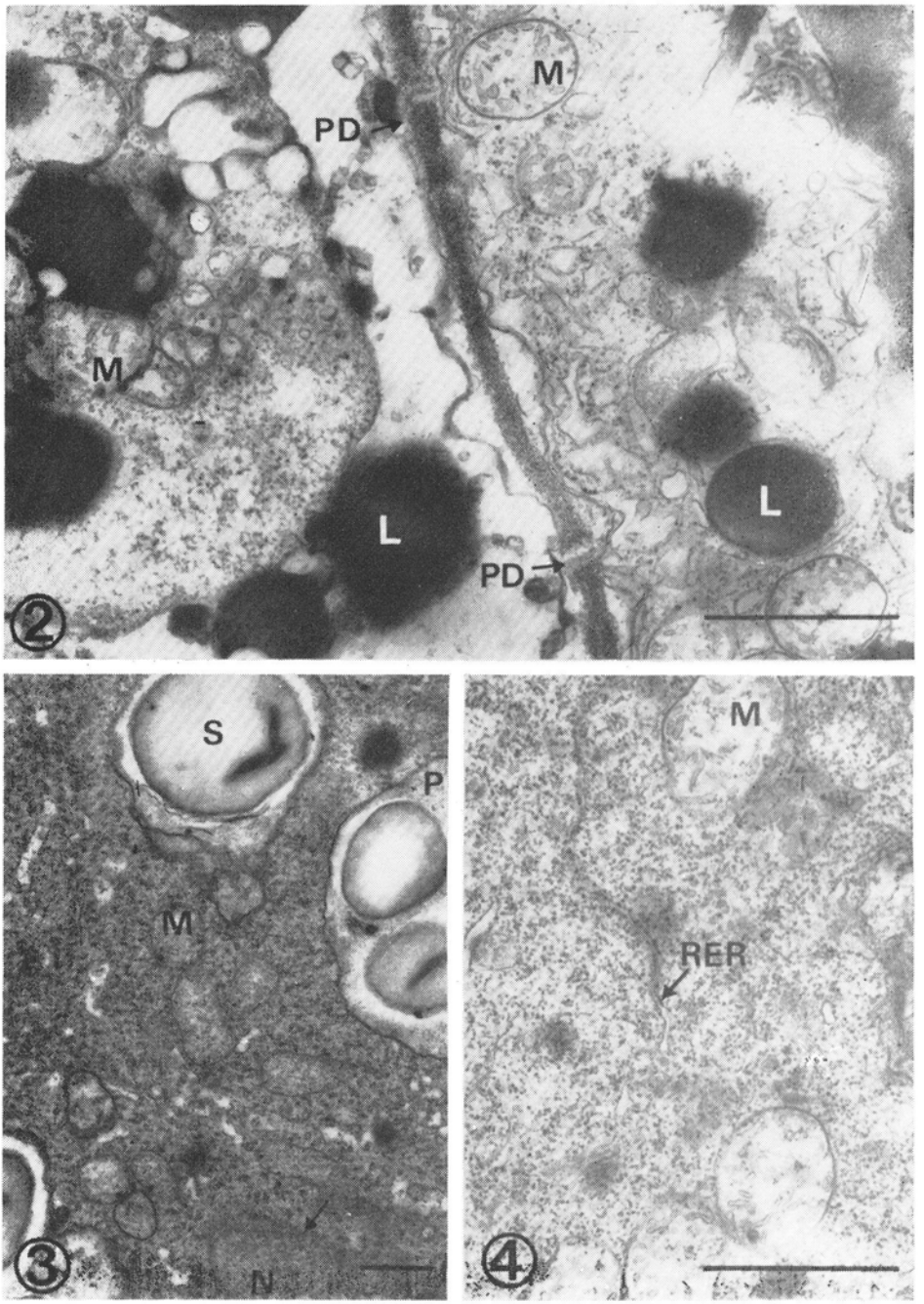

Fig. 2. Cytoplasm of persistent and degenerated synergid. Plasmodesmata are present in their common wall .In degenerated synergid electron-dense material accumulates in mitochondria (27.000 ×). Fig. 3. Chalazal cytoplasm of egg cell. Note the pores in nuclear envelope (arrow) (10.000 ×). Fig. 4. Micropylar cytoplasm of egg cell (27.000 ×).

DS — degenerated synergid, L — lipid body, M — mitochondrium, N — nucleus, P — plastid, PD — plasmodesmata, RER — rough endoplasmic reticulum, S — starch.

During the later part of maturation of the egg cell the initially small strand of micropylar cytoplasm increases in which mitochondria become clustered (Fig. 4).

DISCUSSION

When the constituents of the egg apparatus are organized, each cell is surrounded by a wall (Wilms, 1981), whereas at maturity the walls at the chalazal end are absent. It seems that the cell growth first causes thinning and subsequently, total disappearance of the wall in the chalazal parts, bordering the central cell region. It means that at the fertilizable stage the egg and central cells can be penetrated easily for a brief period by sperm cells, as soon after successful fertilization cell wall formation occurs around the chalazal part of the zygote (Wilms unpublished).

The nuclear and cytoplasmic ultrastructure of the cells of the egg apparatus of spinach differ from most other studied species. The nucleus of the synergid becomes elongated and loses its nucleolus, whereas in other species the nucleus remains spherical (Jensen, 1974; Mogensen, Suthar, 1979). The nucleus of the egg cell however, has an ultrastructure similar to that of cotton (Jensen, 1965). *Capsella* (Schulz, Jensen, 1968b), *Petunia* (Van Went, 1970), *Quercus* (Mogensen, 1972), *Nicotiana* (Mogensen, Suthar, 1979) and others, but the occurrence of myeline-like bodies has not been recorded before the fusion of nuclei. The mitochondria of the synergid are distributed uniformly throughout the cytoplasm, rather than being concentrated near the FA as in cotton (Jensen, 1965), *Capsella* (Schulz, Jensen, 1968a) and *Zea* (Diboll, Larson, 1966). Occurrence of plastids with large starch grains in the egg cell is unusual. Most species have starch only in the central cell (Jensen, 1965; Schulz, Jensen, 1968b; van Went, 1970) or in the synergids (Mogensen, 1972). The tremendous increase of mitochondria in the egg cell shortly before fertilization has also been reported in *Zea mays* (Diboll, Larson, 1966). This might indicate a potential for high metabolic activity generally associated with post-fertilization events.

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